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Hippocampal granule cell loss in human chronic alcohol abusers

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ABSTRACT

Chronic alcohol abuse causes cognitive impairments associated with neurodegeneration and volume loss in the human hippocampus. Here, we hypothesize that alcohol reduces the number of granule cells in the human dentate gyrus and consequently contribute to the observed volume loss. Hippocampal samples were isolated from deceased donors with a history of chronic alcohol abuse and from controls with no alcohol over-consumption. From each case, a sample from the mid-portion of hippocampus was sectioned, immunostained for the neuronal nuclear marker NeuN, and counter stained with hematoxylin. Granule cell number and volume of granular cell layer in the dentate gyrus were estimated using stereology. We found a substantial reduction in granule cell number and also a significantly reduced volume of the granular cell layer of chronic alcohol abusers as compared to controls. In controls there was a slight age-related decline in the number of granule cells and volume of granular cell layer in line with previous studies. This was not observed among the alcoholics, possibly due to a larger impact of alcohol abuse than age on the degenerative changes in the dentate gyrus. Loss of neurons in the alcoholic group could either be explained by an increase of cell death or a reduced number of new cells added to the granular cell layer. However, there is no firm evidence for an increased neuronal death by chronic alcohol exposure, whereas a growing body of experimental data indicates that neurogenesis is impaired by alcohol. In a recent study, we reported that alcoholics show a reduced number of stem/progenitor cells and immature neurons in the dentate gyrus, hence that alcohol negatively affects hippocampal neurogenesis. The present results further suggest that such impairment of neurogenesis by chronic alcohol abuse also results in a net loss of granule cells in the dentate gyrus of hippocampus.

1. Introduction

A common saying is that high alcohol consumption will result in neuronal loss in the brain. It is difficult to identify the origin for this notion, but early radiological studies described cortical atrophy of brains from young alcoholics (Tumarkin et al., 1955). Brewer and Perrett (1971) reported radiologically significant brain atrophy in 70% of alcoholics studied with air-encephalography. Wilkinson and Carlen (1980) also found atrophy in brains from alcoholics by CT imaging and reported that these changes correlated with functional deficits and with age. Most subsequent studies on the possible harmful effects on the brain have used computed tomography (CT) and magnetic resonance imaging (MRI). With increased resolution of the instruments and improved software, more recent studies have examined volume changes of discrete brain regions (Mechtcheriakov et al., 2007; Zahr and Pfefferbaum, 2017), but have not been able to provide detailed information of the impact of chronic alcohol use on specific cell types in the brain.

Many morphological studies have been performed to explore the effects of chronic alcohol exposure in animals on cell numbers in the brain. In rat studies, alcohol exposures have been reported to reduce number of neurons in the neocortex (Mooney and Napper, 2005), cerebellum (Napper and West, 1995a; Pauli et al., 1995), and inferior olive (Napper and West, 1995b). More specifically, Richardson et al. (2009) showed that alcohol dependence in rats resulted in reduced neuronal proliferation and survival in medial prefrontal cortex. A reduction of neurons in the occipital, but not parietal cortex, was observed in rats exposed to repeated administration of alcohol early after birth, suggesting that different cell populations and brain regions may be differently vulnerable to alcohol during brain growth (Mooney and Napper, 2005).

Morphological studies of the human brain in this respect are limited.

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In a study on postmortem brains, Jensen and Pakkenberg (1993) used a stereological method to study the number of neurons in different cortical regions and found no significant difference between alcoholics and controls. Harper et al. (1987) however found a significant reduction of neurons in the superior frontal association cortex (Brodmann's area 8) in alcoholics compared to controls, whereas no changes were seen in motor cortex. These results were later confirmed by the same group (Kril et al., 1997), and suggest that neuronal loss may occur in certain subregions but not in others, and that changes might go undetected when the estimation is averaged for a larger brain area.

Hippocampus, another region important in addiction, has also been investigated regarding alcohol-related changes in neuron numbers. Human alcoholics demonstrate deficits in hippocampus–dependent cognition, such as the episodic-, spatial- and working memory (Green et al., 2010; Pitel et al., 2007a; Pitel et al., 2007b) and this is supposed to make drug-related memories more accessible. It has been suggested that these functional changes are due to loss of the dentate gyrus (DG) granule cells, which are part of the tri-synaptic circuitry and are considered to play a key role for the memory and learning functions in hippocampus (Aimone et al., 2014; Lopez-Rojas and Kreutz, 2016). Cognitive deficits resulting from chronic alcohol overconsumption were shown to improve after short- and long-term abstinence (Sullivan et al., 2000). This improvement may, at least in part, be explained by a restoration of neurogenesis during periods of abstinence (Crews et al., 2005). Repeated alcohol exposure in rats and mice has been shown to produce a loss of neurons and consequently a thinning of the granular cell layer (GCL) (Lukoyanov et al., 2000; Oliveira et al., 2015; Richardson et al., 2009; Satriotomo et al., 2000; Walker et al., 1980). In humans, Bengochea and Gonzalo (1990) observed a reduced number of neurons in hippocampus of alcoholics and a voxel-based morphometric study showed deficits in the grey matter in hippocampus of alcoholics compared to controls (Mechtcheriakov et al., 2007). Some years later, two studies used stereology to count neuron numbers in hippocampus and did not find a significant reduction in alcoholics (Harding et al., 1997; Korbo, 1999). However, both studies had a limited number of cases and did not use immunohistochemistry to identify neurons.

As opposed to most neurons in the brain, the granule cells in the DG are special because they have the ability to renew in adulthood (Altman and Das, 1965; Eriksson et al., 1998; Kornack and Rakic, 1999; Spalding et al., 2013). This means that their numbers either can be reduced by increased cell death or by impaired production. Exposures to high doses of alcohol may cause necrotic cell death of neurons (Obernier et al., 2002), but can also produce increased apoptosis in neurons (Nowoslawski et al., 2005; Rajgopal et al., 2003; Young et al., 2005). However, there are studies that do not support significant neuronal death of either kind (Johansson et al., 2009). Hippocampal neurogenesis has been studied extensively in animals and today it is recognized that this renewal also goes on in the adult human brain (Eriksson et al., 1998; Spalding et al., 2013). A large number of experimental studies have shown that neurogenesis is impaired in addiction (Bayer et al., 2015; He et al., 2005; Richardson et al., 2009; Sudai et al., 2011). We have recently been able to confirm this in a study on deceased human donors; we found that alcoholics show a reduction of neural stem/progenitor cells and immature neurons in the DG (Le Maitre et al., 2017), although it is not clear if this results in a reduction of neurons in the granule cell layer. We therefore decided to perform an immunohistochemical study using a stereological approach on a larger cohort of alcoholics and controls than previously reported in order to assess the possible impact of alcohol abuse on the number of granule cells and the volume of the GCL. However, given that hippocampus is a long, windling structure and comprises several millions of neurons, we decided to focus on exactly the same mid-portion of the hippocampus at the level of lateral geniculate body as we investigated in our previous study (Le Maitre et al., 2017). The total number of neurons and volume of GCL further in this article thus refer to the mid-portion (see Morphometry) of hippocampus.

2. Materials and methods

2.1. Case selection

Brain samples were collected from deceased donors subjected to a forensic autopsy at the Department of Forensic Medicine in Stockholm by KI Donatum, a core facility at the Karolinska Institute providing postmortem samples from well-characterized deceased donors for research purposes.

Information about the subjects was obtained by perusing autopsy protocols, police reports, medical records and from semi-structured interviews with the relatives.

Identification of severe alcohol abusers and controls is straightforward using information from the police, relatives, medical records, and postmortem findings. Further characterization of subjects regarding drug use, somatic and psychiatric illness was based on medical records and interviews with relatives. In addition, we also retrieved data from the Swedish national inpatient register (Ludvigsson et al., 2011), as well as from the Swedish national prescribed drug register (Wallerstedt et al., 2016; Wettermark et al., 2007). None of the controls had any visits for alcohol-related problems. Thirteen of the seventeen alcoholics had repeatedly been hospitalized for alcohol dependency. The remaining four subjects (cases # 17, 23, 24 and 33) had all a history of severe alcohol overconsumption for “several years” according to the interviews with the relatives. Three of them also had alcohol-induced liver disease and one had alcohol cardiomyopathy. According to the register data, some of the alcoholic subjects had been enrolled in disulfiram treatment programs for variable periods, but none had abstained from alcohol for longer times.

Table 1 provides information on age, gender, cause of death, postmortem interval (PMI), brain pH, alcohol and drug use, and presence of depression and liver pathology. In total, 33 cases, 28 males and 5 females, aged 16–74 years, representing 17 alcoholic and 16 control subjects, were included in the study; mean age was 52 (median 53) and 54 (median 60) for alcoholic and control subjects, respectively. Median postmortem interval (PMI) was 51.2 h (range 18–82.5 h) and 41.8 h (range 25.8–84.5 h) for alcoholics and controls, respectively. Median warm time (time before cold storage at 4 °C) was 8.2 h for alcoholics (range 2–26.2 h) and 5.8 h for controls (range 1.1–41.2 h). The average PMI was somewhat long in both groups, but most of the PMI represents cold time (refrigeration), which slows down postmortem tissue degradation. Number of years of alcohol abuse, based on hospital records, is also reported. These data should be considered as a minimum, since it will often take several years until alcohol problems result in hospitalization (e.g. case # 19 was diagnosed with liver cirrhosis at his first hospital visit for alcohol problems).

2.2. Inclusion criteria

Cases selected for this study were long-term alcoholics; this information was obtained from several sources – the police reports, the interviews with relatives and in some cases also confirmed by medical records, when available. All of the alcoholics had been drinking heavily for many years and most of subjects had moderate to severe fatty liver or liver cirrhosis (Table 1), whereas all but three of the controls had normal liver structure; two had minor fatty deposition in liver epithelial cells microscopically and one had moderate steatosis, but this subject was obese, case # 15 (Table 1). Five cases with depression were included in the study; one case in the control group and 4 cases in the alcoholic group. The latter subjects may be representative for a fraction of alcoholics who develop depressive mood as a consequence of the alcoholism. They were therefore not excluded from the study, since there was no history of psychiatric problems prior to the onset of the alcohol abuse. Further, cases included in the study had no other neurodegenerative diseases such as Alzheimer's, Parkinson's or Huntington's disease. One of the alcoholics (case # 28) had received treatment