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Research Report

# Changes in neocortical and hippocampal $GABA_A$ receptor subunit distribution during brain maturation and aging

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ABSTRACT

γ-Aminobutyric acid type A ($GABA_A$) receptors are the most important inhibitory receptors in the central nervous system, playing a pivotal role in the regulation of brain excitability. The pentameric receptor is commonly composed of different α, β, and γ subunits which mediate the function and pharmacology of the receptor and show regional- and temporal-specific expression patterns. Under varying physiological and pathophysiological conditions, this diversity allows a multitude of adaptive changes in subunit composition leading to distinct biological and pharmacological properties of the receptor. Here, we investigated the expression of five major $GABA_A$ receptors subunits (α1, α2, α3, α5, γ2) in early postnatal, adult, and aged rat brains. Immunohistochemistry was performed at postnatal day 10, 30, 60, 90, 180, 360, and 540. Morphological and semi-quantitative evaluations of regional optical densities revealed specific regional and temporal expression patterns for all subunits. The study clearly demonstrated that changes in $GABA_A$ receptor distribution not only occur in the early postnatal cortex and hippocampal formation but also during later periods in the adolescent and aging brain. These findings contribute to a better understanding of age-related changes in brain excitability and further elucidate the distinct pharmacological effects of different GABAergic drugs in young and elderly patients.



## 1. Introduction

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian brain. In the central nervous system, fast inhibitory synaptic interactions are predominantly mediated by $GABA_A$ receptors. These receptors have a pentameric structure composed of different receptor subunits which together build a chloride-selective ion channel. Up to now, at least 19 different subunits of $GABA_A$ receptors (α1–6, β1–3, γ1–3, ρ1–3, π, θ, δ ε) have been characterized (Barnard et al., 1998; Mehta and Ticku, 1999). Most functional $GABA_A$ receptors in the mammalian brain contain two α, one β, and two γ subunits (Baumann et al., 2001; Klausberger et al., 2001).

$GABA_A$ receptors are targets of a variety of pharmacologically and clinically important drugs, such as benzodiazepines, barbiturates, ethanol, neurosteroids, and some general anesthetics (Fritschy and Brunig, 2003; Sieghart, 2000). The

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**Table 1 – Regional optical densities of the immunoreactivity of different $GABA_A$ receptor subunits during brain maturation and aging**

| Subunit | Brain region | Age | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 10 days ($n$ = 6) | 30 days ($n$ = 3) | 60 days ($n$ = 3) | 90 days ($n$ = 3) | 180 days ($n$ = 3) | 270 days ($n$ = 3) | 360 days ($n$ = 3) | 540 days ($n$ = 3) |
| | | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM | Mean ± SEM |
| α1 | Fr | 113 ± 7 | 172 ± 7 | 179 ± 4 | 156 ± 6 | 161 ± 4 | 167 ± 3 | 172 ± 5 | 169 ± 2 |
| | FL | 141 ± 8 | 177 ± 9 | 166 ± 19 | 137 ± 14 | 163 ± 8 | 152 ± 10 | 174 ± 13 | 160 ± 7 |
| | HL | 142 ± 7 | 184 ± 6 | 190 ± 5 | 154 ± 8 | 179 ± 4 | 168 ± 6 | 184 ± 6 | 177 ± 4 |
| | Par1 | 161 ± 4 | 189 ± 6 | 182 ± 6 | 153 ± 8 | 168 ± 4 | 158 ± 6 | 166 ± 4 | 168 ± 3 |
| | Par2 | 129 ± 5 | 178 ± 5 | 167 ± 6 | 149 ± 6 | 161 ± 5 | 157 ± 5 | 165 ± 5 | 169 ± 5 |
| | Hippocampus | 65 ± 6 | 49 ± 6 | 50 ± 5 | 37 ± 7 | 50 ± 5 | 43 ± 5 | 49 ± 5 | 54 ± 6 |
| α2 | Fr | 128 ± 5 | 155 ± 5 | 170 ± 2 | 163 ± 6 | 158 ± 3 | 161 ± 4 | 167 ± 4 | 149 ± 3 |
| | FL | 95 ± 14 | 149 ± 9 | 167 ± 4 | 156 ± 9 | 147 ± 15 | 146 ± 11 | 168 ± 9 | 142 ± 15 |
| | HL | 125 ± 8 | 159 ± 5 | 177 ± 3 | 169 ± 6 | 173 ± 6 | 164 ± 6 | 181 ± 4 | 158 ± 6 |
| | Par1 | 121 ± 6 | 159 ± 4 | 158 ± 6 | 148 ± 6 | 145 ± 4 | 139 ± 9 | 157 ± 5 | 149 ± 6 |
| | Par2 | 118 ± 6 | 169 ± 4 | 157 ± 7 | 158 ± 7 | 155 ± 5 | 152 ± 8 | 172 ± 3 | 166 ± 7 |
| | Hippocampus | 143 ± 14 | 180 ± 7 | 202 ± 2 | 196 ± 7 | 200 ± 6 | 199 ± 4 | 194 ± 6 | 194 ± 6 |
| α3 | Fr | 94 ± 4 | 121 ± 4 | 113 ± 4 | 117 ± 3 | 104 ± 5 | 96 ± 4 | 84 ± 5 | 78 ± 8 |
| | FL | 71 ± 8 | 91 ± 6 | 71 ± 6 | 69 ± 18 | 73 ± 11 | 60 ± 7 | 55 ± 7 | 52 ± 13 |
| | HL | 91 ± 5 | 105 ± 5 | 100 ± 4 | 105 ± 4 | 93 ± 5 | 82 ± 4 | 70 ± 5 | 68 ± 8 |
| | Par1 | 67 ± 5 | 82 ± 3 | 64 ± 5 | 71 ± 2 | 62 ± 3 | 53 ± 4 | 44 ± 4 | 42 ± 6 |
| | Par2 | 77 ± 6 | 123 ± 4 | 94 ± 8 | 95 ± 3 | 95 ± 5 | 78 ± 6 | 75 ± 6 | 66 ± 8 |
| | Hippocampus | 11 ± 3 | 16 ± 3 | 20 ± 1 | 25 ± 4 | 17 ± 3 | 23 ± 5 | 13 ± 4 | 9 ± 2 |
| α5 | Fr | 150 ± 6 | 151 ± 3 | 122 ± 4 | 112 ± 7 | 100 ± 6 | 92 ± 4 | 99 ± 6 | 95 ± 4 |
| | FL | 136 ± 5 | 97 ± 5 | 64 ± 7 | 69 ± 14 | 46 ± 6 | 41 ± 3 | 44 ± 8 | 45 ± 11 |
| | HL | 150 ± 6 | 116 ± 4 | 87 ± 5 | 73 ± 6 | 74 ± 5 | 60 ± 3 | 71 ± 6 | 68 ± 5 |
| | Par1 | 125 ± 5 | 79 ± 3 | 47 ± 2 | 42 ± 5 | 39 ± 4 | 28 ± 2 | 34 ± 4 | 35 ± 3 |
| | Par2 | 126 ± 5 | 104 ± 4 | 68 ± 3 | 68 ± 5 | 60 ± 5 | 51 ± 3 | 62 ± 5 | 60 ± 5 |
| | Hippocampus | 158 ± 8 | 195 ± 2 | 192 ± 2 | 180 ± 5 | 183 ± 3 | 167 ± 4 | 173 ± 5 | 176 ± 6 |
| γ2 | Fr | – | 130 ± 10 | 140 ± 5 | 128 ± 3 | 94 ± 2 | 89 ± 5 | 106 ± 4 | 132 ± 4 |
| | FL | – | 130 ± 13 | 142 ± 9 | 108 ± 9 | 110 ± 8 | 88 ± 9 | 99 ± 11 | 124 ± 20 |
| | HL | – | 146 ± 9 | 148 ± 6 | 132 ± 5 | 115 ± 5 | 95 ± 4 | 112 ± 6 | 133 ± 6 |
| | Par1 | – | 148 ± 9 | 141 ± 3 | 123 ± 4 | 98 ± 2 | 93 ± 4 | 96 ± 5 | 123 ± 4 |
| | Par2 | – | 145 ± 6 | 133 ± 6 | 126 ± 3 | 106 ± 3 | 106 ± 5 | 112 ± 4 | 119 ± 5 |
| | Hippocampus | – | 131 ± 16 | 118 ± 2 | 126 ± 5 | 95 ± 3 | 88 ± 3 | 85 ± 4 | 87 ± 5 |

Data are given as mean ± SEM. Abbreviations: frontal cortex (Fr), forelimb representation cortex (FL), hindlimb representation cortex (HL), primary and secondary somatosensory cortex (Par1, Par2) (Zilles, 1992).

pharmacological and biophysical properties of the receptors are primarily determined by their subunit composition (Hevers and Luddens, 1998; Luscher and Keller, 2004; Mohler et al., 2001). The benzodiazepine (BZ) binding site is localized at the interface of α and γ subunits (Rudolph et al., 2001; Sieghart, 1995; Zezula et al., 1996), whereas the specificity of the BZ is determined primarily by α subunits (Korpi et al., 2002; Kralic et al., 2002). Previous studies have demonstrated that distinct $GABA_A$ receptor subunits mediate specific pharmacological BZ effects. Whereas the α1 subunit is responsible for sedative, amnestic, and anticonvulsant actions, α2, α3, and α5 subunits mediate anxiolytic and other properties (Fritschy and Brunig, 2003; Mohler et al., 2001; Rudolph et al., 1999).

This heterogeneity of $GABA_A$ receptors allows a multitude of adaptive changes during physiological and pathophysiological conditions (Brussaard et al., 1997; Gao et al., 1999) that have to

**Fig. 1 – Age-related changes in hippocampal $GABA_A$ receptor subunit expression in the rat brain. (A) Color-coded images from immunohistochemically processed coronal sections stained with antibodies against the α1,α2,α3,α5, and γ2 subunits. For each subunit, the optical density of the immunoreactivity product was color-coded using a standard 256-level scale, ranging from black for background to violet, blue, green, yellow, and red for the most intense signals. (B) Diagram of the mean optical densities of the immunoreactivity product of each subunit in the frontal cortex (Fr) and the primary somatosensory cortex (Par1). During the first postnatal days, α2 and α5 subunits were abundantly expressed in the cortex whereas only a marginal amount of α1 and α2 subunits was found in this brain region. In the following weeks the expression of α1, α2, α3, and γ2 subunits increased in the cortex and subunit α5 in contrast showed a decrease in optical density. From the age of 3 months a continuous down-regulation of subunits α3, α5, γ2 was observed in the cortex whereas subunits α1 and α2 remained stable at a high expression level. An up-regulation of subunit γ2 was found in the neocortex after 9 months. Data are presented as mean ± S.E.M (bars). Statistically significant differences to 30 day old animals (marked with a black arrow) are indicated with asterisks ($P \leq 0.05$; one-way ANOVA, Bonferroni post-hoc).**