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ORIGINAL PAPER

Reduced volumes of the external and internal globus pallidus in male heroin addicts: a postmortem study

Ulf J. Müller[1,2,3] · Christian Mawrin[2,4] · Thomas Frodl[1,2] · Henrik Dobrowolny[1,2] · Stefan Busse[1] · Hans-Gert Bernstein[1,2] · Bernhard Bogerts[1,2] · Kurt Truebner[5] · Johann Steiner[1,2]

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Abstract

Deep brain stimulation (DBS) of the globus pallidus internus was recently proposed as a potential new treatment target for opioid addiction. DBS requires computer-assisted-3D planning to implant the stimulation electrode precisely. As volumes of brain regions may differ in addiction compared to healthy controls, our aim was to investigate possible volume differences in addicts compared to healthy controls. Volumes of the globus pallidus externus (PE) and internus (PI) in heroin addicts ($n=14$) and healthy controls ($n=12$) were assessed using morphometry of serial whole-brain sections. Total brain volume was larger in the heroin group (mean 1479 ± 62 cm^3 vs. mean 1352 ± 103 cm^3), as the heroin group was more than 10 years younger ($p=0.001$). Despite larger mean whole brain volume, the mean relative volume of the PE and PI was smaller in addicted subjects compared to healthy controls (PE $0.658 \pm 0.183 \times 10^{-3}$ vs. $0.901 \pm 0.284 \times 10^{-3}$; ANOVA $F(1, 24)=6.945$, $p=0.014$, $\eta^2=0.224$; PI $0.253 \pm 0.095 \times 10^{-3}$ vs. $0.345 \pm 0.107 \times 10^{-3}$; ANOVA $F(1, 24)=5.374$, $p=0.029$, $\eta^2=0.183$). These findings were not significantly confounded by age, duration of autolysis, and fixation time. Our results provide further evidence for structural and not only functional deficits of the globus pallidus in addiction. In the context of previous studies, our findings support the idea of shared pathophysiological processes between comorbid depression and impulsivity in opioid addiction.

Keywords Heroin · Addiction · Globus pallidus internus · Globus pallidus externus · Postmortem · Volumetry · Morphometry · Deep brain stimulation · DBS

Introduction

Heroin addiction is a severe, potentially lethal addiction which accounts for up to ten million disability-adjusted life years [1]. In the last one and a half decades, overdose death rates have increased in the United states and the use of licit prescription of opioid medication as well illicit use of prescription opioids and heroin are considered an increasing epidemic [2].

At the same time, deep brain stimulation (DBS) has been introduced [3] and investigated to treat alcohol addiction [4] as well as heroin addiction [5].

In the last years, our group showed reduced nucleus accumbens and hypothalamus volumes in postmortem analyses of brains of male heroin addicts [6, 7]. These studies provided further evidence for a structural and not only functional deficit in heroin addiction and highlighted the importance of volumetric analyzes in addiction, if brain nuclei are considered targets for DBS.

Kurt Truebner and Johann Steiner contributed equally to this work.

✉ Ulf J. Müller
ulfmueller@gmail.com

✉ Johann Steiner
johann.steiner@med.ovgu.de

[1] Department of Psychiatry and Psychotherapy, University of Magdeburg, 39120 Magdeburg, Germany

[2] Center for Behavioral Brain Sciences, Magdeburg, Germany

[3] Department of Psychiatry and Psychotherapy, Saarland University, 66421 Homburg, Germany

[4] Department of Neuropathology, University of Magdeburg, Magdeburg, Germany

[5] Institute of Legal Medicine, University of Duisburg-Essen, Essen, Germany

The globus pallidus consists of the external (PE) and internal (PI) segment and is an essential part of the basal ganglia (BG) network [8]. Historically, the BG were considered to be part of a closed loop connecting cortical motor areas through the basal ganglia back to the frontal cortex. Since then, new studies showed a more complex map of BG connectivity leading to a model in which the striatopallidal system in primates (including humans) plays a crucial role in the transfer from motivation to action/behavior by integrating limbic and cognitive domains with the motor domain [9], thus becoming a target of interest not only in movement disorder neurology but in psychiatry as well.

Recently, a case report showed that a woman with severe opioid as well as alcohol addiction was able to reach sustained remission from alcohol and opioid use after she experienced ischemic lesions to the globus pallidus bilaterally due to a methadone overdose [10]. Based on the fact that DBS of the PI is an approved treatment of dystonias and has been investigated in other neuropsychiatric disorders such as Tourette syndrome, the authors suggested DBS of the PI as a potential new treatment of drug addiction.

Thus, similar to our study of the nucleus accumbens [6], the aim of our current study was to investigate whether volume differences can be detected in the globus pallidus in a postmortem analysis of heroin-addicted patients compared to healthy controls.

Materials and methods

Subjects

All brains were obtained from the Magdeburg Brain Bank. Sampling and preservation of the human brain material were done in accordance with the Declaration of Helsinki, German law and the local institutional review board at the University of Magdeburg. Analysis included 14 chronic male heroin addicts who died from drug overdose (aged 30.9 ± 7.6 years; postmortem interval 50.9 ± 43.7 h) and 12 male controls (aged 44.4 ± 10.5 years; postmortem interval 36.0 ± 24.0 h, see Table 1).

All patients were matched for age and postmortem delay ("duration of autolysis") as closely as possible. Information on clinical characteristics was extracted from the clinical records and by structured interviews with people closely related to the subjects using a psychological autopsy [11]. In addition to heroin, all but one drug addicts had a history of abusing other legal and/or illegal substances, including morphine, cannabis, alcohol, cocaine, barbiturates, benzodiazepines and hallucinogens. However, the tested patients fulfilled addiction criteria only for heroin. An experienced neuropathologist (CM) ruled out qualitative neuropathological changes due to neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Pick's disease), tumors, inflammatory, vascular or traumatic processes, e.g., using samples with Nissl myelin staining, HLA-DR-, beta-amyloid-, and tau-immunostaining. None of the heroin addicts was HIV-positive. A forensic pathologist (KT) established the diagnosis of suicide. A toxicology screen on blood and urine for ethanol and other substances of abuse was performed at each medico-legal autopsy.

Tissue processing

Tissue preparation was performed as previously described [12]. Brains were removed and fixed in toto in 8% phosphate-buffered formaldehyde for at least 2 months. Frontal and occipital poles were separated by coronal cuts anterior to the genu and posterior to the splenium of the corpus callosum. After embedding all parts of the brains in paraffin wax, serial, whole brain coronal sections of the middle block were cut on a large-scale microtome (Balzers, Liechtenstein) at 20 µm and mounted.

Volume shrinkage was determined for each brain before and after dehydration and embedding of tissue. Volume shrinkage factors were calculated using the formula: $VSF = (A1/A2)^{3/2}$ (VSF = volume shrinkage factor; $A1$ = cross-sectional area before processing of tissue; $A2$ = cross-sectional area after processing of tissue).

Morphometric analysis

Histologic and planimetric procedures were performed as previously described by us in detail [13, 14]. For anatomical orientation and morphometric investigations, every 25th serial coronal whole brain section (thickness 20 µm) was stained with a combined cell and fiber staining according to Nissl (cresyl violet) and Heidenhain–Woelcke and sampled [15, 16], resulting in an intersectional distance of 0.5 mm. Measurements of cross-sectional areas of the structures were performed by planimetry from fourfold magnifications of the sections.

As previously described [13, 14], the internal and external parts of the globus pallidus internus (PI)/externus (PE) were clearly demarcated and were measured from their most rostral part situated below the commissura anterior to the most caudal part (see Fig. 1). Total volumes of the PI/PE were calculated by multiplying the respective cross-sectional areas by the distance between the sections and adding up volumes obtained by this procedure along the entire rostro-caudal axis of the globus pallidus. These volumes were multiplied by the tissue shrinkage factor (related to the dehydration and embedding process, see "Tissue processing") to estimate the original volumes of PI and PE.

Table 1 Demographic data of the analyzed patients with heroin addiction ($n = 14$) and healthy control subjects ($n = 12$)

Case number	Age (year)	Duration of autolysis (h)	Fixation time (day)	Total brain volume (cm^3)	Volume shrinkage factor (VSF)	Pallidum externum left (not VSF corrected) (mm^3)	Pallidum externum right (not VSF corrected) (mm^3)	Pallidum internum left (not VSF corrected) (mm^3)	Pallidum internum right (not VSF corrected) (mm^3)	Cause of death
Heroin addicts ($n = 14$)										
1	25	11	1372	1408	1.27	681	717	330	336	Heroin overdose
2	25	30	1426	1446	1.76	778	829	258	293	Heroin overdose
3	33	85	2185	1427	1.63	214	284	119	153	Heroin overdose
4	24	49	2373	1475	2.03	561	613	285	301	Heroin overdose
5	31	10	2914	1475	2.18	499	504	179	191	Heroin overdose
6	40	16	2938	1562	1.60	545	601	185	240	Heroin overdose
7	21	164	2702	1398	1.86	544	641	256	301	Heroin overdose
8	32	16	3202	1543	2.41	556	484	174	193	Heroin overdose
9	47	96	–	1446	1.69	614	580	205	215	Heroin overdose
10	40	81	–	1543	1.62	506	561	173	172	Heroin overdose
11	30	63	–	1456	2.02	529	657	177	283	Heroin overdose
12	32	16	3906	1408	1.58	430	527	189	189	Heroin overdose
13	31	43	3851	1581	1.53	492	428	143	145	Heroin overdose
14	21	33	3815	1533	1.44	526	588	193	277	Heroin overdose
Mean	30.9	50.9	2789	1479	1.76	534	572	205	235	
SD	7.6	43.7	1425	62	0.31	126	130	57	63	
Controls ($n = 12$)										
1	47	24	179	1398	2.66	646	634	230	248	Myocardial infarction
2	47	24	90	1157	1.28	356	447	209	167	Acute respiratory failure (aspiration)
3	56	30	252	1398	1.40	666	649	250	244	Sudden cardiac death
4	38	19	70	1495	2.33	673	622	200	216	Myocardial infarction
5	40	96	180	1495	2.84	642	679	237	237	Myocardial infarction
6	64	35	240	1263	2.68	504	532	200	184	Ruptured aortic aneurysm
7	39	4	330	1354	1.99	535	585	250	247	Pneumonia
8	54	24	250	1379	1.85	557	530	242	247	Pulmonary embolism

Table 1 (continued)

Case number	Age (year)	Duration of autolysis (h)	Fixation time (day)	Total brain volume (cm^3)	Volume shrinkage factor (VSF)	Pallidum externum left (not VSF corrected) (mm^3)	Pallidum externum right (not VSF corrected) (mm^3)	Pallidum internum left (not VSF corrected) (mm^3)	Pallidum internum right (not VSF corrected) (mm^3)	Cause of death
9	46	24	290	1249	2.17	690	838	295	340	Sudden cardiac death
10	45	44	1603	1302	1.59	685	753	204	311	Autoerotic accident (strangulation)
11	28	48	1043	1446	1.48	516	583	169	199	Hemorrhagic shock
12	29	60	808	1292	1.93	509	567	202	202	Myocardial infarction
Mean	44.4	36.0	445	1352	2.02	582	618	224	237	
SD	10.5	24.0	466	103	0.53	102	105	33	50	
Test	*t* test	*t* test	*t* test	*t* test	*t* test	*t* test	*t* test	*t* test	*t* test	
T value	$T=-3.709$	$T=1.101$	$T=7.761$	$T=3.698$	$T=-1.492$	$T=-1.071$	$T=-0.998$	$T=-1.216$	$T=-0.365$	
p value	0.001**	0.284	< 0.001***	0.002**	0.154	0.295	0.328	0.239	0.718	

All tested subjects in this study were male

SD standard deviation, *VSF* volume shrinkage factor

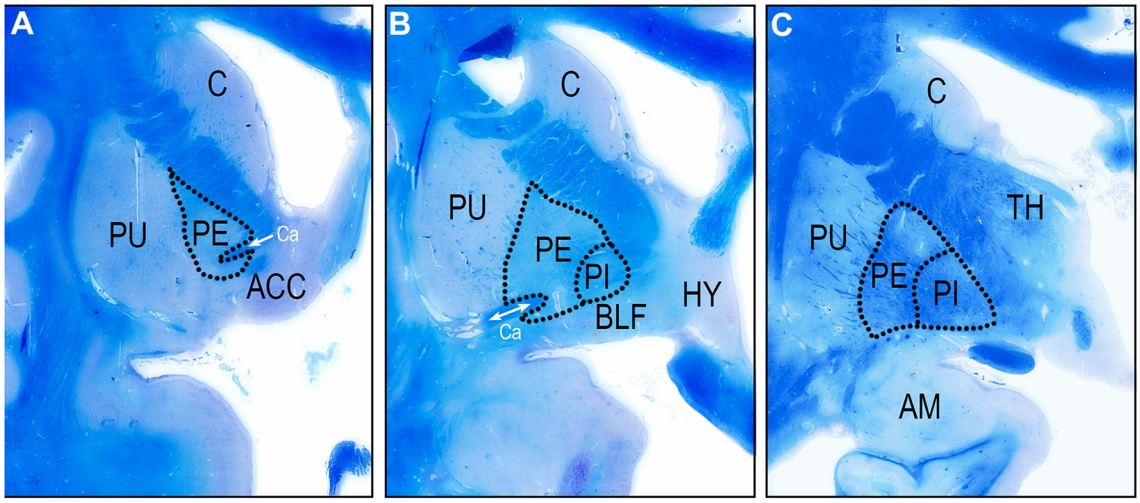

Fig. 1 Delineation of the pallidum in coronal serial Nissl–myelin stained sections. *ACC* nucleus accumbens, *AM* amygdala, *BLF* basal limbic forebrain, *Ca* commissura anterior, *C* caudate, *HY* hypothalamus, *PE* pallidum externum, *PI* pallidum internum, *PU* putamen, *TH* thalamus

Statistical analysis

Statistical analyses were performed with the SPSS 15.0 program (Statistical Package for the Social Sciences, Chicago, IL, USA). Demographic data were compared using *t* tests. Volume data were normally distributed as indicated by Kolmogorov–Smirnov tests. Total brain volume was larger in the heroin group compared to controls (1479 ± 62 cm^3 vs. 1352 ± 103 cm^3; $T = 3.845$; $p = 0.001$), as the heroin group was more than 10 years younger (30.9 ± 7.6 years vs.44.4 ± 10.5 years; $T = -3.806$; $p = 0.001$). To exclude differences in whole brain size as confounding factor, diagnosis-related effects were assessed using the PI/PE volumes (corrected by tissue shrinkage) normalized to whole brain volume (relative volume = volume of the PI or PE divided by the respective whole brain volume). We applied repeated measures analysis of variance (ANOVA) for the analysis of pallidum externum or internum data using "hemisphere" as within-subject factor and "diagnosis" as between-subject factor. We used partial eta-squared (η^2 = between-groups sum of squares divided by total sum of squares) to assess effect sizes. In addition, a more detailed statistical analysis of the potential confounding variables "age", "duration of autolysis", and "fixation time" (see Table 1) was performed by ANCOVA. Pearson correlation coefficient was employed to test if the slight weight changes during the fixation process (i.e., total brain weight after formalin fixation in comparison to fresh non-fixed total brain weight) correlated with the fixation time in our brain bank. All statistical tests were two-tailed, and significance was defined as $p < 0.05$.

Results

Even though subjects in the group of heroin addicts were younger than controls and thus had a larger mean whole brain volume, the mean relative volume of the PE was smaller than in healthy non-addicted controls (PE $0.658 \pm 0.183 \times 10^{-3}$ vs. $0.901 \pm 0.284 \times 10^{-3}$ ANOVA $F(1, 24) = 6.945$, $p = 0.014$, $\eta^2 = 0.224$/medium effect size; Fig. 2). A similar finding was observed regarding the relative volumes of the

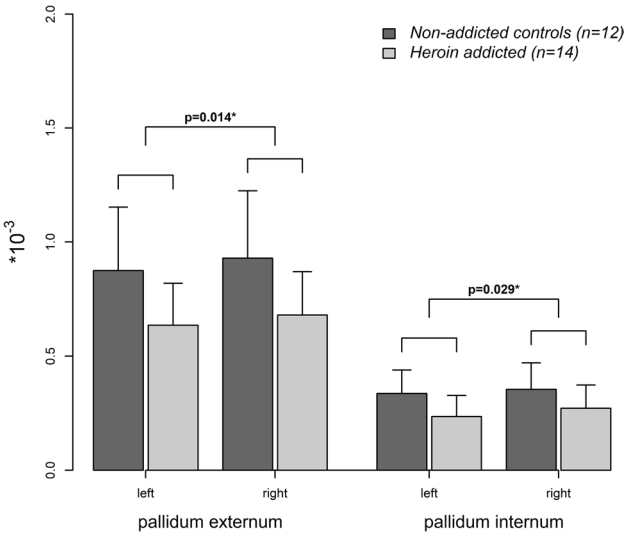

Fig. 2 Relative volumes of the pallidum externum (PE) and pallidum internum (PI) in patients with heroin addiction compared to healthy control subjects. Annotation: volumes of the PE or PI (corrected for tissue shrinkage) were normalized by total brain volume. Data are presented as mean ± standard deviation

PI ($0.253 \pm 0.095 \times 10^{-3}$ vs. $0.345 \pm 0.107 \times 10^{-3}$; ANOVA $F(1, 24) = 5.374$, $p = 0.029$, $\eta^2 = 0.183$/medium effect size; Fig. 2).

In a follow-up analysis, the hemispheres were assessed separately. No interaction of "diagnosis" and "hemisphere" were observed [PE $F(1, 24) = 0.074$, $p = 0.788$; PI $F(1, 24) = 1.119$, $p = 0.301$].

An ANCOVA employing potential confounding variables did not reveal a significant influence of age [PE $F(1, 21) = 0.000$, $p = 0.996$; PI $F(1, 21) = 0.121$, $p = 0.731$], duration of autolysis [PE $F(1, 21) = 0.102$, $p = 0.753$; PI $F(1, 21) = 0.031$, $p = 0.861$], and fixation time [PE $F(1, 21) = 1.685$, $p = 0.203$; PI $F(1, 21) = 2.160$, $p = 0.157$] on the diagnosis-related differences in shrinkage-corrected relative PE and PI volumes. Moreover, fixation times showed no significant correlation with the slight changes in total brain volume during the fixation process ($r = 0.135$, $p = 0.381$).

Discussion

To our knowledge, this is the first study investigating the volume of the globus pallidus in postmortem brains of heroin-addicted individuals. Our result of significantly reduced volumes of the PE and PI raises a few questions.

The aim of this study was to elucidate volumes of the PE and PI, as the globus pallidus was proposed as a possible target for DBS in addiction. If DBS of the PI will be investigated as a potential target in addiction in the future, our morphometric results of reduced volumes of the PI as well as reduced volumes of the PE in addiction should be taken into consideration when calculating activated tissue volumes [17]. After a thorough study of the literature, we did not find any study on volume reduction of the globus pallidus in non-opioid addicts.

While other studies reported bilateral globus pallidus lesions in 5–10% of opiate addicts [18], our neuropathological analysis did not find any evidence of necrosis or neurodegeneration in the globus pallidus. However, random effects may explain this difference compared to other studies by testing rather small cohorts. Evidence of specific neurotoxic effects of heroin is inconsistent and rather sparse (for overviews [19, 20]) although neuronal depletion has been reported in the globus pallidus in heroin addicts [21]. In addition, a recent study by Tolomeo et al. suggests a deteriorating impact of chronic opioid exposure on the globus pallidus volume [22]. Still, as total brain volume was not reduced in our heroin group, it is questionable if the distinct volume reduction is a consequence of chronic heroin intake.

Alternatively—as we have argued before [6]—structural abnormalities in our sample of heroin addicts might be a predisposition for addiction and not a consequence of drug intake and might be connected to mood disturbances leading to the consumption of heroin. This hypothesis is supported by postmortem volumetric analysis as well: decreased volumes of PE and nucleus accumbens were the main findings in a previous study in mood disorders [14]. Alternatively, the relation between heroin addiction, depression and the observed structural abnormalities may be reciprocal, i.e., not only depression may predispose to addiction, but also addiction may predispose to depression-like symptoms, which is a well-known phenomenon in addicted patients [23].

As evidence for specific neurotoxic effects of heroin is inconsistent, currently, we can only speculate on the morphological correlate of the observed volume reduction. One possible mechanism could be neurotoxicity by elevated nitric oxide due to increased expression of neuronal nitric oxide synthase (nNOS). nNOS has already been shown to be increased in the paraventricular nucleus of the left hypothalamus in postmortem brains of heroin addicts [19], and we could show a volume reduction of the hypothalamus in postmortem brains of heroin addicts just recently [7]. However, nNOS has not been studied in the globus pallidus in our tested subjects so far.

Supporting the idea of shared pathophysiological processes between affective disorders and opioid addiction, in depression, reduced activity of the PE has been suggested due to reduced volumes [14] and reduced silver-stained neuronal nucleolar organizer region (AgNOR) area, a marker of cellular transcriptional activity [24] in two previous postmortem studies from our workgroup. Interestingly, particularly those patients with Parkinson's disease, who experience problems with impulse control (IC) show a reduced volume of the right globus pallidus compared to those without IC [25], thus linking reduced impulse control to reduced volume of PE. This finding may be helpful to interpret our results of reduced PE volumes, because increased impulsivity is often a comorbid feature of heroin addiction.

In the last years, knowledge of different globus pallidus territories, its associated complex circuitry, and it role in different aspects of behavior such as motivation, cognition and action has increased and gotten more complex at the same time [26]. As we currently do not know the morphological correlate of the reduced volumes of the PE and PI, it is even more difficult to speculate on the functional effects these volume reductions might have. In an ongoing study, we explore total neuronal cell counts, neuronal AgNOR areas and the GABA-ergic inhibitory interneurons/neuropil in the PE and PI of the cohorts from the study presented here to gain better insight if neuronal loss, reduced neuronal transcriptional activity or a loss of fibers is related to our current findings.

Several factors limit our study: like any postmortem analysis, no longitudinal data can be obtained, the sample size of our study is relatively small and we could only include brains of males, as our brain bank does not contain postmortem brains of female heroin addicts. Due to the limited

clinical records, there are no reliable data on the duration of disease/addiction or on the consumed amount of heroin. Thus, we cannot analyze whether these two variants played a significant role in our main finding of a reduced volume of the globus pallidus externus or whether a reduced PE/PI volume might predispose to addiction itself. Although MRI studies might be suitable to detect possible longitudinal effects, the clinical and behavioral patterns of heroin addiction make it challenging to reliably obtain MRI scans of addicted individuals at different stages of disease.

With regard to the present data, methodologically we cannot rule out a specific effect of the longer postmortem or fixation time in the heroin group specifically on PE/PIa shrinkage, although this appears quite unlikely. Finally, due to the young age of death in the heroin group, we could not match our controls by the same age.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflicts of interest.

Ethical approval Sampling and preservation of the human brain material were done in accordance with the Declaration of Helsinki, German Law and approval by the local institutional review board.

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