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# Noisy Galvanic Vestibular Stimulation Promotes GABA Release in the Substantia Nigra and Improves Locomotion in Hemiparkinsonian Rats

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## Abbreviations

SVS stochastic vestibular stimulation, SN substantia nigra, ST striatum, PPN pedunculopontine nucleus, VM ventromedial thalamus, 6-OHDA 6-hydroxydopamine

## Abstract

**Background:** The vestibular system is connected to spinal, cerebellar and cerebral motor control structures and can be selectively activated with external electrodes. The resulting sensation of disturbed balance can be avoided by using stochastic stimulation patterns. Adding noise to the nervous system sometimes improves function. Small clinical trials suggest that stochastic vestibular stimulation (SVS) may improve symptoms in Parkinson's disease. We have investigated this claim and possible mechanisms using the 6-hydroxydopamine (6-OHDA) hemilesion model of Parkinson's disease.

**Methodology/Principal findings:** Animals were tested in the accelerating rod test and the Montoya staircase test of skilled forelimb use. In 6-OHDA hemilesioned animals, SVS improved rod performance by  $56 \pm 11$  s. At group level L-DOPA treatment had no effect, but positive responders improved time on rod by  $60 \pm 19$  s. Skilled forelimb use was not altered by SVS. To investigate how SVS may influence basal ganglia network activity, intracerebral microdialysis was employed in four regions of interest during and after SVS. In presence of the GABA transporter inhibitor NNC 711, SVS induced an increase in GABA to  $150 \pm 15\%$  of baseline in the substantia nigra (SN) of unlesioned animals, but had no effect in the pedunculopontine nucleus, the striatum or the ventromedial thalamus. Dopamine release remained stable in all areas, as did GABA and amine concentrations in the SN of unstimulated controls. Following SVS, a sustained increase in GABA concentrations was observed in the ipsilesional, but not in the contralesional SN of 6-OHDA hemilesioned rats. In contrast, L-DOPA treatment produced a similar increase of GABA in the ipsi- and contralesional SN.

**Conclusion/Significance:** SVS improves rod performance in a rat model of Parkinson's disease, possibly by increasing nigral GABA release in a dopamine independent way. We propose that SVS could be useful for treating symptoms of Parkinson's disease.

**Keywords:** Stochastic vestibular stimulation; Parkinson's disease; GABA release; 6-hydroxydopamine; substantia nigra; stochastic resonance; Montoya staircase; Rotarod

## Introduction

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4 Standard Parkinson's disease treatments with dopaminergic drugs, and sometimes deep brain  
5 stimulation, often fail to alleviate axial rigidity and gait problems. Axial symptoms are  
6 strongly related to the risk of falls and better treatment options could be of great benefit to a  
7 large group of patients with Parkinson's disease [1].  
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12 Recent findings suggest that a sufficient level of noise may be necessary for normal function  
13 of the central nervous system [2,3,4,5,6]. Under some circumstances, adding noise to a system  
14 will result in improved signal detection or linearity of responses [7,8,9,10]. This phenomenon  
15 can be predicted from mathematical models and is often referred to as stochastic resonance, or  
16 in a wider sense, noise benefit [11]. Several recent studies describe cross-modal noise benefit  
17 in cognitive tasks in healthy subjects [12,13,14,15,16], indicating that noise benefit is not  
18 restricted to signal detection but can be observed also when the outcome is a complex and  
19 abstract behaviour. In a computational model of the degenerating brain, higher levels of  
20 external noise were needed for optimal function under conditions representing aging and loss  
21 of plasticity [4]. This could imply that the effects of neurodegeneration can be counteracted to  
22 some extent by introducing more noise in the CNS.  
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34 Noisy sensory signals can be transmitted to the CNS through any sensory pathway, but  
35 vestibular pathways have some characteristics that may be particularly useful for modulating  
36 motor deficits. Neurons in the vestibular brainstem nuclei influence axial motor functions, eye  
37 movements, autonomic cardiovascular reflexes, as well as spatial perception. Several cortical  
38 regions respond to vestibular stimulation [17] indicating widespread indirect effects. Galvanic  
39 stimulation of the vestibular system using DC current has been used for over a hundred years  
40 and is safe, but leads to disturbed posture and balance. However, by using stochastic current  
41 patterns, it is possible to activate the vestibular system without such adverse effects  
42 [18,19,20,21]. Such stochastic vestibular stimulation (SVS) can improve balance in healthy  
43 subjects [22,23]. In patients with neurodegenerative disease, like Parkinson's disease, there is  
44 some evidence that SVS improves motor functions [19,24], autonomic reflexes and cognitive  
45 executive control [19]. Static postural sway is also reduced by SVS in Parkinson disease  
46 patients [25]. It is not known if there is a general mechanism for noise benefit in higher  
47 functions. A model involving altered dopamine release has been proposed [26], but  
48 experimental evidence to support that is lacking [27].  
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1 We used the 6-hydroxydopamine (6-OHDA) hemilesioned rat model to determine if SVS can  
2 improve motor functions in dopamine-deficient rats. Furthermore, we investigated how SVS  
3 might influence basal ganglia networks under normal and dopamine deficient conditions by  
4 measuring amino acid and dopamine release in intact and 6-OHDA hemilesioned rats.  
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10 Four nuclei that relay or mediate basal ganglia signalling were investigated in intact rats: the  
11 substantia nigra (SN), the striatum (ST), the pedunclopontine nucleus (PPN) and  
12 ventromedial thalamus (VM). Dopamine release in the ST and SN is known to facilitate  
13 movement initiation and to occur during motor activity [28,29,30]. The output from the basal  
14 ganglia is inhibitory and mainly mediated by GABA-ergic neurons projecting from the  
15 internal globus pallidus (entopeduncular nucleus in rodents) and substantia nigra *pars*  
16 *reticulata* to thalamus and brainstem nuclei including the PPN. The PPN is of particular  
17 interest as it responds to a large number of sensory stimuli and is involved in gait initiation  
18 [31]. Furthermore cholinergic/glutamatergic PPN neurons project to the substantia nigra and  
19 influence motor functions [32].  
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31 In line with previous clinical studies, we report improved locomotion in hemilesioned rats  
32 during SVS, but we were unable to demonstrate a change in skilled forepaw use. In  
33 unlesioned rats, SVS increased extracellular GABA concentrations only in the SN. The other  
34 investigated neurotransmitters were not significantly altered in the SN, ST, PPN and VM. In  
35 hemilesioned animals, L-DOPA injections induced similar GABA increases in the ipsi- and  
36 contralesional SN, whereas SVS induced differential effects in the two SN, suggesting that  
37 altered nigral GABA release has a role for the behavioural effects of SVS in 6-OHDA  
38 hemilesioned rats, possibly by decreasing the inhibitory activity of the ipsilesional SN *pars*  
39 *reticulata* neurones.  
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## 49 **Results**

### 50 Effects of SVS and L-DOPA on rotarod performance in 6-OHDA and sham hemilesioned rats

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56 Unilateral 6-OHDA lesion resulted in reduced performance on the rotarod. The total time on  
57 the rod decreased by 48% from 472±80s before the lesion to 266±86s after (-206±45s , paired  
58 t-test,  $p<0.01$ ). In contrast, the sham procedure did not significantly reduce the total time on  
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1 the rod (pre-sham performance:  $544\pm 135$ s, post-sham performance:  $430\pm 112$ s, paired t-test  
2  $p=0.1004$ ).  
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5 Because individual animals differ slightly in performance from day to day the effect of  
6 treatments were evaluated in comparison to a baseline performance obtained immediately  
7 before intervention. Treatment or sham treatment was administered in a balanced  
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9 pseudorandomized order so each animal received either sham treatment or active treatment  
10 first, and the remaining intervention in a separate trial. Baseline performances immediately  
11 before SVS and shamSVS did not differ ( $272\pm 75$ s and  $279\pm 77$ s, respectively, paired t-test,  
12  $p=0,526$ ), and this was the case also with baseline performances immediately before L-DOPA  
13 and NaCl administration ( $265\pm 62$ s and  $244\pm 57$ s, respectively, paired t-test,  $p=0,511$ ).  
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15 6-OHDA hemilesioned animals increased the time spent on rod during SVS compared to  
16 baseline significantly more than during sham SVS ( $\Delta t = 56\pm 11$ s vs.  $\Delta t = 24\pm 11$ s, paired t-test,  
17  $p=0.011$ ). The improvement observed during SVS corresponded to an increase in  
18 performance of  $24\pm 6\%$  of the immediately preceding baseline performance. As changes in  
19 neurotransmission were sustained (see below), we also analyzed if the order of treatment had  
20 an effect. Analysis of variance with treatment and test day order as independent factors  
21 revealed no effect of test day order,  $F(1,8)= 0.0003$ ,  $p=0.987$ , failing to show a carry-over  
22 effect of SVS to the following test session. At group level, L-DOPA treatment did not  
23 improve the mean time spent on the rod relative to baseline and was in that respect not better  
24 than sham treatment with a saline injection (paired t-test,  $p=0.818$ , Fig. 2). At the individual  
25 level, however, animals could be categorized as positive responders to L-DOPA ( $n=3$ ) or  
26 negative responders ( $n=3$ ). The positive responders increased time on rod by  $60\pm 19$ s  
27 compared to the preceding baseline and this was nearly significant ( $p=0.067$ , paired t-test)  
28 compared to saline injection ( $-5\pm 7$ s). In negative responders in contrast time on rod changed  
29 by  $-63\pm 15$ s compared to the preceding baseline in response to L-DOPA and by  $11\pm 30$ s in  
30 response to vehicle (not significant).  
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51 Sham-lesioned animals ( $n=5$ ) did not perform better during SVS than no SVS (paired t-test  
52 for change from preceding baseline,  $p=0.8206$ ,  $n=5$ . Fig. 2). Similar to L-DOPA treated  
53 hemilesioned animals the response to SVS was variable in this group. One animal improved  
54 by 192 s, and another deteriorated by 138s during SVS. The other three retained stable  
55 performance.  
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### Effects of SVS on skilled forelimb use

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4 Forelimb use was evaluated with the Montoya staircase test. As expected there was a  
5 significant decrease in the ratio of pellets picked up with the contralesional forelimb following  
6 a 6-OHDA hemilesion ( $0.93 \pm 0.1$  prelesion vs.  $0.65 \pm 0.1$  postlesion, paired t-test,  $p=0.035$ ).  
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8 No statistical difference was found between the total number of pellets retrieved before and  
9 after the 6-OHDA lesion. Unlike 6-OHDA hemilesioned animals, the sham-lesioned animals  
10 retrieved a higher number of pellets after the lesion procedure than before, suggesting  
11 continued performance improvement, and they did not develop a side preference (Table 2).  
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13 For technical reasons we were unable to administer SVS during the skilled forelimb task, and  
14 we did not observe any change in the total number of retrieved pellets or the ratio retrieved  
15 with the contralesional forelimb when animals were tested immediately after a 30 minutes  
16 SVS-session (Table 2).  
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### Effects of SVS on dopamine and amino acids in un-lesioned animals

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29 We used un-lesioned animals to broadly investigate if SVS induces any detectable changes in  
30 neurotransmitter release in four key brain regions in, or connected to, the basal ganglia (Fig.  
31 3). Extracellular concentrations of dopamine, dopamine metabolites and amino acids were  
32 sampled from microdialysis probes in the substantia nigra (SN), the striatum (ST), the  
33 pedunculopontinenucleus (PPN) and the ventromedial thalamus (VM) before, during and after  
34 SVS. The GABA transporter inhibitor NNC 711 was retrodialysed to amplify synaptic release  
35 of GABA as we have previously demonstrated that rapid increases in GABA transmission can  
36 otherwise go undetected [33].  
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45 Following SVS in un-lesioned animals, dialysate GABA concentrations from the SN  
46 increased to  $150 \pm 15\%$  of baseline (at  $T=150$  min), while GABA concentrations in control  
47 animals that received no SVS were stable (Fig. 4A, Two way ANOVA, main effect of  
48 treatment  $F(1,26)=14.41$ ,  $p=0.0022$ , time  $F(2,26)=3.83$ ,  $p=0.035$  and interaction treatment  $\times$   
49 time  $F(2,26)=3.53$ ,  $p=0.044$ ). The deadspace of tubings introduced a delay of approximately  
50 20-30 minutes in the microdialysis measurements. This means that the observed increase in  
51 GABA started early during SVS-stimulation and persisted for at least 30 minutes after  
52 stimulation was terminated. DA concentrations remained stable in the SN and ST of SVS-  
53 treated animals during SVS and the following 60 min (suppl Fig. S1).  
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1 DA concentrations were below detection levels (0.05 nM) in the PPN and VM throughout the  
2 experiment. No significant changes in GABA concentrations were measured in the PPN, VM,  
3 or the striatum following SVS in un-lesioned animals (Fig. 4B). Concentrations of glutamate,  
4 aspartate, glycine, taurine, serine, alanine, DOPAC and HVA were not significantly affected  
5 by SVS in any of the investigated regions (not shown), but in the SN, there were large  
6 variations in glycine and glutamate concentrations after SVS (suppl Fig. S2).  
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### 13 Effects of SVS and L-DOPA treatment on dopamine and GABA in 6-OHDA hemilesioned 14 animals 15

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20 Based on our findings in unlesioned animals, dopamine and amino acid concentrations were  
21 measured in the bilateral SN of some hemilesioned 6-OHDA animals, during and after SVS  
22 and L-DOPA treatments. These animals were not tested behaviourally, but the degree of  
23 dopamine lesion was assessed after the experiment. The effects of treatments on the nigral  
24 concentrations of DA, GABA and glutamate were evaluated and compared between the  
25 ipsilesional and contralesional SN.  
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32 In the 6-OHDA hemilesioned animals there was a trend towards lower baseline GABA  
33 concentrations in the ipsilesional SN at the beginning of sampling, but the difference was not  
34 significant (ipsi<sub>n=6</sub>: 24±6 nM vs. contra<sub>n=6</sub>: 50±20 nM,  $p=0.353$ , paired t-test. In unlesioned  
35 animals, n=15, absolute baseline concentrations were 50±17 nM).  
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42 Following SVS, the absolute GABA concentration changed differently in the two SN. In the  
43 ipsilesional SN GABA concentrations increased and in the contralesional SN they tended to  
44 fall. This differential effect is expressed as a significant interaction between time and SN side  
45 in a two way ANOVA using the absolute GABA concentrations at t=90 to t=150 minutes as  
46 the independent variable (Fig. 5A,  $F(2,20)=4.89$ ,  $p=0.019$ ).  
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52 In contrast, L-DOPA treatment on day two produced roughly parallel changes in absolute  
53 GABA concentrations in the two SN, despite apparent asymmetries in the dopamine  
54 concentrations in the two SN following the L-DOPA injection (Fig. 5B). The relative changes  
55 in GABA concentrations in the bilateral SN following L-DOPA treatment (Fig. 6) was of  
56 similar magnitude to that observed after SVS in intact animals (Figs. 4, 6) and in the  
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1 ipsilesional SN, GABA concentrations (percent of baseline) were significantly increased  
2 compared to untreated control animals at T=120 (Fig. 6). Due to tubing deadspace, that  
3 timepoint corresponds to approximately 30-40 minutes after L-DOPA administration.  
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7 Like in unlesioned animals SVS did not alter DA concentrations in the bilateral SN of 6-  
8 OHDA hemilesioned animals (Fig. 5C). After the L-DOPA administration the DA  
9 concentrations increased in both ipsi- and contralesional SN and peaked in the first  
10 succeeding sample (T=90, Fig. 5D). This indicates that DA levels peaked within 10 minutes  
11 after the L-DOPA injection. The increase in DA following L-DOPA treatment was in  
12 absolute terms more than two times larger in the ipsilesional SN (ipsi<sub>n=4</sub>:  $+2.0\pm 0.3$  nM,  
13 contra<sub>n=5</sub>:  $+0.9\pm 0.2$  nM,  $p=0.028$ , unpaired t-test), but peak values were not significantly  
14 higher in the ipsilesional SN (ipsi<sub>n=4</sub>:  $2.3\pm 0.4$  nM, contra<sub>n=5</sub>:  $1.5\pm 0.2$  nM,  $p=0.119$ ). For  
15 technical reasons however, only two measurements could be obtained from the ipsilesional  
16 SN at T=90, so peak values from the ipsilesional SN may be underestimated.  
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27 Concentrations of the amino acid glutamate in dialysates from the two SN of 6-OHDA  
28 hemilesioned animals were also analysed. Following SVS, as well as L-DOPA injection, no  
29 significant changes or differences between the two sides were observed (suppl Fig 3.).  
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## 34 Discussion

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38 This is to our knowledge the first study to evaluate motor effects of stochastic vestibular  
39 stimulation (SVS) in rodents, and the first to explore the neurochemical effects of SVS *in*  
40 *vivo*. Stochastic resonance in CNS function has previously mainly been studied as a  
41 phenomenon, without trying to understand how activity patterns in the brain may change. This  
42 study demonstrates that SVS improves rod performance in hemilesioned rats. SVS was  
43 associated with increased GABA concentrations in the substantia nigra, but not with altered  
44 dopamine transmission in normal animals. In hemilesioned rats SVS increased GABA in the  
45 ipsilesional SN by a magnitude similar to that observed after L-DOPA treatment. As  
46 described previously [34], L-DOPA treatment increased nigral DA release. In contrast, SVS  
47 was not associated with altered dopamine release in lesioned animals.  
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56 For technical reasons we used two different stimulation setups and stimulation currents were  
57 not identical (suppl Fig. S5). The current used in behavioural experiments and microdialysis  
58 experiments in hemilesioned animals contained more frequencies over 30 Hz than the current  
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1 used in microdialysis in unlesioned animals. Although vestibular neurons can support activity  
2 frequencies well over 100Hz, the vestibular system is optimized for frequencies up to ~ 30Hz  
3 [35]. The low frequency content of the signal can therefore be expected to have a higher  
4 impact on brainstem activity. The neurochemical effects of the two currents were however  
5 similar, and importantly both protocols stimulated vestibular afferents in a way that did not  
6 cause observable nystagmus or balance deficits.  
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### 10 *Behavioural effects of SVS*

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18 Stochastic resonance (SR) is characterized by a biphasic response curve shaped as an inverted  
19 U [5]. The design of this study did not include more than one noise level per animal, so we  
20 cannot know if the observed improvement in rod performance involves SR. A SR  
21 phenomenon has been demonstrated previously for balance improvement with SVS [22], and  
22 adding noise with small amplitude, both electrical and mechanical, to proprioceptive  
23 pathways improves balance [36,37]. The observation that 6-OHDA hemilesioned, but not  
24 sham lesioned animals, improved rod performance can be interpreted as support for the notion  
25 that brains with impaired function display more noise benefit than the normally working brain  
26 [4,26,27]. The lack of improvement in sham lesioned animals is not likely to be only a ceiling  
27 effect, as the group response included both large improvement and impairment in response to  
28 SVS. Although SR can serve as a theoretical framework for understanding the effect of noisy  
29 sensory stimulation like SVS, it remains an unproved hypothesis that the effects of external  
30 noise on CNS functions involve SR. SVS could alternatively be viewed as a method to  
31 specifically activate the vestibular system without the adverse effects associated with  
32 stimulation with regular pattern.  
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47 SVS improved rod performance which is an integral measure of balance and locomotion.  
48 Improvements in time on an accelerating rod indicate not only improved endurance but also  
49 an increase in motor and balance functions, because the difficulty of the task increases  
50 gradually. The effect of SVS on rod performance was of similar magnitude as the effect of a  
51 single L-DOPA injection in positive responders, suggesting that the efficacy of SVS in terms  
52 of improved rod performance is not marginal. Furthermore, unlike L-DOPA treatment, SVS  
53 improved the rod performance in all lesioned animals.  
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1 The vestibular pathways are mainly influencing axial motor systems and a stronger effect of  
2 SVS on balance and locomotion than on appendicular motor control can therefore be  
3 expected. As there are reports of cross modal function improvements following noisy sensory  
4 stimulation [e.g. 16,38], we hypothesized that skilled forelimb use would also improve in  
5 response to SVS. We were, however, unable to demonstrate any effect of SVS in the Montoya  
6 stair case test. It therefore appears that noise benefit did not spread to forepaw motor function.  
7 Because SVS could not be administered in the Montoya box, an alternative explanation is that  
8 the effect of SVS wanes off rapidly. However, microdialysis measurements show that the  
9 increase in nigral GABA release is sustained for at least 30 minutes after SVS, making that  
10 explanation for the lack of observed effects on skilled forelimb use less likely. Future studies  
11 should also evaluate neurotransmission in the entopeduncular nucleus, the rodent equivalent  
12 of globus pallidus *pars interna*, an output structure which is analogue to substantia nigra *pars*  
13 *reticulata* and in more direct control of appendicular motor programs in humans [39].  
14 A positive effect of SVS on axial muscle functions may have some clinical implications  
15 because axial rigidity and imbalance in Parkinson's disease are often difficult to control with  
16 L-DOPA and subthalamic DBS [40,41].  
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### 31 *The effects of SVS on neurotransmission*

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34 The detection of rapid increases in GABA release with microdialysis sampling is improved by  
35 using a GABA re-uptake inhibitor (NNC 711) in the perfusion fluid [33]. One concern with  
36 this approach is that the re-uptake inhibitor may prolong and extend the increase in GABA  
37 concentrations and that this could lead to secondary changes in network activity. The increase  
38 in nigral GABA release following SVS is, however, not a long term effect of NNC 711  
39 retrodialysis, because GABA concentrations remained stable in retrodialysed animals that did  
40 not receive SVS but were only retrodialysed with NNC 711 (Fig. 4A). Furthermore, the  
41 increase occurred selectively in the SN and not in the other investigated nuclei following the  
42 vestibular stimulation.  
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52 The microdialysis measurements did not reveal any other significant changes in  
53 neurotransmitter concentrations following SVS than the increase in nigral GABA. Because  
54 microdialysis measurements of amino acids do not necessarily reflect synaptic release activity  
55 [42], at least not without using a reuptake inhibitor or stimulated release, this does not exclude  
56 altered activity and release of neurotransmitters other than dopamine and GABA in the  
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1 investigated regions. In microdialysates from the SN, mean values of glutamate and glycine  
2 increased following SVS, but the change was not significant. It may be possible to determine  
3 if this represents an increase in afferent activity by measuring stimulated release or reuptake  
4 inhibitors. Immediate early gene expression may also be informative regarding by which  
5 mechanism nigral GABA release increases following SVS.  
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10 Nigrostriatal dopamine lesions lead to increased activity of the indirect loop and decreased  
11 activity of direct loop [43,44], and a decrease in GABA release in the SN can therefore be  
12 expected (Fig. 3). Increasing GABA release in the SN disinhibits the activation of motor  
13 programs and counteracts Parkinsonism. This is illustrated by the increase in nigral GABA  
14 concentrations that was observed following L-DOPA treatment, a treatment that is known to  
15 ameliorate Parkinsonism. There are obvious similarities between the nigral GABA increases  
16 observed after SVS in intact animals and the nigral increases in GABA after L-DOPA  
17 treatment of hemilesioned rats, not least regarding the timing of the GABA increase. Because  
18 SVS was not associated with altered dopamine release in ST (or SN) it appears that the SVS-  
19 induced increase in nigral GABA release is not mediated by the action of dopamine on  
20 striatonigral GABA neurons. It is beyond the scope of this article to determine which  
21 pathways may mediate the observed nigral GABA increase following SVS, but as indicated in  
22 Fig. 3, vestibular neurons project to the cerebellum and the *formatio reticularis*, and possibly  
23 the PPN; all structures that may directly or indirectly alter neurotransmission in the SN. The  
24 subthalamic nucleus was not investigated in this study but may be of particular interest as  
25 DBS of the subthalamic nucleus has well documented antiparkinsonian effects, and increases  
26 nigral GABA and glutamate release [45].  
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44 Surprisingly, SVS did not induce similar changes in GABA release in the ipsi- and  
45 contralesional SN. Despite the bilateral nature of SVS the changes in GABA release in the  
46 two SN were in opposite direction, towards a more balanced GABA release between the two  
47 SN. Although we have no explanation for this phenomenon, it may have some relevance for  
48 the consistent improvement in rod performance that was observed after SVS, but not after L-  
49 DOPA treatment. Because L-DOPA increases nigral GABA release bilaterally, it may not  
50 reduce the imbalance in locomotor functions that is detrimental for rod performance. This  
51 could perhaps also explain why the initial laterality of Parkinsonism remains clearly  
52 observable throughout the course of the disease also in optimally treated Parkinson's disease  
53 patients.  
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## Conclusion

SVS improves rod performance in 6-OHDA hemilesioned rats. We observed a prominent and sustained increase in GABA release in the SN following SVS, but no change in dopamine release in the SN or ST. We propose that vestibular stimulation leads to a dopamine independent disinhibition of basal ganglia output that could promote movement initiation. We suggest that it should be evaluated as a treatment alternative for Parkinson's disease, in particular with prominent axial involvement.

## **Materials and methods**

### Animals

Experimental design and procedures on unlesioned animals were carried out in compliance with the UK Animal (Scientific Procedures) Act 1986 (unlesioned animals) and approved by the UK Home Office under project license no 60/3334. Experimental design and procedures for experiments involving hemilesioned and sham lesioned animals were carried out in compliance with the European Communities Council Directive of November 24th, 1986 and approved by *Göteborgs djurförsöksetiska nämnd*, the local ethics committee in Gothenburg, Sweden, under project license no. 331/10. Experiments on unlesioned animals were performed using 18 male Lister-Hooded rats (120-150g, age 5-6 weeks, Charles River Ltd, UK), 10 of these received microdialysis probes in ST/SN and 8 received probes in PPN/VM. Experiments with 6-OHDA and sham-lesioned animals were performed using 32 female Sprague-Dawley rats (150-200g, age 6-8 weeks, Charles River, Germany). For the microdialysis tests 12 of these were lesioned and 4 were sham lesioned. The other 16 animals were used for behavioural tests subsequent to a hemilesion in 11 of them and a sham-hemilesion in 5 of them. Animals were housed 4-6 per cage under standard controlled environmental conditions. After implantation of microdialysis guides they were single-caged for the remainder of the experiment.

## Surgical procedures

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3 All procedures were performed in deep surgical anaesthesia (1.7-2.5% isoflurane). The  
4 surgical area was shaved and disinfected before lidocaine (1%) was infused subcutaneously  
5 for pre-emptive analgesia. Ketoprofen (5 mg/kg, s.c.) was administered as needed for post  
6 surgical analgesia.  
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## 6-hydroxydopamine hemilesion

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12 The animal was placed in a stereotactic frame with bregma and lambda in the horizontal  
13 plane. After exposure of the skull, a hole was drilled over the medial forebrain bundle (4.2  
14 mm posterior and +/- 1.2 mm lateral to bregma). A 150 µm diameter fused silica capillary was  
15 lowered 8 mm below the dura mater and 10 µg of 6-OHDA (Sigma Aldrich) dissolved in  
16 0.9% NaCl, 0.3% ascorbate, 5 µg/µl, was injected over 2 minutes. The capillary was slowly  
17 removed after another minute and the brain surface was covered with periosteal membrane  
18 before closing the wound. Sham-treated animals received the saline ascorbate vehicle only.  
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## Implantation of vestibular electrodes

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32 Bilateral vestibular stimulation electrodes were attached by securing the 1 mm peeled end of a  
33 teflon coated stainless steel wire (0.2 mm diameter) over the horizontal canals of the two  
34 labyrinths by pushing short wire loops through the most ventral ends of the bilateral petrosal  
35 crests. The electrode wires were fixed to an acrylic cement foundation on the skull and  
36 externalized. Animals receiving microdialysis implants were implanted with electrodes in the  
37 same surgical session.  
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## Microdialysis probe implantation

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49 The unlesioned animals were stereotactically implanted with guide cannulae two days before  
50 microdialysis experiments. The skull was exposed and holes were drilled over two of the  
51 target nuclei. Targets were identified in the Paxinos and Watson rat brain atlas [46] and the  
52 coordinates relative to bregma were for striatum: +1.0 mm anterior, 2.8 mm lateral, for  
53 substantia nigra: 5.3 mm posterior, 2.1 mm contralateral, for the pedunculopontine nucleus:  
54 8.0 posterior, 1.8 mm lateral, and for the ventromedial thalamus 2.6 posterior, 1.5 mm  
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contralateral). Holes were also drilled in the parietal bones to fasten two stainless steel jewellers' screws. The dura was opened and the underlying pial membrane was opened by pulling it gently with fine forceps. Microdialysis guides (MAB 4.15.IC, Microbiotech, Sweden) were slowly implanted to a depth immediately above the target nucleus (6.0 mm from the brain surface for the pedunclopontine nucleus and ventromedial thalamus, 3.2 mm and 6.2 mm for the striatum and the substantia nigra, respectively). The guide cannulae were secured to the skull with acrylic dental cement.

Some 6-OHDA hemilesioned animals were chronically implanted with in-house constructed microdialysis probes positioned bilaterally in the substantia nigra as described previously [47] and provided with vestibular electrodes as described above. Animals were allowed to recover for 24-48h before the first microdialysis experiment.

### Microdialysis

Two days after implanting the microdialysis guide cannulae, the unlesioned animals were briefly anaesthetized to insert bilateral microdialysis probes (Cuprothane membranes extending 1 mm, or with striatum guides, 3 mm, below the guide, MAB 4.15, Microbiotech, Sweden). The probes were continuously perfused with a Ringer solution containing 140 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl<sub>2</sub> and 1.0 mM MgCl<sub>2</sub> and a GABA reuptake inhibitor, NNC 711 (30 µM, Tocris Cookson Ltd, UK). The animals were allowed to recover and were then kept in a standard microdialysis bowl, with the fluid lines from the microdialysis probes connected to the external equipment via a swivelled balance arm. The perfusion rate was kept at 4.0 µl/min for one hour and was then lowered to 1.5 µl/min. Baseline sampling commenced after 2 hours of perfusion. Microdialysis samples were collected every 30 minutes. After two baseline samples, stochastic vestibular stimulation commenced. Microdialysis perfusion continued throughout the 30 minute stimulation period, and for another 60 minutes, providing a total of 5 microdialysis samples from each probe. The samples were split and analyzed for amino acid content as described previously [33], and for amine content by two-dimensional HPLC with electrochemical detection as described by Lindgren et al. [34].

The 6-OHDA hemilesioned animals were subjected to a repeat microdialysis experiment on the following day. The experiment protocol was as described above, but instead of a 30

1 minutes stimulation period, they were injected with L-DOPA and benserazide (6 mg/kg and  
2 12 mg/kg, respectively, i.p.) after the second sampling period.  
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5 After the last microdialysis experiment animals were euthanized and the brain was removed  
6 and fixed in paraformaldehyde. The brains were sectioned and probe locations were  
7 determined with reference to the Paxinos and Watson rat brain atlas [46]. Results from probes  
8 with less than 50% of the active membrane in the target nucleus were excluded. This reduced  
9 the final n of investigated brain areas to 5 for PPN and VM and 10 for ST, 9 for SN  
10 respectively in intact animals. In 6-OHDA lesioned animals, a total of 6 probes were included  
11 in the lesioned SN, and 6 in the unlesioned. The positions of the active membranes of all  
12 included probes are indicated in supplementary Fig. S4.  
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### 21 Stimulation protocol

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25 Current was delivered through the bilateral electrodes using two setups. The stochastic  
26 waveforms delivered with these setups were similar to each other. Unlesioned animals were  
27 stimulated using two stimulus isolators (NeuroLog NL800, Digitimer Ltd. Hertfordshire, UK)  
28 connected to a biphasic pulse buffer unit (NeuroLog NL512) and a stimulus pattern fed from a  
29 voltage source pulse generator or a DAT recorder with a prerecorded stochastic stimulation  
30 (<30 Hz) pattern. For lesioned animals a bipolar analogue stimulus isolator (model 2200 A-M  
31 Systems, Sequim, Washington, U.S.A ) was used, and the stimulus pattern was fed from a low  
32 pass (<30 Hz) filtered voltage source generator (BitScope 100 with BitGen coprocessor,  
33 Metachip Pty. Ltd., Australia), which was programmed to generate sinusoid or white noise  
34 patterned voltages.  
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45 Bipolar 1 Hz sinusoid stimulation pattern was used to determine an appropriate maximum  
46 current. The amplitude was slowly increased until a 1 Hz rocking of the animals head was  
47 clearly visible. The lowest amplitude A that reproducibly produced head rocking was used as  
48 maximum amplitude during the stochastic vestibular stimulation period of the experiment.  
49 The mean maximum stimulation amplitudes are given in Table 1, and were typically between  
50 0.2 and 0.6 mA. The stochastic stimulation protocol consisted of a 30 minutes sequence  
51 containing bipolar current with random amplitude between (-A) and (+A) and random  
52 frequencies <30 Hz. Examples of the stimulation currents are given in supplemental Fig S5.  
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### Motor behaviour – Rotarod

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Animals were trained to run on a 6 cm accelerating rod. Training and a set of prelesion baseline tests took place before any surgical procedure, and animals were re-tested three weeks after sham/6-OHDA lesion procedures. Each baseline test and experimental test episode consisted of four 10 minute long accelerations (5-40 rpm). The mean time on rod during each test episode was determined as previously described [28]. Three to five days after implantation of bilateral vestibular electrodes the animals were baseline tested and after 30 minutes tested again with or without vestibular stimulation.

Stimulation was started 30 minutes before the first of four rod session, and continued throughout testing. This procedure was repeated for two days in a counterbalanced order so that each animal was tested one day with and one day without stimulation. The same procedure was carried out over an additional two day period, this time with either an L-DOPA and benserazide intraperitoneal injection (6 mg/kg and 12 mg/kg respectively) or a sham injection (NaCl 0,9%) given in a counterbalanced order (Fig. 1). The change in performance invoked by the SVS or no SVS condition was calculated with reference to the baseline performance on the same day. 6-OHDA-lesioned animals with less than 70% dopamine depletion (n=5), as revealed by port-mortem analysis, were excluded from the Rotarod data analysis.

### Motor behaviour - Montoya staircase test

Two weeks before the hemilesion procedure, rats were food restrained to 90-95% of free feeding weight and trained to retrieve sugar pellets (45 mg each; BioServ, Frenchtown, NJ, U.S.A.) in a Montoya staircase apparatus (9x6x30 cm, Campden Instruments Ltd, Loughborough, UK). Training took place between 9am – 1pm, over a period of 5 days and consisted of 2x15 min sessions in the box with 1h 45 min rest between sessions. The box was covered with a dark cloth to maintain a dark and constant environment throughout training and testing. Animals which after initial training retrieved at least 9 pellets per side were included in the experiment. Seven days after implantation of vestibular electrodes the rats were food restrained and baseline tested. They were tested again over the following two days with and without vestibular stimulation in a counterbalanced order. Stimulation was applied for 30 minutes immediately before the animal was transferred to the test box. 6-OHDA

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lesioned animals with less than 70% dopamine depletion in the post mortem analysis were excluded from analysis.

### Statistical analysis

When appropriate, microdialysate concentrations were transformed to percent of baseline to reduce the effects of recovery variations. The treatment effect on neurotransmitter concentrations was evaluated by repeated measure two-way ANOVA with treatment and time as independent factors or with one sample t-tests with 100% as the theoretical mean, as appropriate. Locomotion effects were evaluated with paired t-tests comparing the change in time (s) on rod observed during stimulation or no-stimulation conditions. On the Montoya staircase, the overall number of sugar pellets consumed, total number of pellets on each side as well as the ratio between number of pellets eaten from impaired (contralesional) side and non-impaired (ipsilesional) side after vestibular stimulation or after no stimulation, were evaluated by paired t-tests. Data are given as mean±SEM, and  $p \leq 0.05$  was considered statistically significant.

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## References

- 1  
2  
3 1. Bloem BR, Hausdorff JM, Visser JE, Giladi N (2004) Falls and freezing of gait in Parkinson's  
4 disease: a review of two interconnected, episodic phenomena. *Mov Disord* 19: 871-884.
- 5  
6 2. Aihara T, Kitajo K, Nozaki D, Yamamoto Y (2010) How does stochastic resonance work within the  
7 human brain? - Psychophysics of internal and external noise. *Chemical Physics* 375: 616-624.
- 8  
9 3. Hospedales TM, van Rossum MC, Graham BP, Dutia MB (2008) Implications of noise and neural  
10 heterogeneity for vestibulo-ocular reflex fidelity. *Neural Comput* 20: 756-778.
- 11  
12 4. Li SC, von Oertzen T, Lindenberger U (2006) A neurocomputational model of stochastic resonance  
13 and aging. *Neurocomputing* 69: 1553-1560.
- 14  
15 5. McDonnell MD, Abbott D (2009) What is stochastic resonance? Definitions, misconceptions,  
16 debates, and its relevance to biology. *PLoS Comput Biol* 5: e1000348.
- 17  
18 6. Yu XL, Lewis ER (1989) Studies with spike initiators: linearization by noise allows continuous  
19 signal modulation in neural networks. *IEEE Trans Biomed Eng* 36: 36-43.
- 20  
21 7. Funke K, Kerscher NJ, Worgotter F (2007) Noise-improved signal detection in cat primary visual  
22 cortex via a well-balanced stochastic resonance-like procedure. *Eur J Neurosci* 26: 1322-1332.
- 23  
24 8. Wells C, Ward LM, Chua R, Timothy Inglis J (2005) Touch noise increases vibrotactile sensitivity  
25 in old and young. *Psychol Sci* 16: 313-320.
- 26  
27 9. Zeng FG, Fu QJ, Morse R (2000) Human hearing enhanced by noise. *Brain Res* 869: 251-255.
- 28  
29 10. Collins JJ, Imhoff TT, Grigg P (1996) Noise-enhanced tactile sensation. *Nature* 383: 770-770.
- 30  
31 11. Kosko B (2006) *Noise: Viking Adult*.
- 32  
33 12. Smith PF, Geddes LH, Baek JH, Darlington CL, Zheng Y (2010) Modulation of memory by  
34 vestibular lesions and galvanic vestibular stimulation. *Front Neurol* 1: 141.
- 35  
36 13. Usher M, Feingold M (2000) Stochastic resonance in the speed of memory retrieval. *Biological*  
37 *Cybernetics* 83: L11-16-L11-16.
- 38  
39 14. Wilkinson D, Ko P, Kilduff P, McGlinchey R, Milberg W (2005) Improvement of a face  
40 perception deficit via subsensory galvanic vestibular stimulation. *Journal of the International*  
41 *Neuropsychological Society: JINS* 11: 925-929.
- 42  
43 15. Wilkinson D, Nicholls S, Pattenden C, Kilduff P, Milberg W (2008) Galvanic vestibular  
44 stimulation speeds visual memory recall. *Exp Brain Res* 189: 243-248.
- 45  
46 16. Wilkinson D, Zubko O, Degutis J, Milberg W, Potter J (2010) Improvement of a figure copying  
47 deficit during subsensory galvanic vestibular stimulation. *J Neuropsychol* 4: 107-118.
- 48  
49 17. Lobel E, Kleine JF, Bihan DL, Leroy-Willig A, Berthoz A (1998) Functional MRI of galvanic  
50 vestibular stimulation. *J Neurophysiol* 80: 2699-2709.
- 51  
52 18. Mian OS, Day BL (2009) Determining the direction of vestibular-evoked balance responses using  
53 stochastic vestibular stimulation. *J Physiol* 587: 2869-2873.
- 54  
55  
56  
57  
58  
59  
60  
61  
62  
63  
64  
65

19. Yamamoto Y, Struzik ZR, Soma R, Ohashi K, Kwak S (2005) Noisy vestibular stimulation improves autonomic and motor responsiveness in central neurodegenerative disorders. *Ann Neurol* 58: 175-181.
20. Pavlik AE, Inglis JT, Lauk M, Oddsson L, Collins JJ (1999) The effects of stochastic galvanic vestibular stimulation on human postural sway. *Exp Brain Res* 124: 273-280.
21. Scinicariello AP, Inglis JT, Collins JJ (2002) The effects of stochastic monopolar galvanic vestibular stimulation on human postural sway. *J Vestib Res* 12: 77-85.
22. Mulavara AP, Fiedler MJ, Kofman IS, Wood SJ, Serrador JM, et al. (2011) Improving balance function using vestibular stochastic resonance: optimizing stimulus characteristics. *Exp Brain Res* 210: 303-312.
23. Scinicariello AP, Eaton K, Inglis JT, Collins JJ (2001) Enhancing human balance control with galvanic vestibular stimulation. *Biol Cybern* 84: 475-480.
24. Pan W, Soma R, Kwak S, Yamamoto Y (2008) Improvement of motor functions by noisy vestibular stimulation in central neurodegenerative disorders. *J Neurol* 255: 1657-1661.
25. Pal S, Rosengren SM, Colebatch JG (2009) Stochastic galvanic vestibular stimulation produces a small reduction in sway in Parkinson's disease. *J Vestib Res* 19: 137-142.
26. Sikström S, Söderlund G (2007) Stimulus-dependent dopamine release in attention-deficit/hyperactivity disorder. *Psychol Rev* 114: 1047-1075.
27. Pålsson E, Söderlund G, Klamer D, Bergquist F (2010) Noise benefit in prepulse inhibition of the acoustic startle reflex. *Psychopharmacol* 214: 675-685.
28. Andersson D, Nissbrandt H, Bergquist F (2006) Partial depletion of dopamine in substantia nigra impairs motor performance without altering striatal dopamine neurotransmission. *Eur J Neurosci* 24: 617-624.
29. Bergquist F, Shahabi HN, Nissbrandt H (2003) Somatodendritic dopamine release in rat substantia nigra influences motor performance on the accelerating rod. *Brain Res* 973: 81-91.
30. Lappin JM, Reeves SJ, Mehta MA, Egerton A, Coulson M, et al. (2009) Dopamine release in the human striatum: motor and cognitive tasks revisited. *J Cereb Blood Flow Metab* 29: 554-564.
31. Winn P (2008) Experimental studies of pedunculopontine functions: are they motor, sensory or integrative? *Parkinsonism Rel Disord* 14 Suppl 2: S194-198-S194-198.
32. Andersson DR, Bjornsson E, Bergquist F, Nissbrandt H (2010) Motor activity-induced dopamine release in the substantia nigra is regulated by muscarinic receptors. *Exp Neurol* 221: 251-259.
33. Bergquist F, Ludwig M, Dutia MB (2008) Role of the commissural inhibitory system in vestibular compensation in the rat. *J Physiol* 586: 4441-4452.
34. Lindgren HS, Andersson DR, Lagerkvist S, Nissbrandt H, Cenci MA (2010) L-DOPA-induced dopamine efflux in the striatum and the substantia nigra in a rat model of Parkinson's disease: temporal and quantitative relationship to the expression of dyskinesia. *J Neurochem* 112: 1465-1476.

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64  
65
35. Dakin CJ, Son GML, Inglis JT, Blouin JSb (2007) Frequency response of human vestibular reflexes characterized by stochastic stimuli. *J Physiol* 583: 1117-1127.
  36. Gravelle DC, Laughton CA, Dhruv NT, Katdare KD, Niemi JB, et al. (2002) Noise-enhanced balance control in older adults. *Neuroreport* 13: 1853-1856.
  37. Priplata AA, Patrilli BL, Niemi JB, Hughes R, Gravelle DC, et al. (2006) Noise-enhanced balance control in patients with diabetes and patients with stroke. *Ann Neurol* 59: 4-12.
  38. Manjarrez E, Mendez I, Martinez L, Flores A, Mirasso CR (2007) Effects of auditory noise on the psychophysical detection of visual signals: cross-modal stochastic resonance. *Neurosci Lett* 415: 231-236.
  39. Baker KB, Lee JY, Mavinkurve G, Russo GS, Walter B, et al. (2010) Somatotopic organization in the internal segment of the globus pallidus in Parkinson's disease. *Exp Neurol* 222: 219-225.
  40. Ostergaard K, Sunde NA (2006) Evolution of Parkinson's disease during 4 years of bilateral deep brain stimulation of the subthalamic nucleus. *Mov Disord* 21: 624-631.
  41. Umemura A, Oka Y, Okita K, Toyoda T, Matsukawa N, et al. (2010) Predictive factors affecting early deterioration of axial symptoms after subthalamic nucleus stimulation in Parkinson's disease. *Parkinsonism Rel Disord* 16: 582-584.
  42. Timmerman W, Westerink B (1997) Brain microdialysis of GABA and glutamate: what does it signify? *Synapse* 27: 242-261.
  43. DeLong MR, Wichmann T (2007) Circuits and Circuit Disorders of the Basal Ganglia. *Arch Neurol* 64: 20-24.
  44. Kravitz AV, Freeze BS, Parker PR, Kay K, Thwin MT, et al. (2010) Regulation of parkinsonian motor behaviours by optogenetic control of basal ganglia circuitry. *Nature* 466: 622-626.
  45. Windels F, Bruet N, Poupard A, Urbain N, Chouvet G, et al. (2000) Effects of high frequency stimulation of subthalamic nucleus on extracellular glutamate and GABA in substantia nigra and globus pallidus in the normal rat. *Eur J Neurosci* 12: 4141-4146.
  46. Paxinos G, Watson C (1998) *The Rat Brain in Stereotaxic Coordinates*, Fourth Edition: Academic Press.
  47. Bergquist F, Jonason J, Pileblad E, Nissbrandt H (1998) Effects of local administration of L-, N-, and P/Q-type calcium channel blockers on spontaneous dopamine release in the striatum and the substantia nigra: a microdialysis study in rat. *J Neurochem* 70: 1532-1540.
  48. Bostan AC, Dum RP, Strick PL (2010) The basal ganglia communicate with the cerebellum. *Proc Natl Acad Sci U S A* 107: 8452-8456.
  49. Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL (2005) The cerebellum communicates with the basal ganglia. *Nat Neurosci* 8: 1491-1493.

## Figure legends

### Figure 1

#### Schematic illustration of the experimental design for the Rotarod testing procedure.

The black arrows represent training episodes for each animal. Rod performance during SVS or no SVS was compared to the baseline measurement (BL, 4x10 min before either condition) on the day of the experiment. The stimulated condition (30 min SVS in cage followed by 4x10 min testing during SVS) or non stimulated condition (30 min rest in cage followed by 4x10 min testing during no SVS) was carried out in a counterbalanced order after the BL measurements. L-DOPA testing was carried out in the beginning of the following week in counterbalanced order; BL testing followed by either an L-DOPA or NaCl injection, rest in cage for 30 min followed by testing for 4x10 min.

### Figure 2

#### Rotarod performance after 6-OHDA or sham-hemileisions

$\Delta t$  represents the change in time on rod (s, mean $\pm$ SEM) compared to baseline performance on the day of the experiment. Stochastic vestibular stimulation (SVS) or no stimulation (No SVS) was administered in a counterbalanced order. In the following week a repeat experiment was performed with 6-OHDA hemilesioned animals and the effect of L-DOPA treatment or a vehicle injection (NaCl) was evaluated in counterbalanced order. P-value for paired t-test.

### Figure 3

#### Vestibular pathways (dashed arrows) that may influence basal ganglia transmission

A) SVS is expected to activate multiple pathways from the vestibular nuclear complex (VN). Of particular interest may be pathways that connect the cerebellum and the basal ganglia over the subthalamic nucleus and thalamus [48,49]. We chose to sample the substantia nigra (SN), the striatum, the pedunculopontine nucleus (PPN) and the ventromedial thalamus for dopamine and amino acid concentrations before, during and for 60 minutes after stochastic vestibular stimulation in unlesioned animals. Panel B indicates the activity of the direct and indirect loop projections to the SN in normal intact rats and after nigral dopamine cell degeneration. Loss of nigrostriatal dopamine neurons lead to hyperexcitation of SN *pars reticulata* neurons (SNr), which can be counteracted by increased GABA release following L-

DOPA treatment. SVS also increases nigral GABA concentrations, but the pathways involved in this effect remains to be elucidated. RF: reticular formation, Thal: thalamus.

Figure 4

Microdialysate concentrations of GABA during SVS in intact animals and untreated intact controls.

Percent of baseline, mean±SEM is shown. The GAT-inhibitor NNC 711 was included in the dialysates throughout the experiment. SVS stimulation period is indicated by a horizontal bar.

A. Nigral GABA concentration in SVS treated intact animals (n=9) and untreated controls (n=6). P-values are from Bonferroni corrected post hoc tests following two-way ANOVA for T=90-150 minutes, indicating significant interaction between treatment and time  $F(2,26)=3.53$ ,  $p=0.044$ . B. GABA concentrations in the PPN, VM and striatum of intact animals following SVS.

Figure 5

Absolute concentrations of GABA and dopamine in the ipsi- and contralesional SN of hemilesioned 6-OHDA animals

Panel A-B shows the GABA concentrations and panel C-D the simultaneous dopamine (DA) concentrations following SVS and L-DOPA treatment (nM, mean±SEM). Panel A and C are measurements from day 1 and B and D from day 2. NNC 711 (30  $\mu$ M) was present throughout the experiment and left in the microdialysis tube that was re-sealed over night. SVS treatment is indicated by a horizontal bar, and the L-DOPA injection by an arrow.

Figure 6

Relative changes in GABA concentrations in the ipsi- and contralesional SN of hemilesioned 6-OHDA animals following L-DOPA treatment

The microdialysate concentrations of GABA (percent of baseline, mean±SEM, n=5-7) in the bilateral SN of 6-OHDA hemilesioned rats following an i.p. injection of L-DOPA are compared to GABA concentrations in the SN of untreated control animals (n=6). Two-way repeated measure ANOVA for t=90 - t=150 with L-DOPA treatment and time as main factors indicated a significant effect of treatment  $F(2,30)=4.68$ ,  $p=0.026$ . \*  $p<0.05$  in post hoc Bonferroni corrected t-tests comparing ipsilesional SN in treated animals to control SN.

Supplementary Figure S1

Figure shows dopamine (DA) concentrations (percent of baseline, mean±SEM) in dialysates from the substantia nigra (SN) and the contralateral striatum (ST) of 9 un-lesioned animals subjected to stochastic vestibular stimulation for 30 minutes (horizontal bar). Concentrations remained stable throughout the stimulation period and the following hour. For clarity, measurements from untreated control animals (n=6) are omitted from the figure. NNC 711 (30 mM) was present in the perfusate throughout the experiment.

Supplementary Figure S2

Panel A shows the relative concentrations (percent of baseline, mean±SEM) of glutamate and glycine from the substantia nigra (SN) of un-lesioned animals subjected to stochastic vestibular stimulation (SVS, horizontal bar, n=9) or no SVS (ctrl, n=6) for 30 minutes. Over this time there were no significant difference in relative concentrations (Two-way repeated measure ANOVA for t=90 - t=150 with SVS treatment and time as main factors, Glutamate:  $F_{\text{treat}}(1, 24)=2.76$ ,  $p=0.12$ ,  $F_{\text{time}}(2, 24)=0.5$ ,  $p=0.61$ ,  $F_{\text{interact}}(2,24)=1.01$ ,  $p=0.38$ , Glycine  $F_{\text{treat}}(1, 26)=1.36$ ,  $p=0.26$ ,  $F_{\text{time}}(2, 26)=1.63$ ,  $p=0.21$ ,  $F_{\text{interact}}(2,26)=1.34$ ,  $p=0.28$ ). The higher mean values and large variability in samples t=120 and t=150 coincided with increased exploratory behaviour in the cage. Taurine and glutamine levels remained stable following SVS (Panel B, two-way repeated measure ANOVA for t=90 - t=150 with SVS treatment and time as main factors, Taurine:  $F_{\text{treat}}(1, 26)=1.88$ ,  $p=0.19$ ,  $F_{\text{time}}(2, 26)=1.63$ ,  $p=0.22$ ,  $F_{\text{interact}}(2,26)=0.44$ ,  $p=0.65$ , Glutamine  $F_{\text{treat}}(1, 26)=1.71$ ,  $p=0.21$ ,  $F_{\text{time}}(2, 26)=1.81$ ,  $p=0.18$ ,  $F_{\text{interact}}(2,26)=0.30$ ,  $p=0.75$ ). NNC 711 (30 mM) was present in the perfusate throughout the experiment.

Supplementary Figure 3

Figure shows glutamate (GLU) concentrations (percent of baseline, mean±SEM) in dialysates from the ipsi- and contralateral substantia nigra (SN) before, during and after stochastic vestibular stimulation (SVS, horizontal bar, 30 minutes) in panel A and in response to L-DOPA treatment (6 mg/kg, i.p., arrow) in panel B. NNC 711 (30 mM) was present in the perfusate throughout the experiment.

Supplementary Figure 4

Placement of microdialysis probes in intact and hemilesioned animals. Unlesioned animals are shown schematically in panel A (striatum and substantia nigra) and panel B (ventromedial thalamus and the

pedunculopontine nucleus). Panel C shows the bilateral nigral locations in hemilesioned animals. Solid lines indicate the estimated location of the active dialysis membrane.

Supplementary Figure 5

Panel a shows the stimulation current used in normal animals and panel b the current used in hemilesioned rats. The time base is 200 ms per division, and the upmost part of each panel shows the frequency distribution.

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Figure 1  
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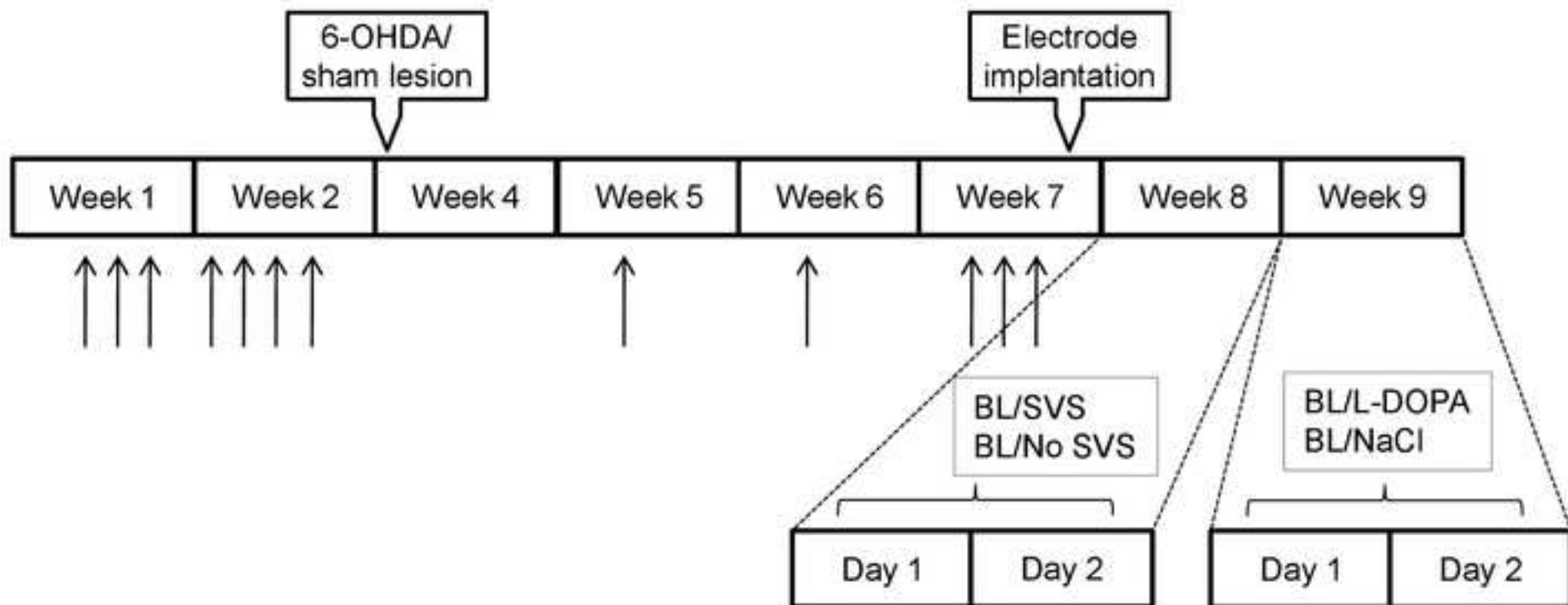
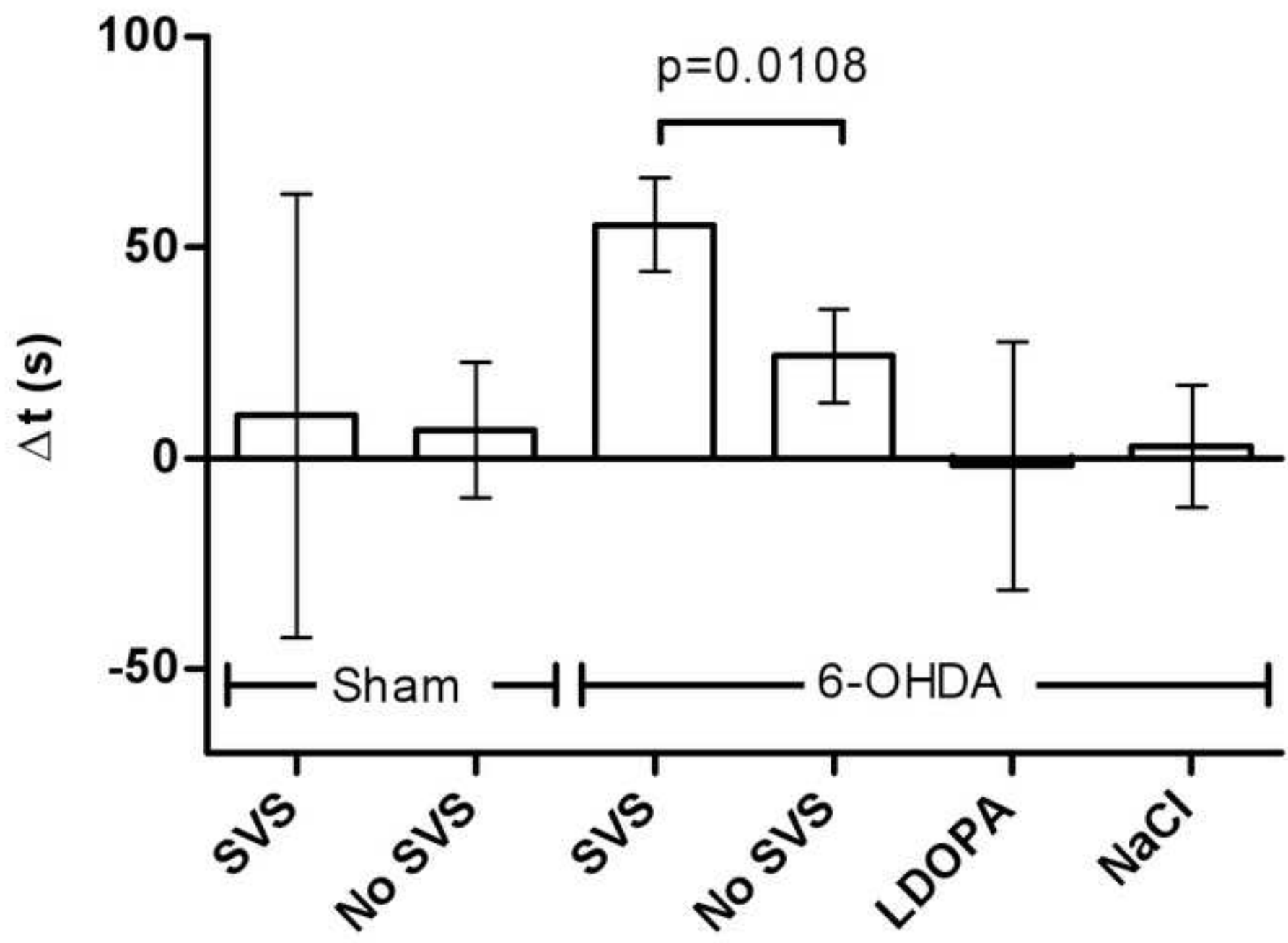


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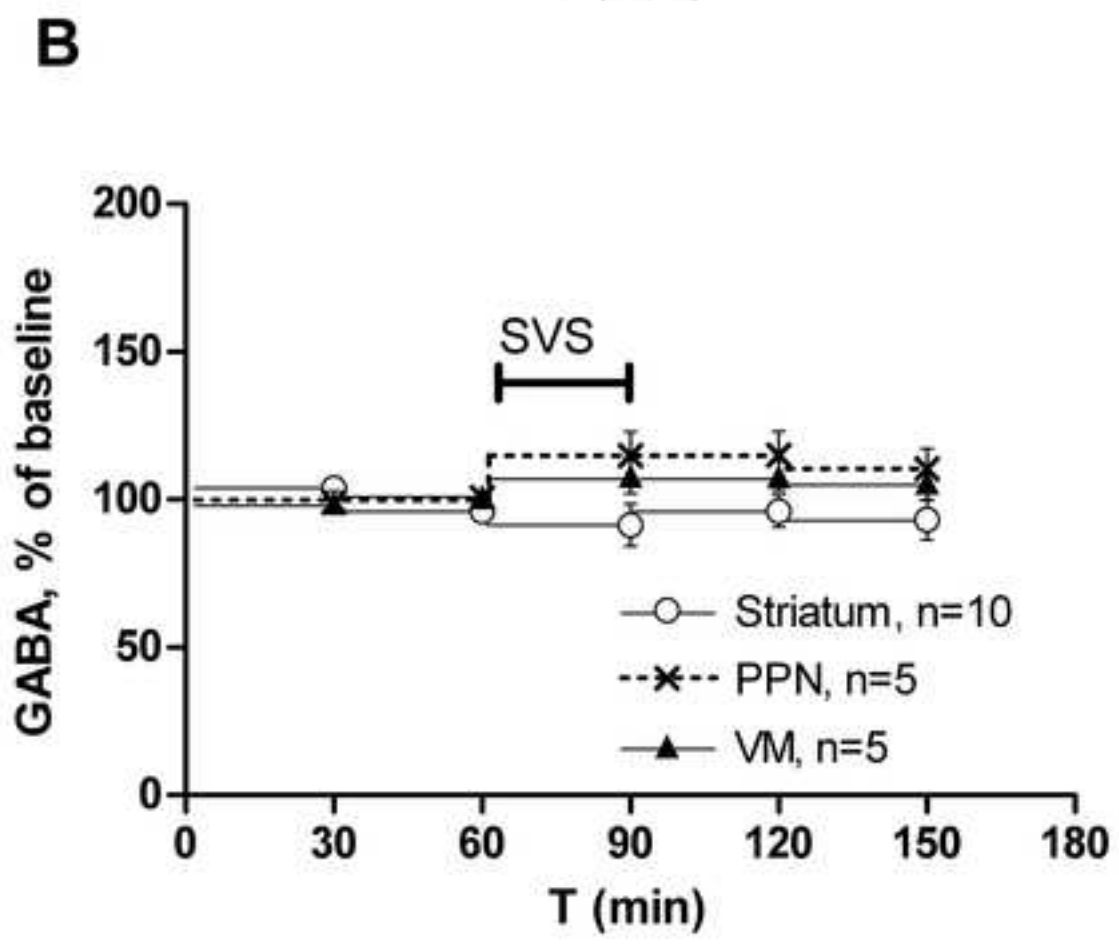
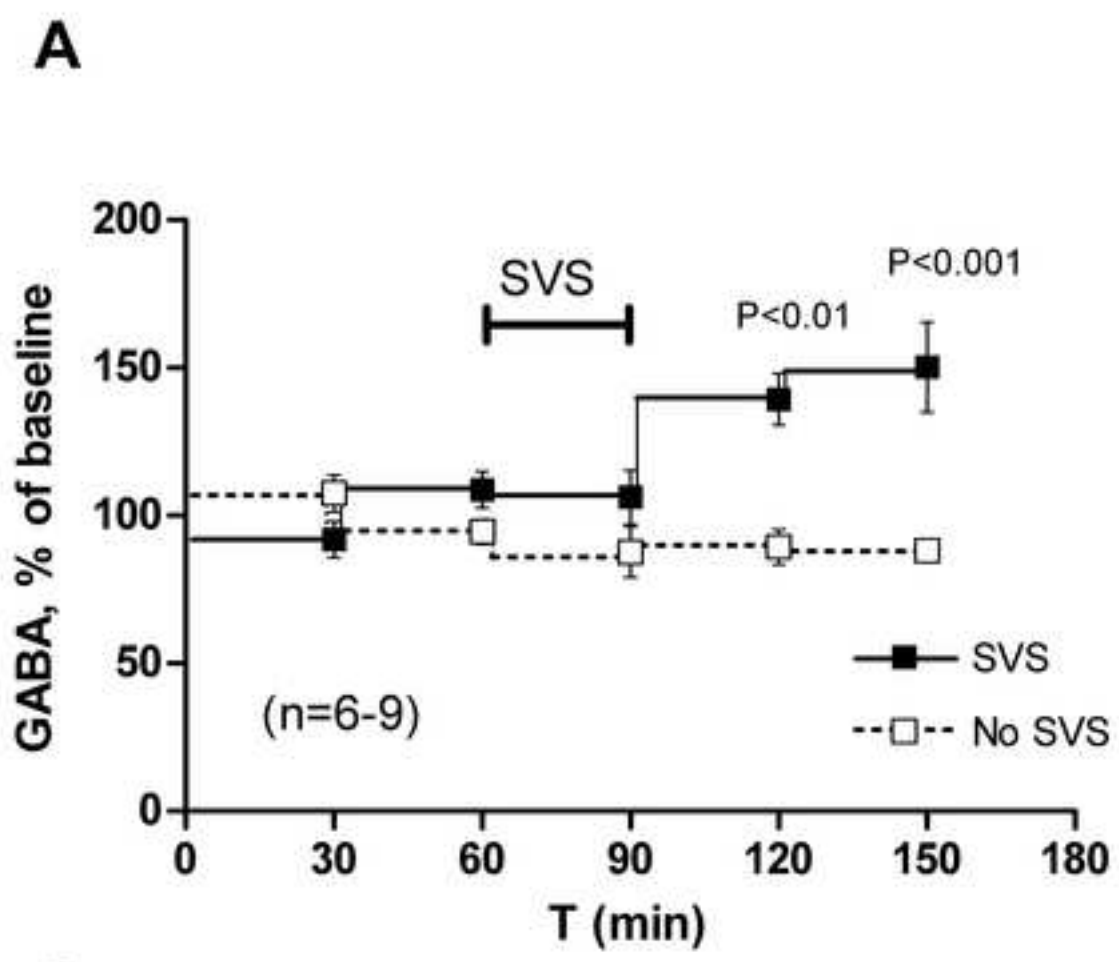


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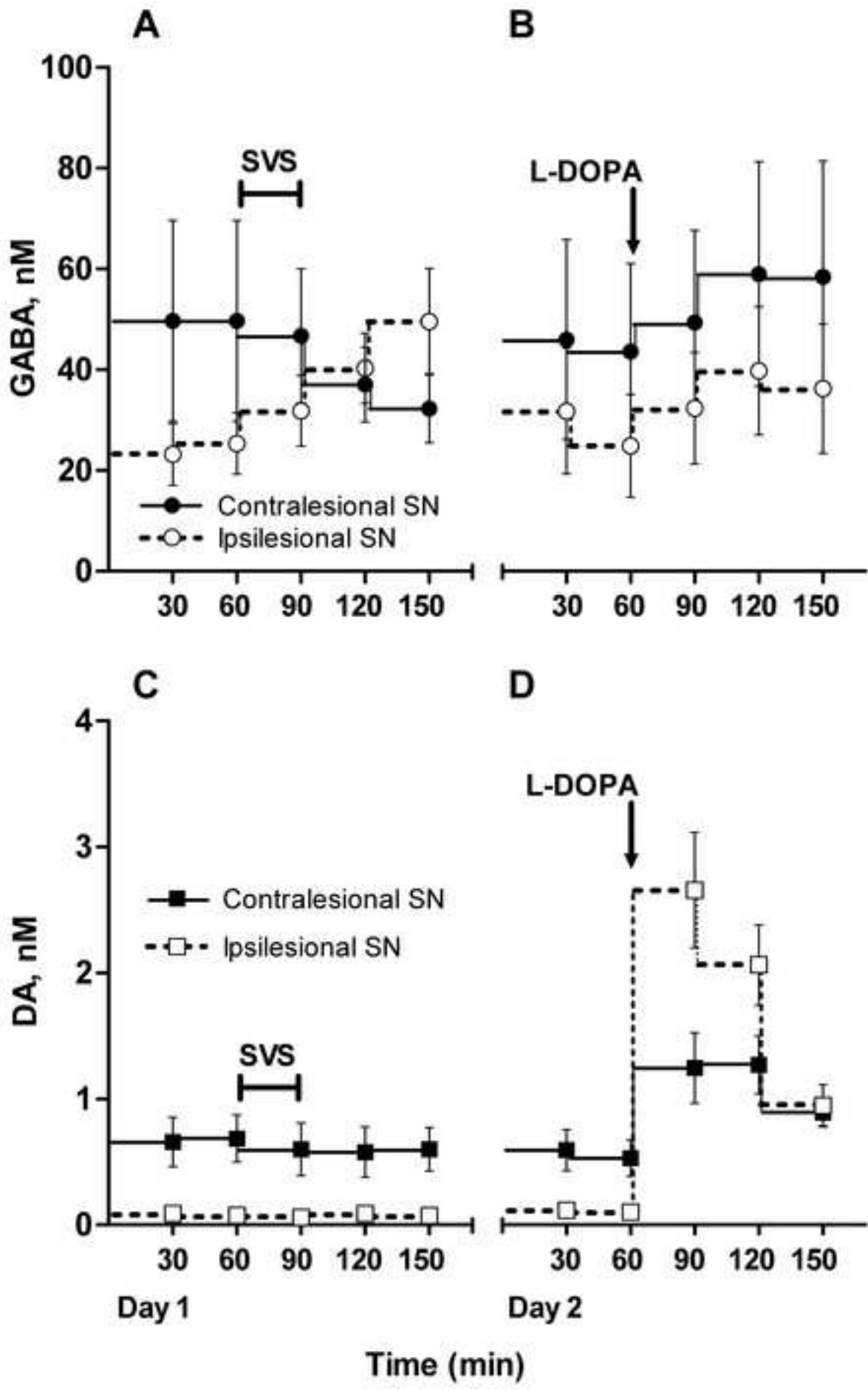
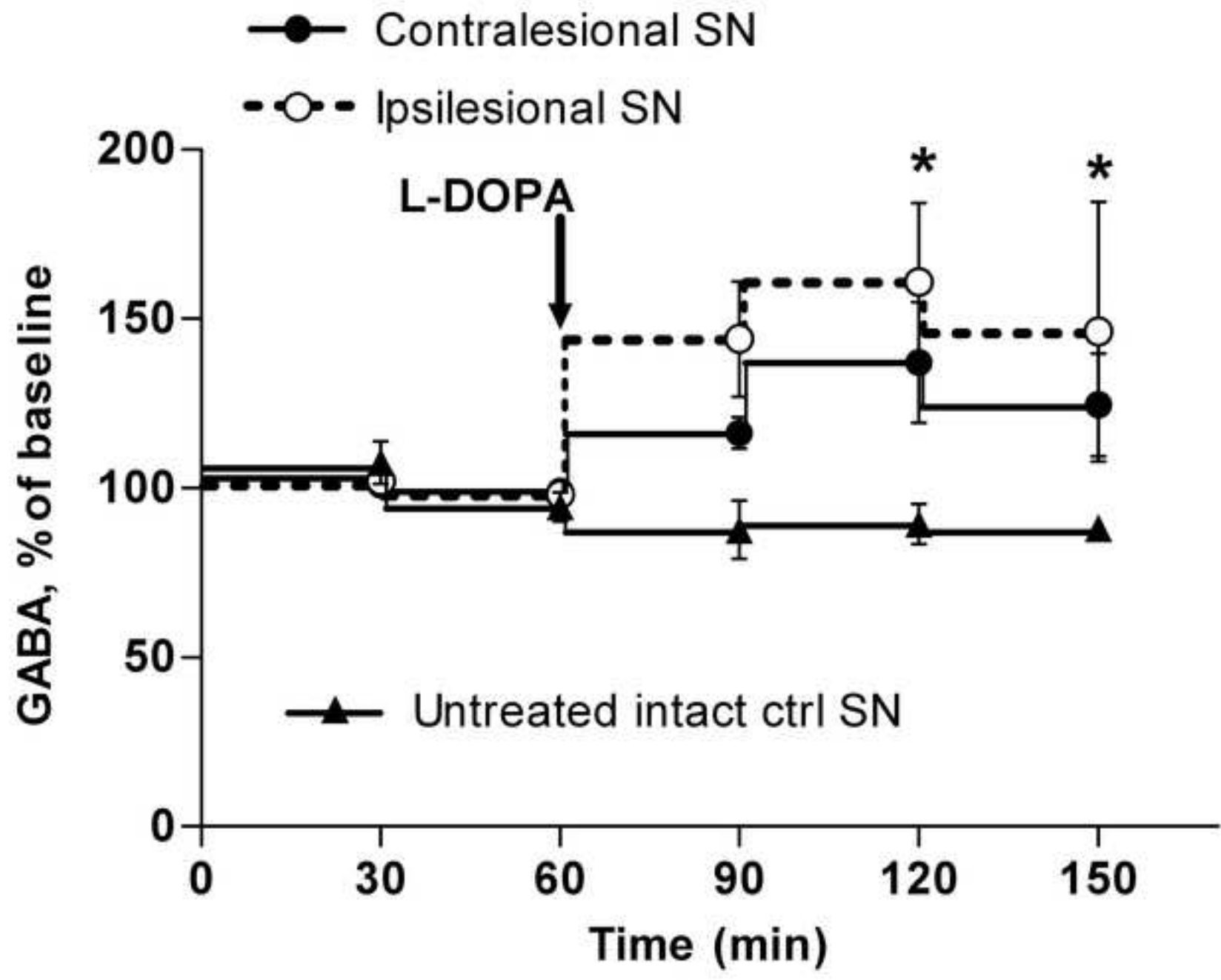


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**Tables**Table 1 Maximum stimulation amplitudes (mA, mean±SEM) in the different experiments

Experiment		Mean±SEM (mA)
Rotarod	6-OHDA hemiles (n=6)	0.30±0.02
	Sham hemiles (n=5)	0.27±0.03
Montoya staircase	6-OHDA hemiles (n=5)	0.23±0.05
	Sham hemiles (n=3)	0.27±0.03
Microdialysis	SN/ST, unlesioned (n=9)	0.48±0.02
	PPN/VM, unlesioned (n=5)	0.41±0.03
	Bilat SN, 6-OHDA hemiles (n=7)	0.47±0.08

Stimulation amplitudes were determined by subjecting the animals to a sinusoid current that was increased until head rocking was just visible. During stochastic stimulation the amplitude never exceeded  $\pm$  this amplitude. A different current delivery system was used for the microdialysis experiments in unlesioned animals.

Table 2 Montoya staircase performance before and two weeks after a hemilesion procedure

		Pre lesion	Post les	<i>p-value</i>	No SVS	SVS	<i>p-value</i>
<b>6-OHDA</b> (n=5)	<i>Total</i>	51±2	45±3	0.377	45±3	46±3	0.753
	<i>Ratio</i>	0.93±0.1	0.65±0.1	0.035	0.58±0.1	0.54±0.1	0.618
<b>Sham</b> (n=3)	<i>Total</i>	40±3	50±3	0.005	50±4	46±3	0.497
	<i>Ratio</i>	1.23±0.2	1.05±0.1	0.239	1.25±0.3	1.10±0.2	0.520

Total number of pellets retrieved and the contralesional/ipsilesional ratio is given (mean±SEM) Three weeks post lesion the effect of stochastic vestibular stimulation (SVS) or no stimulation (No SVS) was evaluated on different days in a counterbalanced order. P-values for paired t-tests.

Suppl Fig S1

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