NMDA receptors in frontal cortex and hippocampus of alcohol consumers

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ABSTRACT

Specific binding of [³H]MK801 to N-methyl-D-aspartate (NMDA) receptors in the frontal cortex and hippocampus (CA1 and gyrus dentatus) was measured by receptor autoradiography in 16 Caucasian chronic alcohol consumers free of clinical manifestations of alcoholism, and compared with 16 Caucasian control subjects. Binding densities were not significantly different between heavy and moderate drinkers, neither between alcohol consumers that were abstinent or non-abstinent before death, nor between ethanol drinkers and controls. Continued alcohol consumption, in the absence of hepatic, neurologic or psychiatric disorders related to alcoholism, does not alter the binding properties of NMDA receptors in the brain areas studied.

Keywords Alcoholism, glutamate receptors, human brain, MK801, radioligand binding, receptor autoradiography.

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A number of studies have suggested that, at least partially, alcohol-induced brain damage associated to chronic alcohol consumption is mediated through changes in glutamatergic N-methyl-D-aspartate (NMDA) receptors. This is supported by the effects of drugs such as acamprosate, a partial NMDA antagonist used in relapse prevention in alcohol-dependent patients (Hammarberg et al. 2009). Only few studies have addressed the analysis of glutamate receptors in relation to alcohol abuse in humans and have provided controversial results (Cummins, Sack & von Hungen 1990; Dodd et al. 1992; Freund & Anderson 1996, 1999; Ridge et al. 2008). Our hypothesis is that permanent neuronal damage produced by chronic alcohol consumption is in part due to progressive changes in NMDA receptors, and that these changes might develop before the beginning of clinical manifestations of chronic alcoholism, including dependence, craving and abstinence. Thus, we assessed here the density of NMDA receptors in frontal cortex and hippocampus—brain areas known to be notably affected by chronic alcohol consumption—in a group of 16 alcohol consumers (AC) (16 male, mean age 58 ± 12 years, post-mortem delay 19 ± 8 hours) in comparison to a control group of 16 non-drinkers (14 male and 2 female, mean age 60 ± 12 years, post-mortem delay 17 ± 5 hours) using autoradiographic techniques with the specific NMDA-antagonist [³H]MK801. AC subjects included had not suffered any hepatic, neurologic or psychiatric disorders related to alcoholism. They were classified as moderate consumers (40–80 g of absolute alcohol daily for at least 10 years) or heavy consumers (more than 80 g of absolute alcohol daily for at least 10 years, enough to develop chronic alcoholic pathology). We also discriminated between AC cases that were abstinent (alcohol withdrawal minimum 1 week before death) or non-abstinent (last ethanol ingestion less than 12 hours before death). In consequence, it is unlikely that these subjects would undergo a withdrawal situation that could eventually involve acute changes in NMDA receptors (see Supporting Information and Table S1 for additional data concerning the cases studied).

Densities of [³H]MK-801 binding were measured by microdensitometry on film autoradiograms in frontal cortex (layers II-VI), and CA1 (orients and pyramidal layers) and gyrus dentatus (molecular layer) of the hippocampus (Fig. 1), and are summarized in Table 1. Statistical analysis (unpaired t-test, GraphPad Prism) revealed the absence of significant differences between the different groups analyzed. Covariance analysis (SPSS
for Windows) indicated no influence of liver fibrosis in our sample (see Supporting Information for detailed data).

Our results suggest that binding to frontal cortex and hippocampal NMDA receptors remains unaltered after chronic consumption of high doses of alcohol in the absence of clinical manifestations of alcoholism. Previous studies on hippocampal homogenates reported higher densities of $[^3]$Hglutamate binding in five alcoholics compared with three controls, with concomitant decreases in specific NMDA binding (Michaelis et al. 1990, 1993). Other authors (Dodd et al. 1992) found no differences in $[^3]$HMK801 binding in cortical membrane preparations between 10 alcoholics and 6 controls. Finally, Ridge et al. (2008) detected decreases in the expression of NR1, NR2A and NR2B NMDA receptor subunits in cerebral cortex only in cirrhotic alcoholics, but not in alcoholics without liver disease. Using receptor autoradiography, Freund & Anderson (1996, 1999) found significant increments in binding in frontal cortex (13 abstinent alcoholics and 13 controls) when using $[^3]$Hglutamate and the selective NMDA antagonist $[^3]$HCGP39653. On the contrary, Cummins et al. (1990) showed reduced $[^3]$Hglutamate binding in hippocampus from 10 abstinent alcoholics.

Different reasons could explain the discrepancies between those data and our study. Clinical variations related to co-morbidity such sudden death, cirrhosis, acute hypoxia or pneumonia could eventually alter glutamate binding (Piggott et al. 1992; Freund & Anderson 1996; Ridge et al. 2008). In our sample, we did not find an influence of liver fibrosis (a necropsy finding without previous clinical manifestations), supporting the study of Ridge et al. (2008) that suggests that hepatopathy, but not alcohol per se, could be the cause of changes in NMDA receptors.

Another possible explanation is related to the ligand used. MK-801 binds inside the previously opened ion channel of the NMDA receptor and it has been demonstrated that ethanol diminishes $[^3]$HMK801 binding only in the absence of glycine, a modulator of channel aperture (Freund & Anderson 1996). Since we used intact, fresh-frozen brain tissue, it is possible that glycine present in our sections might interfere with the binding of $[^3]$HMK801 and therefore mask putative alterations present in samples from chronic alcohol consumers.

It might be considered that ethanol could interact with NMDA receptors in a manner not measurable by auto-radiographic techniques. For instance, a decrease in specific NMDA receptor subunits could eventually be compensated by an accelerated synthesis of mRNA encoding the proteins involved. Finally, it could be speculated that ethanol modifies other cellular events, such as the migration of receptor subunits from extrasynaptic sites in the plasmatic membrane to synaptic functional loci, or receptor assembly, among others.

![Figure 1](image.png)

**Figure 1** Autoradiographic images showing the total (a) and non-specific (b) binding of $[^3]$HMK801 in the hippocampus of a control subject. CA1 = CA1 field, DG = dentate gyrus, PH = parahippocampal gyrus, wm = white matter. Bar = 4 mm.

**Table 1** Densities of $[^3]$HMK 801 binding in controls and alcohol consumers.

<table>
<thead>
<tr>
<th>Group</th>
<th>Frontal cortex</th>
<th>Dentate gyrus</th>
<th>CA1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>71.62 ± 17.89 (n = 16)</td>
<td>147.83 ± 14.94 (n = 15)</td>
<td>173.18 ± 49.65 (n = 15)</td>
</tr>
<tr>
<td>Alcohol consumers (AC) all</td>
<td>82.94 ± 20.57 (n = 15)</td>
<td>139.26 ± 38.97 (n = 13)</td>
<td>191.66 ± 70.19 (n = 13)</td>
</tr>
<tr>
<td>&gt;80 g/day consumers</td>
<td>81.16 ± 16.25 (n = 11)</td>
<td>131.28 ± 36.91 (n = 11)</td>
<td>187.18 ± 75.94 (n = 11)</td>
</tr>
<tr>
<td>40–80 g/day consumers</td>
<td>87.86 ± 32.39 (n = 4)</td>
<td>183.14 ± 6.92 (n = 2)</td>
<td>216.29 ± 4.61 (n = 2)</td>
</tr>
<tr>
<td>Abstainers</td>
<td>79.71 ± 4.35 (n = 3)</td>
<td>124.36 ± 18.93 (n = 4)</td>
<td>165.75 ± 42.56 (n = 4)</td>
</tr>
<tr>
<td>Non-abstainers</td>
<td>83.75 ± 23.05 (n = 12)</td>
<td>145.88 ± 44.53 (n = 9)</td>
<td>203.17 ± 78.90 (n = 9)</td>
</tr>
</tbody>
</table>
Thus, a number of fundamental questions remain open, and further studies on a greater number of human brain samples are necessary. Combined studies of histological assessment with autoradiography with different ligands and in situ hybridization can yield information on the effects of chronic ethanol consumption on functional mature NMDA receptors and some of their subunits.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 Summary of cases.

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References


