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Research Report

Quantitative analysis of astrogliosis in drug-dependent humans

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ABSTRACT

Drug addiction is a chronic, relapsing disease caused by neurochemical and molecular changes in the brain. In this human autopsy study qualitative and quantitative changes of glial fibrillary acidic protein (GFAP)-positive astrocytes in the hippocampus of 26 lethally intoxicated drug addicts and 35 matched controls are described. The morphological characterization of these cells reflected alterations representative for astrogliosis. But, neither quantification of GFAP-positive cells nor the Western blot analysis indicated statistical significant differences between drug fatalities versus controls. However, by semi-quantitative scoring a significant shift towards higher numbers of activated astrocytes in the drug group was detected.

To assess morphological changes quantitatively, graph-based representations of astrocyte morphology were obtained from single cell images captured by confocal laser scanning microscopy. Their underlying structures were used to quantify changes in astroglial fibers in an automated fashion. This morphometric analysis yielded significant differences between the investigated groups for four different measures of fiber characteristics (Euclidean distance, graph distance, number of graph elements, fiber skeleton distance), indicating that, e.g., astrocytes in drug addicts on average exhibit significant elongation of fiber structures as well as two-fold increase in GFAP-positive fibers as compared with those in controls.

In conclusion, the present data show characteristic differences in morphology of hippocampal astrocytes in drug addicts versus controls and further supports the involvement of astrocytes in human pathophysiology of drug addiction. The automated

Abbreviations: BAC, blood alcohol concentration; CNS, central nervous system; cLSM, confocal laser scanning microscope; GFAP, glial fibrillary acidic protein; IR, immunoreactivity; MDMA, 3,4-methylendioxy-N-methylamphetamine; NF, neurofilament; PMI, post mortem interval; THC, Δ^9-tetrahydrocannabinol

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quantification of astrocyte morphologies provides a novel, testable way to assess the fiber structures in a quantitative manner as opposed to standard, qualitative descriptions.

1. Introduction

Astrocytes are integral components in normal brain physiology and have been proved to play important roles, e.g., in homeostasis preservation, blood brain barrier maintenance as well as in responding to neuronal activity and plasticity (Araque and Navarrete, 2010; Franke et al., 2012; Kimelberg and Norenberg, 1989). Furthermore, it was suggested that astrocytes contribute to both initiation and propagation of many (if not all) neurological diseases (Verkhratsky et al., 2012). The activation of astrocytes (astrogliosis) is a complex phenomenon and is morphologically characterized by extensive hypertrophy, increased expression of intermediate filaments and in some cases by proliferation. Various pathological processes such as acute or chronic disease (e.g., epilepsy, ischemia, trauma, Alzheimer's disease) induce the activation of astrocytes and an up-regulation of glial fibrillary acidic protein (GFAP). This protein is specifically located at astrocytes and represents the major constituent of their intermediate filaments in the mature brain. It is involved in cellular differentiation and fiber formation, in the regulation of structural stability and in responses to injury, neuronal activity and plasticity (for references see Araque and Navarrete (2010), Franke et al. (2003a), Aronica et al. (2012); Kimelberg and Norenberg (1989)). Therefore, changes in the GFAP-expression have been proposed as a biomarker of astrocytic reactions in the CNS (O'Callaghan, 1993, 1994).

Apart from CNS diseases, chemicals also influence GFAP-immunoreactivity (IR) in a substance-specific manner (Malhotra and Shnitka, 1994; Norenberg, 1994; O'Callaghan, 1993). Striking changes in the expression of GFAP have been observed in different animal models also after drug administration of, e.g., alcohol (Franke, 1995), morphine (Beitner-Johnson et al., 1993), amphetamine (Armstrong et al., 2004; Franke et al., 2003a), cannabinoids (Suarez et al., 2000) or cocaine (Fattore et al., 2002).

For example, chronic morphine treatment in rats has been associated with a marked increase (>70%) in the GFAP level specifically in the ventral tegmental area, indicative for structural and functional changes in this region. However, no alterations in GFAP content were observed in several other regions of the CNS studied (Beitner-Johnson et al., 1993). After a single cocaine injection in the dentate gyrus (DG) in mice the GFAP-expression was two-fold enhanced, still significantly higher after seven consecutive daily administrations, but not statistically significant after prolonged drug treatment for two weeks (Fattore et al., 2002). Prenatal exposure to cocaine in mice caused neuronal misaddressing among neocortical layers, abnormal gliogenesis, as well as defective neurite formation and bundling, whereas methadone does not produce detectable alterations in the developing brain (Nassogne et al., 1998). Furthermore, differences in relative preference for, e.g., morphine and other drugs of abuse were shown among distinct strains of rats (Beitner-Johnson et al., 1993). These morphological analyses in animal models revealed damage of diverse cellular and subcellular targets as well as time- and region-dependent differences in astroglial modifications (number, cell size, shape) suggesting that drug exposure differently affects the expression of GFAP in animal brain.

Only a few studies were targeted at the characterization of neuropathological alterations in human drug addicts and the particular effects of drugs (e.g., heroin, cocaine, methadone, morphine) on astrocytes are still controversial. Furthermore, in the brains of polydrug abusers, a significant neuronal loss (direct reaction) and a reduced number of GFAP-positive astrocytes (indirect reaction) have been described (Büttner and Weis, 2006). In brains of heroin addicts a decreased neuronal density has been found in the globus pallidus (Pearson et al., 1976) as well as nerve cell loss, e.g., in the hippocampal formation (Oehmichen et al., 1996). In the hippocampus of 80% of this non-human immunodeficiency virus-infected drug addicts an enhanced expression of GFAP was observed (Oehmichen et al., 1996). In contrast, marked reductions of total neurofilament (NF) proteins were measured in the prefrontal cortex of opioid addicts, but the immunodensities of GFAP, α-internexin and synaptophysin were found to be unchanged (Ferrer-Alcon et al., 2000). The exact etiology of the different neuropathological alterations associated with heroin abuse and the general astrocytic response to drugs of abuse in human is still unclear. Therefore, this study aimed at a quantification of astroglial changes as well as a concrete morphometrical characterization of these changes. The astroglial response in the human CA4 subfield of the hippocampal formation, which is considered sensitive for ischemia and external stimuli, was examined in post-mortem brain samples from drug addicts by histology, immunohistochemistry and Western blot technique (Diemer et al., 1993; Kitamura, 1994). The morphology of astroglial fibers was quantified by automated image analyses.

2. Results

2.1. *Pathomorphology*

2.1.1. *Goldner's Masson trichrome (GM) labeling*

More generally, typical findings in brains of drug addicts are eosinophilia of the nerve cells, decomposing of Nissl substance as well as shrinkage of the cytoplasm and the nucleus of cells are predominantly in areas sensitive to hypoxia, e.g., the CA1-subfield of the hippocampus and Purkinje cells in the cerebellum (data not shown). Such morphological alterations may reflect hypoxic nerve cell damage. In some cases, they were accompanied by severe edema, but almost all cases showed brain edema similar to that of control cases as