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Research report

Demonstration of disturbed activity of external globus pallidus projecting neurons in depressed patients by the AgNOR staining method

Tomasz Gos [a,b,*], Dieter Krell [b], Hendrik Bielau [b], Johann Steiner [b], Kurt Trübner [c], Ralf Brisch [b], Hans-Gert Bernstein [b], Zbigniew Jankowski [a], Bernhard Bogerts [b]

[a] Institute of Forensic Medicine, Medical University of Gdańsk, Poland
[b] Department of Psychiatry, Otto-von-Guericke-University, Magdeburg, Germany
[c] Institute of Legal Medicine, University of Duisburg-Essen, Germany

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ABSTRACT

Background: The external globus pallidus (EGP) is thought to play the most important integrating and conveying role in the striatopallidal system involved in the transfer from motivation to action. The aim to find a morphological biomarker of disturbed EGP activity in depression was approached by the karyometric analysis of large projecting neurons.
Methods: The study was performed on paraffin-embedded brains from 19 depressed patients from both the major depressive disorder (MDD) and the bipolar disorder (BD) diagnostic groups encompassing 10 suicides and from 24 controls. The karyometric parameters of EGP neurons bilaterally were evaluated by argyrophilic nucleolar organiser (AgNOR) silver staining method.
Results: A significantly decreased AgNOR area was found in the left EGP neurons in depressed patients compared to controls. The distinctness of the diagnostic groups and suicidal vs non-suicidal patients was not shown in the statistical comparisons. The AgNOR parameter which was decreased correlated positively with the mean dose of benzodiazepines in non-suicidal patients.
Limitations: A major limitation of this study is the relatively small number of cases. A further limitation is given by the lack of data on drug exposure across the whole lifespan of patients.
Conclusion: The results suggest disturbed, most likely decreased, activity of the left EGP projecting neurons in depressed patients, a disturbed activity that should hypothetically be counteracted by the applied pharmacotherapy in non-suicidal patients.

1. Introduction

The striato-pallidal system, the largest part of the basal ganglia, is inextricably involved in subcortical–cortical loops (limbic–cortical–striatal–pallidal–thalamic circuits) that constitute the neural networks thought to modulate human mood, cognitive states and behaviour (Haber, 2003; Smith et al., 2004; Graybiel, 2005, 2008; Bonelli et al., 2006; Person and Perkel, 2007; McNab and Klingberg, 2008; Schiff, 2008). The limbic, association and sensorimotor cortical areas project in a segregated manner onto three distinct striatal regions and this organisation is maintained throughout, however strongly collateralised, striatopallidal efferents (Parent and Hazrati, 1995a,b; Parent et al., 2000; Levesque and Parent, 2005). The anatomical and functional properties of the system provide the propagation of activity from limbic through associative to sensorimotor parts in the performance of behavioural tasks. Therefore, the entire striatopallidal system is believed to be involved in the transfer from motivation to action (Haber, 2003). The experimental evidence from habit learning supports the holistic view on the system function (Graybiel, 2008). The

* Corresponding author. Institute of Forensic Medicine, Medical University of Gdańsk, ul. Dębowa 23, 80-204 Gdańsk, Poland. Tel.: +48 58 349 1252; fax: +48 58 341 0485.
E-mail address: gost@amg.gda.pl (T. Gos).

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Table 1
Characteristics of the subjects.

	No./sex/ age (years)	Psychiatric diagnosis (DSM-IV)	Cause of death	PMI (h)
Controls	1/M/47		CF	24
	2/F/52		Ovarian carcinoma	24
	3/F/48		PE	48
	4/M/47		Respiratory insufficiency	24
	5/M/40		CF	72
	6/F/64		Sepsis	24
	7/F/33		CF	72
	8/M/38		CF	19
	9/F/50		Dissecting aortic aneurysm	72
	10/M/64		CF	35
	11F/48		PE	26
	12/M/49		CF	72
	13/F/65		HF	24
	14/F/30		PE	48
	15/F/61		HF	8
	16/M/63		HF	48
	17/F/38		PE	24
	18/M/39		Peritonitis	4
	19/F/61		HF	24
	20/F/67		SCD	24
	21/M/54		RHF	24
	22/M/46		Lymphoma	24
	23/F/66		RHF	24
	24/F/39		MI	48
Non-suicide disease group	1/F/63	MDD (296.34)	PE	17
	2/F/62	BD-D (296.54)	PE	72
	3/F/60	BD-D (296.54)	Bronchopneumonia	14
	4/M/39	BD-D (296.54)	PE	14
	5/F/61	MDD (296.34)	SCD	70
	6/M/39	BD-D (296.54)	CF	56
	7/M/69	BD-D (296.54)	PE	48
	8/M/69	BD-D (296.54)	Bronchopneumonia	24
	9/F/65	BD-D (296.54)	HF	52
Suicide disease group	10/M/47	BD-D (296.53)	Stab wound	24
	11/F/39	BD-D (296.53)	Overdose of medication	48
	12/F/53	MDD (296.34)	Hanging	47
	13/F/53	MDD (296.33)	Hanging	46
	14/F/26	MDD (296.33)	Fall from the height	22
	15/F/46	BD-D (296.54)	Overdose of medication	4
	16/M/42	BD-D (296.54)	Hanging	17
	17/F/59	BD-D (296.54)	Overdose of medication	72
	18/M/35	MDD (296.33)	Incision of the radial artery	15
	19/M/36	MDD (296.34)	Hanging	42

M = male; F = female; PMI = postmortem interval; MDD = major depressive disorder; BD = bipolar disorder; BD-D = bipolar disorder depressed; CF = coronary failure; HF = heart failure; MI = myocardial infarction; PE = pulmonary embolism; RHF = right heart failure; SCD = sudden cardiac death.

external globus pallidus (EGP) should play the most important integrating and conveying role in the striatopallidal system in primates, including humans (Parent and Hazrati, 1995b; Parent et al., 2000; Sato et al., 2000; Levesque et al., 2003; Levesque and Parent, 2005).

Both postmortem and neuroimaging studies have revealed structural abnormalities in this system in mood disorders (Husain et al., 1991; Krishnan et al., 1992, 1993; Parashos et al., 1998; Baumann et al., 1999; Strakowski et al., 1999, 2002; Beyer et al., 2004; Bielau et al., 2005a). However, the involvement of the limbic part of the striatopallidal system has been predominantly accentuated (for a review see: Bonelli et al., 2006; Drevets et al., 2008).

The nucleolar organising regions (NORs) are genetic loci on the chromosomes composed of ribosomal DNA (rDNA) and proteins, some of which are argyrophilic. In human interphase cell, the silver-stained NORs (AgNORs) clustered together in the nucleolus represent the site of ribosomal RNA synthesis and transcriptionally active NORs. Therefore, the cellular activity in terms of transcriptional activity of rDNA can be evaluated by measuring the AgNOR parameters (Ploton et al., 1986, 1992; Ploton, 1994; Rüschoff et al., 1995; Derenzini, 2000; Garcia-Moreno et al., 2001; Raska et al., 2006; Sirri et al., 2008). The relatively simple AgNOR staining method is well-established in neuropathology (Figarella-Branger et al., 1991; Reusche, 1991; Abe et al., 1994; de Wolde et al., 1997; Büttner et al., 2001; Janczukowicz, 2003). It has also been used in forensic research and practice (Rebolledo Godoy et al., 2000; Büttner et al., 2001). Recently it has been also employed in depression research (Bielau et al., 2005b, Gos et al., 2007, 2008).

2. Materials and methods

2.1. Characteristics of the subjects

Brains were collected postmortem from 19 patients with mood disorders and 24 controls free from psychiatric disorders autopsied at several institutes of forensic medicine and pathology. The age of the patients (9 men and 10 women) ranged from 26 to 69 years. Ten subjects died by suicide (4 men and 6 women, age range: 26 to 59 years). The methods of suicide comprised medication overdose (3) and violent measures, such as hanging (4), incision of the radial artery (1), self-stabbing (1) and fall from the height (1). The diagnosis of suicide was established by a forensic pathologist. The remaining 9 subjects (4 men and 5 women, age range: 39 to 69 years), died a natural death. Control brains were collected from 10 men and 14 women (age range: 30 to 67 years) who died a natural death. The postmortem interval (PMI) ranged from 4 to 72 h in both depressed patients and controls. A toxicology blood and urine screen for ethanol, other substances of abuse, many antidepressant and antipsychotic drugs as well as their metabolites were performed at each of the forensic autopsies.

The study was performed in compliance with the policies of the ethic committee of the University of Magdeburg, Germany, and the Declaration of Helsinki of 1975. In accordance with the German autopsy laws, informed consent for an autopsy, dissections of the brains and for using clinical information for research purposes was obtained from the relatives of all the deceased. Two independent psychiatrists (H.B., J.S.) established, in consensus meetings, that the DSM-