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Ricardo Jorge Nogueira Rodrigues da Rocha

Effect of chronic alcohol consumption and withdrawal on the hippocampal formation

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Nome: RICARDO JORGE NOGUEIRA RODRIGUES DA ROCHA

Endereço electrónico: RESINO@GMAIL.COM

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Assinatura: Ricardo Jorge Rocha

Effect of chronic alcohol consumption and withdrawal on the hippocampal formation

Ricardo Jorge Nogueira Rodrigues da Rocha

Department of Anatomy, Porto Medical School, Alameda Hernâni Monteiro, 4200-319 Porto, Portugal.

Corresponding author. Department of Anatomy, Porto Medical School, University of Porto,
Alameda Hernâni Monteiro, 4200-319, Porto, Portugal
Tel: ++351-22-5513616
Fax: ++351-22-5513617
E-mail address: besiro@gmail.com

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Abstract

Alcohol is the second most common psychotropic drug consumed worldwide and alcoholism represents a major nowadays social and medical problem, especially in a wine-producing country like ours. Chronic alcohol abuse is known to have primary effects in learning and memory brain functions, which are deeply related with the hippocampal formation. Therefore, several research groups have been studying the main effects of chronic alcohol consumption and withdrawal in this brain area, so that a better understanding of its underlying molecular mechanisms is possible. This review will discuss current knowledge on the morphologic and functional changes, as well as, its consequent behavioral implications in the hippocampal formation caused by the chronic alcohol consumption and withdrawal.

Keywords: Hippocampal formation; Chronic alcohol consumption; Alcohol withdrawal

Introduction

Throughout ancient times, alcoholism has been a serious problem to mankind (Phillips, S. C. and Cragg, B. G. 1983). Recent numbers indicate that alcohol is one of the most common psychotropic drugs consumed worldwide and chronic alcohol abuse is seen as an important social and medical problem (Casswell, S. and Thamarangsi, T. 2009), with many known deleterious effects, not only for the individual himself, but also for society, representing a pattern of addictive behavior with a very negative connotation (Grønbaek, M. 2009; Spanagel, R. 2009). However, in Portugal, a great wine-producing country, alcoholism constitutes an important concern for physicians. Due to the acceptance of social drinking, many people cannot restrain the consumption of alcohol, and what was supposed to be a leisure or a social event soon turns out to addiction (Spanagel, R. 2009).

Despite the well established relationship between chronic alcohol consumption and liver cirrhosis and cardiovascular impairment (Grønbaek, M. 2009), there is still much to discover about the molecular basis of the consequences of alcohol abuse in the central nervous system (Campanella, S., Petit, G., Maurage, P., Kornreich, C., Verbanck, P. and Noël, X. 2009). In what concerns brain function, clinical practice has shown that learning and memory skills are the most affected, together with some cognitive deterioration (Nolte, J. 2002).

The field of Neuroscience has evolved greatly throughout the past decades and it is now clear that the hippocampal formation plays a pivotal role in learning and memory processes (Nolte, J. 2002). Basic science studies constitute a powerful tool to understand signaling pathways and molecular mechanisms. Therefore, many research groups have been devoting their work to learning the effects, at the cellular and molecular levels, of chronic alcohol on the brain structure and function.

The important role of the hippocampal formation in learning and memory is probably on the basis of the particular attention that this region of the brain has received in investigations addressing the effects of excess alcohol on brain structure and function. Also, its highly organized and laminar arrangement makes the hippocampal formation a convenient model for studying morphology and synaptic actions *in vivo* and *in vitro* (Nolte, J. 2002). Due to these facts, this review will focus on the morphologic, functional and behavioral changes in the hippocampal formation caused by the chronic alcohol consumption and withdrawal.

The hippocampal formation

The hippocampal formation is a curved sheet of cortex that is folded into the medial surface of the temporal lobe. Some authors consider that it consists of three distinct zones: the dentate gyrus, the hippocampus proper (also known as *cornu ammonis*) and the subiculum (Nolte, J. 2002). However, other authors state that the entorhinal cortex should be included as a component of the hippocampal formation (Lavenex, P. and Amaral, D. G. 2000). The entorhinal cortex is part of the medial temporal lobe and appears to be necessary for the establishment of long-term declarative memory (Lavenex, P. and Amaral, D. G. 2000). In particular, it may be involved in the "consolidation" of information in higher-order associational cortices, perhaps through feedback projections (Lavenex, P. and Amaral, D. G. 2000). Indeed, associational connections within the perirhinal, parahippocampal, and entorhinal cortices enables a significant amount of integration of unimodal and polymodal inputs, so that only highly integrated information reaches the remainder of the hippocampal formation (Lavenex, P. and Amaral, D. G. 2000). The feedback efferent projections from the perirhinal and parahippocampal cortices to the neocortex largely reciprocate the afferent projections from the neocortex to these areas (Lavenex, P. and Amaral, D. G. 2000). These

observations are particularly important for models of hippocampal-neocortical interaction and long-term storage of information in the neocortex (Lavenex, P. and Amaral, D. G. 2000).

The hippocampus proper and the dentate gyrus are three-layered areas, with a superficial molecular layer and a deep polymorphic layer, both similar to the layers of the neocortex (Nolte, J. 2002). The intermediate stratum is a granule cell layer in the dentate gyrus and a pyramidal cell layer in the hippocampus proper (Nolte, J. 2002). The molecular layer is mainly occupied by the dendrites of the dentate granule cells and by the fibers of the perforant pathway that originates in the entorhinal cortex (Nolte, J. 2002). In addition, it contains a small number of interneurons and fibers originating in several other regions of the brain (Nolte, J. 2002).

In the dentate gyrus, the cell-containing layer, the granule cell layer, is largely made up of densely packed granule cells (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007). It also contains a small number of other neurons that are located at the border between the granule cell layer and the polymorphic layer (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007). The cell bodies of the dentate pyramidal basket cells, for example, are often located just within the granule cell layer at its border with the polymorphic layer (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007). The granule cell layer encloses a cellular region, the polymorphic cell layer – also known as the hilus of the dentate gyrus –, which constitutes the third layer of the dentate gyrus (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007). A number of cell types are located in the polymorphic layer, but the most prominent are the mossy cells (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007). The granule cells give rise to distinctive unmyelinated axons which Ramón y Cajal called mossy fibers (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007). The mossy fibers have unusually large boutons that form en passant synapses with the mossy cells of the polymorphic layer and with the CA3 pyramidal cells of the hippocampus (Amaral, D. G., Scharfman, H. E. and Lavenex, P. 2007).

The main input to the hippocampus, the perforant pathway (Fig. 1), arises from the entorhinal cortex and projects the dentate gyrus (Nolte, J. 2002). In turn, the axons of the granule cells of dentate gyrus, the mossy fibers, project to the CA3 hippocampal region (Fig. 1) (Nolte, J. 2002). CA3 sends connections to CA1 pyramidal cells via the Schaffer collaterals (SC) and to the contralateral hippocampus via commissural fibers (Nolte, J. 2002). The final output from the hipocampus proper reaches the subiculum (Fig. 1), which projects via the the alveus, fimbria and fornix to other regions of the brain (Nolte, J. 2002). This circuit (diagrammatically represented in Fig. 1), is known as the trisynaptic circuit of the hippocampal formation and uses glutamate as its major neurotransmitter (Nolte, J. 2002). The fornix fibres arch under the corpus callosum, and at the level of the interventricular foramina and in close proximity with the anterior commissure, they split off to form the pre-and the precommissural fornix (Nolte, J. 2002). Fibers incorporating the precomissural fornix terminate in the septal region, ventral striatum, and orbital and cingulate cortices, whereas those in the postcommisural fornix end in the anterior thalamic nucleus and the mammillary body (Nolte, J. 2002).

The entorhinal cortex receives inputs from the cingulate, temporal and orbital cortices as well as from the amygdala and olfactory bulb (Nolte, J. 2002). Some other inputs originate in the septal nuclei and hypothalamus (Nolte, J. 2002).

Animal models used to assess the effects of alcohol consumption

Human disease and biomedical research frequently require the use of animal models, in order to help the understanding of the molecular processes involved in pathogenesis and, furthermore, help developing drug targets to prevent or minimize damage for the patient. The study of alcoholism is no exception. In addition to epidemiological studies that can be made

in some cohorts of people chronically consuming alcohol, animal research plays a pivotal role to assess the effects of chronic alcohol consumption and withdrawal.

Even though the experimental models of alcohol consumption vary, depending on research group, most of the studies performed in the hippocampal formation have been done using a model in which a 20% ethanol solution was the only liquid source available and in which rats had free access to solid food (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999).

In order to evaluate the effects of chronic alcohol consumption, three animal groups are required. One, is a control-group (A, Fig. 2), which has no access to ethanol throughout the study. Another, is a pair-fed control group. The inclusion of this group in the studies of chronic alcohol consumption is important to avoid bias resulting from the reduced caloric intake of alcohol-consuming rats. This group consists of rats that consume an amount of solid food that provides the same amount of calories that ethanol-treated rats receive from the solid diet and from the alcohol they consume. The third group (B, Fig. 2), is composed of rats that start a progressive diet of alcohol intoxication, usually starting with 5% (v/v) ethanol and an every-day increase of 1% until a maximum of 20% (v/v). The whole study usually takes place during 12 to 18 months and usually the rats are said to be fully intoxicated at about 6 months of the referred diet. This time point is crucial, because then the group will be split in two, in order to assess withdrawal, meaning that when the study ends, one will have three groups to evaluate. The withdrawal group (C, Fig. 2), usually follow a progressive ethanol consumption decrease 20%, 15%, 10%, 5%, 0% until the animals are no longer provided with ethanol and are only provided with water, like in the control group (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999). However, two important remarks must be done at this point. This experimental situation does not recreate the commonly known withdrawal syndrome in humans. In order to do so, rats after chronic exposure to ethanol should be abruptly deprived

from any ethanol consumption (D, Fig 2) and it has been proven that the resulting effect is an early death by seizures, hence reinforcing the clinical importance of a proper and immediate management of a withdrawal syndrome in patients and also stands as an important experimental set up useful for drug evaluation before clinical use in patients with withdrawal syndrome (Gonzalez, L. P. 1985). In other words, the purpose of assessing withdrawal in rats is to evaluate if the there are some positive or negative morphological and functional changes in the brain, after the interruption of ethanol consumption (Gonzalez, L. P. 1985).

It should be recalled that there is variation between different types of animal alcohol consumption/exposure. Several methods have been described and include: ethanol-containing liquid diets, ethanol gastric gavage, ethanol inhalation and intraperitoneal injection of ethanol (Piano, M. R., Artwohl, J., Kim, S. D. and Gass, G. 2001). For historic reasons, one cannot forget to mention the Lieber-DeCarli method, because it alerted the researcher for the habituation phase of the liquid diet. The rodents are aversive to taste of ethanol, and the introduction of alcohol is associated with low food intake and weight loss, potentially important non-experimental variables (Piano, M. R., Artwohl, J., Kim, S. D. and Gass, G. 2001). It has been proven that moderately restricted food intake has been reported to affect the secretion patterns of hormones and can impair physiological function in organs such as the heart, liver and central nervous system (Piano, M. R., Artwohl, J., Kim, S. D. and Gass, G. 2001). Therefore, for most experimental studies of chronic alcohol consumption, the liquid diet technique is an efficient tool to study the effects of ethanol under controlled nutritional conditions (Lieber, C. S., DeCarli, L. M. and Sorrell, M. F. 1989). It allows for alcohol consumption of clinical relevance and offers flexibility to adjust to special experimental or physiologic needs by allowing for various substitutions required for a particular experimental design, including changes in lipids, proteins or other dietary constituents (Lieber, C. S., DeCarli, L. M. and Sorrell, M. F. 1989). The technique also facilitates the comparison with

controls by simplifying the pair feeding and is the best procedure available for the study of the toxic effects of alcohol and their interactions with deficiency or excess of various nutrients (Lieber, C. S., DeCarli, L. M. and Sorrell, M. F. 1989).

Finally, when choosing an animal model for an experiment to assess chronic alcohol consumption and withdrawal, the researcher should be aware that the available methods serve different purposes: some are more suitable to evaluate liver damage, other endocrine dysfunction and other central nervous system impairment (Lieber, C. S., DeCarli, L. M. and Sorrell, M. F. 1989).

The hippocampus proper and alcohol

Investigations carried out in the hippocampus proper have shown that the thickness of the CA3 pyramidal layer and the numerical density of its neurons are both significantly reduced in alcohol-fed groups than in age-matched controls (Cadete-Leite, A., Tavares, M. A., Alves, M. C., Uylings, H. B. and Paula-Barbosa, M. M. 1989). It has also been reported that the numerical density of the CA3 pyramidal cells is smaller, although not significantly, in withdrawn than in age-matched alcohol-treated groups (Cadete-Leite, A., Tavares, M. A., Alves, M. C., Uylings, H. B. and Paula-Barbosa, M. M. 1989). Conversely, the total number of CA3 pyramidal was found to be reduced, by approximately 18%, in alcohol-treated animals (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999; Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D. and Paula-Barbosa, M. M. 2000).
Moreover, in withdrawn rats there was a further decay in the total number of pyramidal neurons, which amounted to 15% relative to ethanol-treated animals (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999).

Studies carried out in Golgi-impregnated material showed that there were no significant differences in the number of dendrites per cell in alcohol-fed groups, as opposed to withdrawn rats that had significantly fewer dendrites per cell than did the controls (Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993). The total dendritic length was significantly greater in alcohol-fed animals and significantly reduced in the withdrawal group (Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993). The radial distance of the terminal segments was significantly greater after 6 months of alcohol intake than in controls (Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993). In the neuropil of the *stratum lucidum* no significant differences were found in the numerical density of the MF-CA3 synapses when 6-month and 12-month alcohol-fed groups were compared with respective controls, but after 18 months of experiment there was a significant reduction in the number of synapses in both the alcohol-fed and withdrawal groups (Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993). The percentage of mossy fiber plasmalemma occupied by postsynaptic densities was greater in the alcohol-fed and withdrawn rats than in controls (Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993). It was also shown that the total number of synapses between the mossy fibers and CA3 pyramids was unaffected by alcohol treatment (Cadete-Leite, A., Tavares, M. A., Pacheco, M. M., Volk, B. and Paula-Barbosa, M. M. 1989; Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D. and Paula-Barbosa, M. M. 2000). However, in ethanol-treated rats, the proportion of the mossy fiber plasmalemma occupied by the synapses there was an increase (Cadete-Leite, A., Tavares, M. A., Pacheco, M. M., Volk, B. and Paula-Barbosa, M. M. 1989).

In the CA1 region, the thickness of the pyramidal cell layer and the numerical density of its neurons were significantly lower in alcohol-fed groups than in age-matched controls (Fig. 3 and Fig. 4) (Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A.

1993). Also, in the alcohol-treated animals, there was a noticeable loss (of approximately 20%) of CA1 pyramidal cells (Paula-Barbosa, M. M., Borges, M. M., Cadete-Leite, A. and Tavares, M. A. 1986; Cadete-Leite, A., Tavares, M. A., Pacheco, M. M., Volk, B. and Paula-Barbosa, M. M. 1989). In withdrawn rats, there was a further decay in the total number of pyramidal neurons, which amounted to 15% relative to ethanol-treated animals (Fig. 5) (Lukoyanov NV, Madeira MD and Paula-Barbosa MM 1999). In other study, the authors found that the number of cells in a section of hippocampus was unchanged in field CA1 by alcohol exposure but was reduced 9% during withdrawal from alcohol (Phillips, S. C. and Cragg, B. G. 1983). In the same work, the authors measured the spine heads and they were smaller in the alcohol group than in the control group, being too small to be visible with the light microscope (Phillips, S. C. and Cragg, B. G. 1983). Multivesicular bodies with diameters up to 4.5 micrometers were observed in the hippocampal pyramidal cells of rats submitted to chronic alcohol consumption, showing a significant increase in the volumetric density in CA1 pyramidal cells (Paula-Barbosa, M. M., Borges, M. M., Cadete-Leite, A. and Tavares, M. A. 1986).

The dentate gyrus and alcohol

Stereological methods were applied in a considerable number of studies to evaluate the damage inflicted by alcohol intake in the structure of the hippocampal formation (Cadete-Leite, A., Tavares, M. A. and Paula-Barbosa, M. M. 1988; Cadete-Leite, A., Tavares, M. A., Uylings, H. B. and Paula-Barbosa, M. M. 1988; Cadete-Leite, A., Tavares, M. A., Alves, M. C., Uylings, H. B. and Paula-Barbosa, M. M. 1989; Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993; Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D. and Paula-Barbosa, M. M. 2000).

Research proved that, in some cases, after 6 months of alcohol consumption, the thickness of the dentate gyrus granular layer and the relative number of dentate granule cells were significantly decreased when compared with controls (Paula-Barbosa MM, Brandão F, Madeira MD and Cadete-Leite A 1993), and even less in the withdrawn group (Cadete-Leite, A., Tavares, M. A., Uylings, H. B. and Paula-Barbosa, M. M. 1988; Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993). Others revealed the same decrease after 18 months compared to controls (Cadete-Leite A, Tavares MA and Paula-Barbosa MM 1988; Cadete-Leite, A., Tavares, M. A. and Paula-Barbosa, M. M. 1988). In a different study, a more dramatic reduction was observed in a group rats submitted to 6 months of withdrawal after 12 months of alcohol consumption (Cadete-Leite, A., Tavares, M. A. and Paula-Barbosa, M. M. 1988). Subsequent studies have shown that in alcohol-treated animals there was a 10% loss of granule cells in the dentate gyrus (Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D. and Paula-Barbosa, M. M. 2000). It was further shown that withdrawal from alcohol leads to a further decrease of the relative number of granule cells in the dentate gyrus (Cadete-Leite, A., Tavares, M. A., Alves, M. C., Uylings, H. B. and Paula-Barbosa, M. M. 1989). The study of the dendritic arborizations of the dentate granule cells revealed that the total dendritic length was reduced in withdrawn relative to ethanol-treated rats (Cadete-Leite A, Tavares MA, Alves MC, Uylings HB and Paula-Barbosa MM 1989). Interestingly, the dendritic length was increased in alcohol-treated rats (Cadete-Leite, A., Tavares, M. A., Uylings, H. B. and Paula-Barbosa, M. M. 1988).

Investigations focused in the hilus of the dentate gyrus showed that the numerical density of hilar cells was significantly lower in alcohol-treated and withdrawn animals than in controls (Andrade, J. P., Fernando, P. M., Madeira, M. D., Paula-Barbosa, M. M., Cadete-Leite, A. and Zimmer, J. 1992; Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993), and that subsequent withdrawal provoked an increase in the

numerical density to controls levels (Andrade, J. P., Fernando, P. M., Madeira, M. D., Paula-Barbosa, M. M., Cadete-Leite, A. and Zimmer, J. 1992; Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993).

Lipofuscin

Some investigations suggested that chronic alcohol consumption accelerated a number of age-related changes in the hippocampal formation (Borges, M. M., Paula-Barbosa, M. M. and Volk, B. 1986; Paula-Barbosa, M. M., Brandão, F., Pinho, M. C., Andrade, J. P., Madeira, M. D. and Cadete-Leite, A. 1991). So, some authors studied the pattern of lipofuscin deposition in alcohol-fed rats for periods of 1, 3, 6, 12 and 18 months and compared the results with those obtained in the respective pair-fed controls (Borges, M. M., Paula-Barbosa, M. M. and Volk, B. 1986). A precocious and progressive deposition of lipofuscin pigment was found in the pyramidal cells of CA1 and CA3 hippocampal fields, after 3 and 6 months of alcohol feeding (Borges, M. M., Paula-Barbosa, M. M. and Volk, B. 1986). A study, stated in addition, that it was also found that alcohol withdrawal did not reverse the changes (Paula-Barbosa, M. M., Brandão, F., Pinho, M. C., Andrade, J. P., Madeira, M. D. and Cadete-Leite, A. 1991). On the contrary, most of the alterations observed during alcohol consumption worsened as happens with the increased lipofuscin formation (Paula-Barbosa, M. M., Brandão, F., Pinho, M. C., Andrade, J. P., Madeira, M. D. and Cadete-Leite, A. 1991).

Neurochemical changes

The major amino acid transmitter systems of the central nervous system - the inhibitory gamma-aminobutyric acid (GABA) and the excitatory glutamate - have been

widely studied over the past years. There is now general consensus that acute ethanol consumption facilitates GABAergic transmission, by enhancing chloride conductance through the GABA-A receptor, and inhibits glutamatergic function by decreasing cationic conductance through the NMDA receptor. Conversely, the development of tolerance associated with chronic ethanol consumption leads to reduced GABAergic activity and to increased glutamatergic function (reviewed in Nevo, I. and Hamon, M. 1995).

A number of ligands of the dopaminergic, serotonergic and opioidergic receptors involved in ethanol consumption-related behaviors have been recognized for their effects in reducing ethanol preference and/or alleviating the symptoms of the ethanol withdrawal syndrome in various animal models (reviewed in Nevo, I. and Hamon, M. 1995).

Some behavioral studies have demonstrated the ability of GABA mimetic drugs to potentiate sedative and incoordinating effects of alcohol in rodents, whereas GABA antagonists and inverse agonists have been shown to attenuate these effects (Allan A. M. and Harris R. A. 1987; reviewed in Nevo, I. and Hamon, M. 1995). In some studies, the number of hilar somatostatin immunoreactive, a major neuronal subpopulation of GABAergic interneurons, was found to be significantly reduced in both alcohol-fed and withdrawn rats when compared with the respective age-matched controls (Andrade, J. P., Fernando, P. M., Madeira, M. D., Paula-Barbosa, M. M., Cadete-Leite, A. and Zimmer, J. 1992; Paula-Barbosa, M. M., Brandão, F., Madeira, M. D. and Cadete-Leite, A. 1993).

Research concerning the central cholinergic system in alcoholics has provided clues to the mechanisms underlying the deleterious effects of ethanol on learning and memory, and evidence of a reduced central cholinergic activity has been reported in alcohol-dependent patients (Arendt T., Allen Y., Marchbanks R. M., Schugens M. M., Sinden J., Lantos P. L. and Gray J. A. 1989; reviewed in Nevo, I. and Hamon, M. 1995). Interestingly, acetylcholine-rich grafts and cholinomimetic drugs have been found to ameliorate ethanol-induced

behavioral deficits in alcohol-treated rats (Hodges H., Sinden J., Mitchell S. N., Arendt T., Lantos P. L. and Gray J. A. 1991; reviewed in Nevo, I. and Hamon, M. 1995).

Other studies examined the effects of chronic ethanol exposure and withdrawal on the sensitivity of the hippocampus to local injection of physostigmine, an inhibitor of acetylcholine metabolism (Gonzalez, L. P. 1985). While intrahippocampal physostigmine elicited hippocampal seizure activity in 80% of the animals tested during withdrawal from chronic exposure to low levels of ethanol, seizure activity was elicited in only 30% of ethanol-naive subjects (Gonzalez, L. P. 1985).

Another investigation tried to elucidate if the cholinergic neurochemical functions were altered by long-term ethanol consumption in rats (Pelham, R. W., Marquis, J. K., Kugelmann, K. and Munsat, T. L. 1980). It was found that 18 weeks of ethanol consumption in a liquid diet reduced rat striatal and mammillary body choline acetyltransferase (ChAT) by 53% and 58%, respectively, compared to controls (Pelham, R. W., Marquis, J. K., Kugelmann, K. and Munsat, T. L. 1980). In these same regions, the density of muscarinic cholinergic receptors was increased by 117% and 12%. Nevertheless, these alterations were not observed in the hippocampal formation (Pelham, R. W., Marquis, J. K., Kugelmann, K. and Munsat, T. L. 1980). Although there were no alterations following 2 weeks of alcohol consumption, they were apparent after 4 weeks of withdrawal. After 13 months of ethanol consumption, the number of neuroleptic binding sites in striatum was diminished by 29% (Pelham, R. W., Marquis, J. K., Kugelmann, K. and Munsat, T. L. 1980).

It was also reported that chronic ethanol treatment decreases the cholinergic facilitation of population spikes in the CA1 hippocampal region, without affecting cholinergic inhibition of field EPSPs, indicating that alcohol appears to selectively disrupt a subset of cholinergic effector systems within hippocampal neurons (Rothberg B. S., Yasuda R. P., Satkus S. A., Wolfe B. B. and Hunter B. E. 1993; reviewed in Nevo, I. and Hamon, M. 1995).

Another study investigated the sensitivity to NMDA in ethanol-treated animals by evaluating the damage provoked by intrahippocampal injections of NMDA (Davidson, M. D., Wilce, P. and Shanley, B. C. 1993). ChAT and glutamate decarboxylase (GAD) specific activities were used as markers of cholinergic and gamma-aminobutyric acid neurons, respectively (Davidson, M. D., Wilce, P. and Shanley, B. C. 1993). Ethanol-dependent animals were more liable to die following intrahippocampal injection of either 120 or 240 nmol of NMDA (Davidson, M. D., Wilce, P. and Shanley, B. C. 1993). There was a significantly greater decrease in hippocampal GAD but not ChAT specific activities in the surviving animals (Davidson, M. D., Wilce, P. and Shanley, B. C. 1993).

Behavioral changes

The neurodegenerative changes that occur in the hippocampal formation are of special relevance given the involvement of this limbic structure in the process of memory formation, which is known to be impaired in human alcoholics and ethanol-treated animals (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999).

According to some studies that tested the reference memory task, significant cognitive deficits were not observed in rats continuously exposed to ethanol, whereas withdrawn animals showed an obvious impairment of their overall performance (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999), particularly in the probe trial (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999). In fact, these animals required significantly longer swimming distances to approach the former position of the platform when compared with controls and alcohol-consuming animals (Lukoyanov, N. V., Madeira, M. D. and Paula-Barbosa, M. M. 1999).

On the other hand, another study found that alcohol consumption does not affect the performance of rats in the reference memory task as indicated by the measures derived from the acquisition trials and from the probe-trial, which were similar for alcohol-fed and control animals (Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D. and Paula-Barbosa, M. M. 2000). In these studies, working memory was not significantly altered in alcohol-treated animals (Lukoyanov, N. V., Brandão, F., Cadete-Leite, A., Madeira, M. D. and Paula-Barbosa, M. M. 2000).

Some other studies investigated if chronic ethanol ingestion in mice produced long-term deficits in learning and memory after the cessation of ethanol (Farr, S. A., Scherrer, J. F., Banks, W. A., Flood, J. F. and Morley, J. E. 2005). It was found that ethanol consumption resulted in deficits in learning and long-term memory and that 12 weeks after the alcohol withdrawal, there was still behavioral impairments (Farr, S. A., Scherrer, J. F., Banks, W. A., Flood, J. F. and Morley, J. E. 2005). Nevertheless, short-term memory was not affected (Farr, S. A., Scherrer, J. F., Banks, W. A., Flood, J. F. and Morley, J. E. 2005).

Decrements of long-term potentiation (LTP) in the hippocampal formation are thought to be involved in the long-term impairment of learning and memory resulting from chronic ethanol treatment. (Peris, J., Anderson, K. J., Vickroy, T. W., King, M. A., Hunter, B. E. and Walker, D. W. 1997). Treatment during 28 weeks affected the rat hippocampal formation and decreased the magnitude of LTP, an effect that can last as long as 7 months after ethanol withdrawal (Peris, J., Anderson, K. J., Vickroy, T. W., King, M. A., Hunter, B. E. and Walker, D. W. 1997). It appears that NMDA receptor number in the hippocampal formation is unchanged after chronic ethanol consumption, which suggests a more pronounced role for changes in GABAergic and cholinergic synaptic transmission in determining the influence of chronic alcohol consumption in the induction of LTP in hippocampus (Peris, J., Anderson, K. J., Vickroy, T. W., King, M. A., Hunter, B. E. and Walker, D. W. 1997). In particular,

changes in presynaptic modulation of neurotransmitter release in the hippocampal formation may be one mechanism by which chronic ethanol consumption inhibits LTP (Peris, J., Anderson, K. J., Vickroy, T. W., King, M. A., Hunter, B. E. and Walker, D. W. 1997). Thus, the mechanisms underlying the effect of ethanol consumption on LTP are a result of changes in a number of neurotransmitter systems in hippocampal formation (GABAergic and cholinergic) rather than in glutamate transmission alone (Peris, J., Anderson, K. J., Vickroy, T. W., King, M. A., Hunter, B. E. and Walker, D. W. 1997).

Considerations and perspectives

A vast amount of studies have been quite conclusive concerning the deleterious effects observed in the hippocampal formation related to the chronic exposure to alcohol and also withdrawal. From the morphological point of view, it is clear that chronic alcohol consumption leads to neuronal degeneration and dendritic alterations. Regarding neurotransmitters and synaptic pathways it has been shown that there are damaging effects, which culminate in impaired learning and memory abilities. In addition, it has been shown that withdrawal has an even more negative repercussion in all these parameters. These observations may cause one to speculate whether, at some point in chronic alcohol consumption, the neuronal damage becomes irreversible and most likely self-triggered, leading to a chain of events which can hardly be stopped.

The magnitude of the damage caused by alcohol consumption emphasizes the need of developing drugs that can prevent the neuronal death or, at least, slow the progression of neuronal damage associated with alcohol abuse. In this respect, the neuroprotective drug piracetam has been used for some years, with some success (Paula-Barbosa, M. M., Brandão, F., Pinho, M. C., Andrade, J. P., Madeira, M. D. and Cadete-Leite, A. 1991; Brandão, F.,

Paula-Barbosa, M. M. and Cadete-Leite, A. 1995; Brandão, F., Cadete-Leite, A., Andrade, J. P., Madeira, M. D. and Paula-Barbosa, M. M. 1996). Also, flavonoids, which are known anti-oxidative compounds, may also play an important role in the prevention of lipofuscin accumulation, which was shown to be highly correlated with chronic alcohol consumption and withdrawal (de Freitas, V., da Silva Porto, P., Assunção, M., Cadete-Leite, A., Andrade, J. P. and Paula-Barbosa, M. M. 2004). In addition, some recent and exciting new studies have been made in the field of regenerative medicine, with the use of stem cells in alcoholic patients (Nixon, K., Morris, S. A., Liput, D. J. and Kelso, M. L. 2010).

In conclusion, despite the major importance of translational research and of a permanent dialogue between physicians and researchers, further bench work will still be needed for better understanding the pathogenesis underlying the deleterious effects of chronic alcohol consumption and withdrawal in the hippocampal formation. Next goals of research may include the understanding of the patient susceptibility to develop alcohol addiction and neuronal damage, likely related to gene/environment complex interactions (Ryabinin, A. E. 1998; Heidbreder, C. A. and Newman, A. H. 2010; Schwandt, M. L., Lindell, S. G., Chen, S., Higley, J. D., Suomi, S. J., Heilig, M. and Barr, C. S. 2010).

A final remark should be addressed to the physicians, as there is a need to complement basic science studies with field work, such as epidemiological studies including large cohorts of alcoholics, in order to fully assess the bio-psycho-social dimension of a patient, who is inserted in a proper socio-economical scenario, which is impossible to reproduce in the laboratory.

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Figure legends

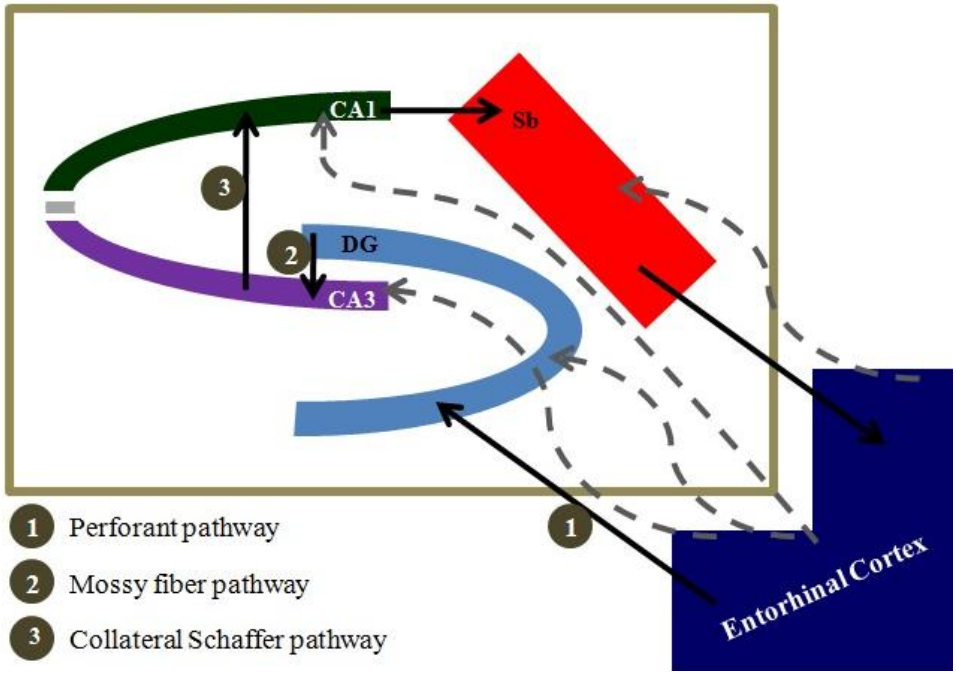
Fig.1. Schematic overview of the trisynaptic circuit of the hippocampal formation.

Fig. 2. Pair feeding experimental model used, in the Center of Experimental Morphology, Faculty of Medicine, University of Porto, for the assessment of chronic alcohol consumption and withdrawal in the hippocampal formation. A: control group, B: chronic alcohol consumption group, C: withdrawal group; t0: time point zero – beginning of the study, *: time point where withdrawal is begun in the study, tF: final time point – end of the experimental study; D: experimental model used to recreate human withdrawal syndrome)

Fig. 3. Photomicrograph of a thick Giemsa-stained section from the CA1 pyramidal cell layer of the hippocampal formation of a control rat.

Fig. 4. Photomicrograph of thick Giemsa-stained sections from the CA1 pyramidal cell layer of the hippocampal formation of an alcohol-treated rat.

Fig. 5. Photomicrograph of thick Giemsa-stained sections from the CA1 pyramidal cell layer of the hippocampal formation of a withdrawn rat.


CA1
Sb
3
2
DG
CA3
1
Perforant pathway
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Mossy fiber pathway
3
Collateral Schaffer pathway
1
Entorhinal Cortex

Figure 1

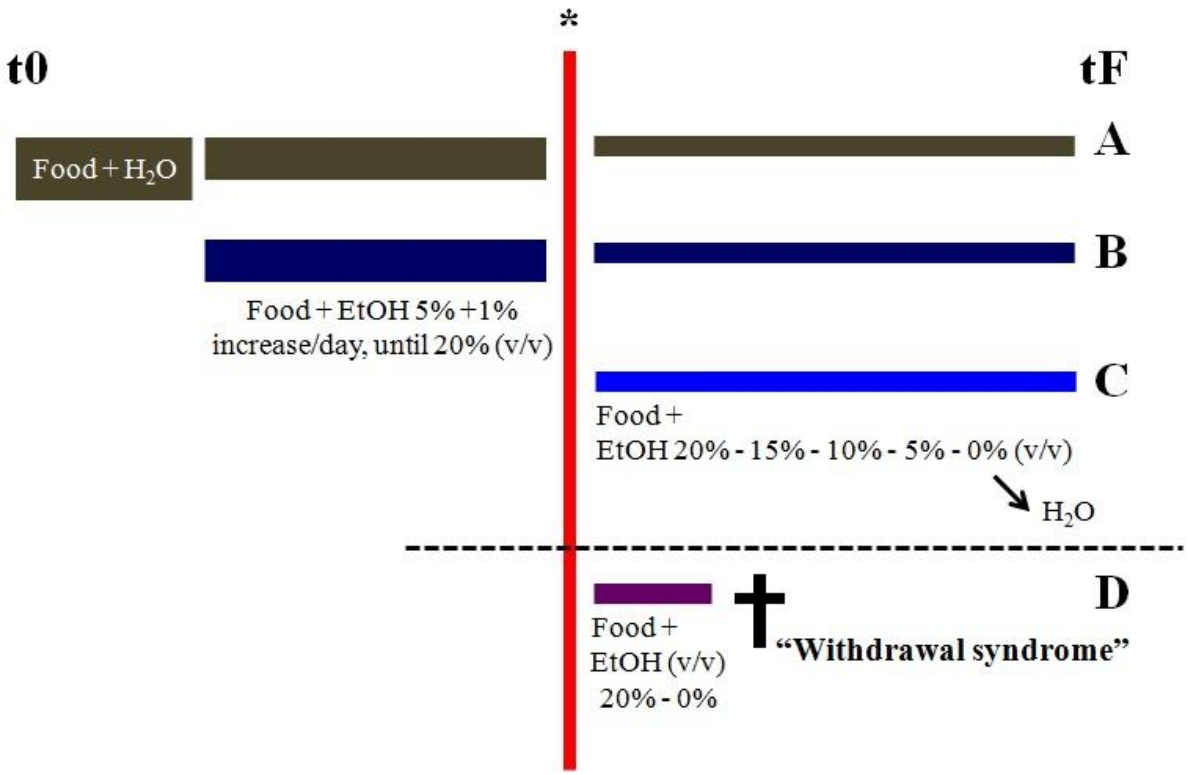
*
t0
tF
Food + H2O
A
B
Food + EtOH 5% +1%
increase/day, until 20% (v/v)
C
Food +
EtOH 20% - 15% - 10% - 5% - 0% (v/v)
H2O
D
Food +
EtOH (v/v)
20% - 0%
"Withdrawal syndrome"

Figure 2

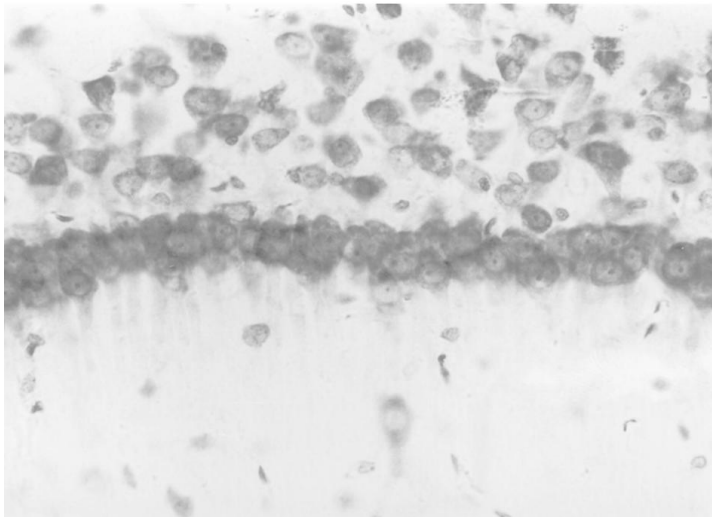

Figure 3

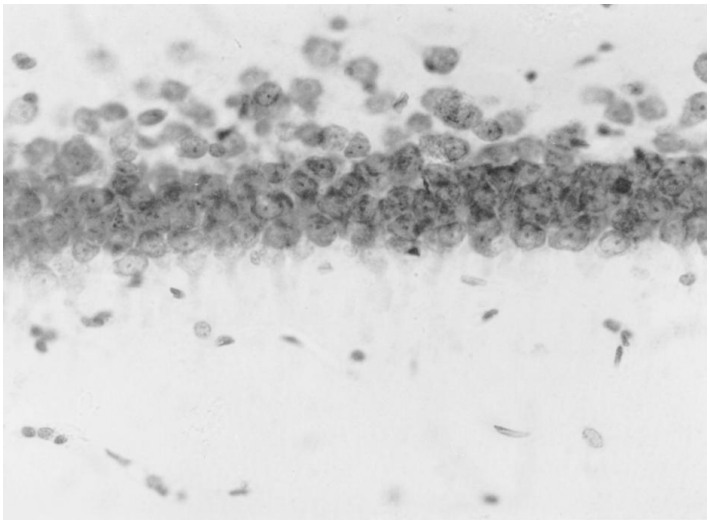

Figure 4

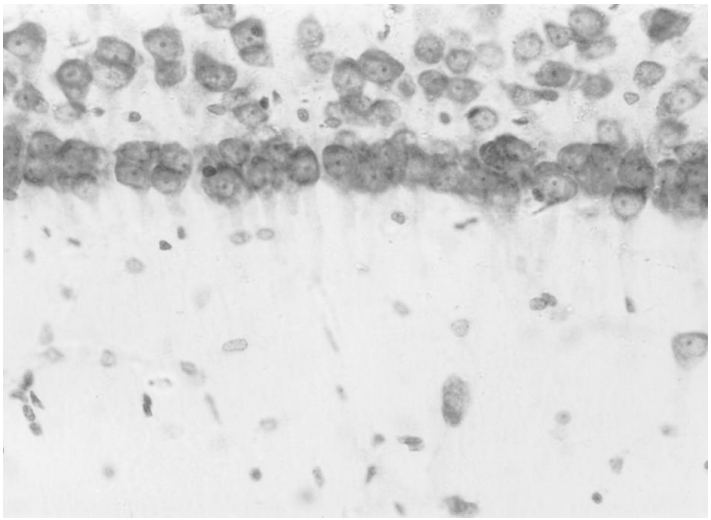

Figure 5