GABA<sub>a</sub> RECEPTORS IN RAT WHISKER BARREL CORTEX:
EFFECTS OF SENSORY DEPRIVATION

THESIS

Presented to the Graduate Council of the
University of North Texas in Partial
Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

by

Eduardo Salazar, B.S.
Denton, Texas
August, 1995
GABA$_A$ RECEPTORS IN RAT WHISKER BARREL CORTEX: EFFECTS OF SENSORY DEPRIVATION

THESIS

Presented to the Graduate Council of the University of North Texas in Partial Fulfillment of the Requirements For the Degree of

MASTER OF SCIENCE

by

Eduardo Salazar, B.S.

Denton, Texas

August, 1995
Salazar, Eduardo, GABA$_A$ receptors in rat whisker barrel cortex: effects of sensory deprivation. Master of Science (Biology), August, 1995, 23 pp., 1 Table, 4 illustrations, references, 49 titles.

The GABAergic system in adult sensory cortex is affected by sensory deprivation, but little is known about how this predominant inhibitory system is affected during ontogeny. The present study investigates developmental effects of whisker trimming on GABA$_A$ receptors in rat barrel cortex. Rats trimmed for 6 wk beginning at birth and adulthood showed similar decreases in [3H]muscimol binding in deprived relative to non-deprived barrels, suggesting absence of a critical period. Effects persisted in rats deprived from birth to 6 wk and analyzed after 10 wk without deprivation, compatible with enduring physiological signs of disinhibition (Simons and Land, '87). Whisker trimming from birth to day 10 resulted in only a small decrease, suggesting that whisker stimulation is not necessary for the developmental peak in GABA$_A$ receptors. Cytochrome oxidase staining showed deprivation effects in all of the groups trimmed for 6 wk.
ACKNOWLEDGMENTS

I am especially thankful to everyone who participated in this study: to Dr. Fuchs, whose continuous creative suggestions during the experimental and manuscript stages, as well as her patience, positive spirit and eagerness to obtain financial support to the Neurochemistry lab through her grant proposals, brought solid foundation to this research; to Dr. Schwark, who readily shared his scientific expertise in order to solve technical, statistical, computational and any other problems found in this process; to Terry Austin, who assisted the TAMS students in the preliminary parts of this study, and whose time and experience were always available; to the pioneers of the techniques of 'cortex flattening' and 'whisker trimming': Steve Grady and Adrianna Garcia; to the "TAMS-students crew": Joseph Ayoub, Clay Carpenter, Seema Mody and Rachel Miller, who creatively, meticulously, and patiently, worked on several stages of the project. Finally, I want to thank the indirect, but unconditional support from my family and the Dominican friars, who gave continuous insight to this work.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>iii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>5</td>
</tr>
<tr>
<td>Subjects</td>
<td>5</td>
</tr>
<tr>
<td>Whisker trimming</td>
<td>5</td>
</tr>
<tr>
<td>Histology</td>
<td>6</td>
</tr>
<tr>
<td>[³H]Muscimol binding</td>
<td>6</td>
</tr>
<tr>
<td>Autoradiography</td>
<td>6</td>
</tr>
<tr>
<td>Data analysis</td>
<td>7</td>
</tr>
<tr>
<td>RESULTS</td>
<td>8</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>14</td>
</tr>
<tr>
<td>Effects of whisker trimming on GABA&lt;sub&gt;A&lt;/sub&gt; binding: Comparison among groups</td>
<td>14</td>
</tr>
<tr>
<td>Effects of sensory deprivation on GABAergic systems</td>
<td>15</td>
</tr>
<tr>
<td>Cytochrome oxidase activity</td>
<td>15</td>
</tr>
<tr>
<td>Possible functional implications</td>
<td>16</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>18</td>
</tr>
</tbody>
</table>
INTRODUCTION

Sensory deprivation has been a productive approach to studying the effects of environmental stimuli on the developing brain. The ultimate goal of this genre of studies is to understand the factors that influence the development of sensory pathways. One of the first studies clearly demonstrating effects of sensory deprivation on a specific cortical neurotransmitter system was done by Hendry and Jones ('86) in the visual cortex of adult monkeys. They found that monocular deprivation decreases levels of the predominant CNS inhibitory neurotransmitter, GABA (γ-aminobutyric acid), in the deprived ocular dominance columns. These areas also showed a reduction in GABA\textsubscript{A} receptors after visual deprivation (Hendry et al., '90) and in glutamic acid decarboxylase (GAD), the enzyme that synthesizes GABA (Hendry and Jones, '86). It is not known, however, whether the GABAergic system responds differently to deprivation during development.

Developmental effects of sensory deprivation have been widely studied in the rodent primary somatosensory cortex (SI). Due to the precise one-to-one correspondence between the five rows of major vibrissae and the five rows of cortical 'barrels' in layer IV of SI (Woolsey and Van der Loos, '70), it has been possible to study the effects of whisker stimulation and deprivation on physiology (Welker, '71, '76; Simons, '78; Simons and Woolsey, '79) and cytoarchitecture (Woolsey and Van der Loos, '70; Welker and Woolsey, '74; Van der Loss and Woolsey, '73) of barrel cortex.

Marked reductions in barrel size occur after neonatal deprivation that involve
damage of peripheral nerves (Van der Loos and Woolsey, '73; Weller and Johnson, '75; Killackey et al., '78; Belford and Killackey, '80; Pidoux et al., '80; Jeanmonod et al., '81; Durham and Woolsey, '84). Reducing sensory stimulation without damaging nerves has only been recently studied by trimming the whiskers (Simons and Land, '87, '94; Akhtar and Land, '91). Without affecting cytoarchitecture, this procedure leads to changes in $[^{14}\text{C}]2$-deoxyglucose uptake (Hand, '82), cytochrome oxidase activity (Akhtar and Land, '91), and GAD immunoreactivity (Akhtar and Land, '91). Moreover, trimming whiskers from birth to adulthood results in reduced specific signs of inhibition in deprived barrel neurons, such as increased spontaneous activity and reduced ability to discriminate among different angles of whisker deflection (Simons and Land, '87). A diminished effectiveness of the intracortical inhibitory mechanisms that normally depress spontaneous activity was suggested to underlie those changes (Simons and Land, '87). Therefore, in order to test this hypothesis it is important to find the source of those inhibitory changes.

GABA is the main inhibitory neurotransmitter of the central nervous system (Curtis and Johnson, '74; Krmjevic and Schwartz, '67; Roberts et al., '76). Physiological effects of applying GABA agonists and antagonists include changes in receptive field size (Dykes et al., '84; Kaneko and Hicks, '88; Alloway, '89). Early participation of GABAergic systems in brain development is also suggested by its appearance in fetal life before synaptogenesis and in early postnatal life (Chronwall and Wolff, '80; Lauder et al., '86, Van Eden et al., '89; Cobas et al., '91, Fiszman et al., '93).

GABA neurons have been described in SI of various mammals, including the monkey, rat and mouse (Hendry and Jones, '81; Lin et al., '85; Keller and White, '86;
Warren et al., '89). Immunocytochemistry was used to localized GABA for the first time in 1984 (Ottersen and Storm, '84). In normal adult rat SI cortex, GABA-immunoreactive neurons appear in all layers (I-VI), but the numerical density of somata and synapses (puncta) is greatest in layer IV barrel cortex (Chmielowska et al., '88; Beaulieu '93). GAD-positive neurons are also densest in layer IV (Hendrickson et al., '81; O'Hara et al., '83; Ottersen and Storm, '84; Lin et al., '85, Akhtar and Land, '91). Autoradiography of \[^3H\]muscimol binding has been used to localize high affinity GABA\_A receptors in neocortex. Again, lamina IV is enriched in GABA\_A receptor binding (Palacios et al., '81; Penny et al., '81).

GAD-immunocytochemistry was used to search for the chemical correlates of the neurophysiological changes found by Simons and Land ('87) after neonatal deprivation (Akhtar and Land, '91). GAD-immunoreactivity decreased only in adult deprived barrels, while neonatally trimmed rats showed normal levels of GAD in barrel neurons. This finding did not explain the decrease in inhibition observed in rats deprived from birth. However, autoradiographic studies from this lab show a decline in GABA\_A receptor binding after whisker trimming from birth on (Fuchs, '93, '95; Salazar et al., '94).

In the present study, effects of whisker trimming on GABA\_A receptors were studied, with the following aims: (1) to determine if whisker stimulation contributes to the transient peak in GABA\_A receptor binding (Fuchs, '93, '95) that occurs around postnatal day 10 (P10), by assessing effects of whisker trimming from birth (P0) to P10; (2) to examine whether there is a developmental critical period for the effects of whisker trimming, by comparing rats trimmed for 6 wk, beginning on P0 versus beginning on week...
6; (3) to see if the decline in GABA$_A$ receptor binding is reversible if the whiskers are clipped from birth for 6 wk and then allowed to grow out (4 wk for full regrowth, and additional 6-wk period with whiskers intact). In addition, this study evaluates the decrease in cytochrome oxidase activity in the same sections.
MATERIALS AND METHODS

Subjects

The subjects were 73 Long-Evans hooded rats (Simonsen, Gilroy, CA), distributed in four deprivation groups. In the first group, whiskers were trimmed from birth to postnatal day 10 (P0-P10; \( N = 15 \)), to test whether the developmental peak in \( \text{GABA}_A \) receptors requires input from intact whiskers. In the second (\( N = 17 \)), whiskers were kept trimmed for six weeks, from P0 - 6 wk (P0 - P41). In the third (\( N = 19 \)), whiskers were also clipped for 6 wk, but from wk 6 - 12 (P42 - P83); this and the second group were compared to test for a critical period. In the fourth group (\( N = 22 \)), whiskers were kept trimmed for 6 wk after birth, followed by 10 wk without clipping, to test whether the effect is reversible.

Whisker trimming

Whiskers were trimmed daily with iridectomy scissors under a dissecting microscope from birth until 6 wk of age. After this age, clipping took place every other day. No anesthetic was used. All experimental rats had the mystacial vibrissae in either row C or rows ABDE clipped on one side of the face.

Histology

Unperfused rats were sacrificed by decapitation. The deprived barrel region was
dissected out from the brain, and was flattened and frozen at -44°C with the heat
dissipator of a cryostat (2800 Frigocut N, Reichert-Jung). Sections 16μm thick were cut
at -20°C tangentially to the pial surface, and were thaw-mounted onto gelatin subbed
slides. They were air dried for 0.5 to 3 h and then stored desiccated at -80°C. Following
the ligand binding and autoradiography, the sections were stained for cytochrome oxidase
activity (Wong-Riley, '79).

[^3H]Muscimol binding

GABA<sub>A</sub> receptors were assessed with [³H]muscimol (15 Ci/mmol, DuPont NEN)
as the ligand. Methods were based on those described previously (Mower et al., '86).
Sections stored at -80°C were thawed and dried. In order to remove endogenous GABA,
sections were preincubated 20 min at 4°C in 0.31 M Tris-citrate buffer (pH 7.1). Then
they were incubated 40 min at 4°C in the ligand solution, consisting of 10 nM
[^3H]muscimol in 0.31 M Tris-citrate buffer (pH 7.1). Sections were then sequentially
washed in ice cold rinses: two 30-sec Tris-citrate buffer rinses and one 1-sec rinse in
distilled water. Brain sections were immediately dried in a stream of air.

Autoradiography

The brain sections and tritium standards (Microscale, Amersham) were exposed
simultaneously in the same cassette to tritium-sensitive Hyperfilm (Amersham). Following
a 3-6 month exposure period, the film was developed with Kodak D19 and processed
according to the manufacturer's instructions.
Data analysis

[^3]H]Muscimol binding was quantitatively analyzed using a video-based computerized image analysis system (MCID, Imaging Research, St. Catherines, Ont., Canada). Tritium standards were used to calibrate autoradiographic densities. Samples were taken within a computer-generated circle centered over each barrel. For each section, the size of the circle was large enough to cover most of each barrel without extending outside the barrel boundary. In tangential sections,[^3]H]muscimol binding levels were measured for individual barrels in C versus B and D. The usefulness of comparing non-deprived barrelfields as controls is limited by section-to-section variability and marked change in[^3]H]muscimol binding with cortical depth. However, to test the possibility that an apparent decrease in receptor binding in a deprived row is actually an increase in the adjacent row, additional comparison were made between rows A versus B, or D versus E, in 11 subjects C-deprived from P0 - 6 wk. Within each section, a ratio was calculated as the mean of the non-deprived barrels divided by the mean of the deprived barrels. A ratio for each subject was then calculated by averaging the ratios for each section. The group average was converted to percent decrease in the deprived rows. For each group, t-tests were used to test the null hypothesis that the ratio was not different from 1.0. Ratios were then compared across groups by analyses of variance and Bonferroni post hoc tests, using 0.05 as the significance level. In the cytochrome oxidase stained sections, density readings were calibrated to optical density standards, and the same procedures for data collection and analysis were used.
RESULTS

[$^3$H]Muscimol autoradiographs showed a clear decrease in binding density in the deprived barrel rows both in the group with whiskers trimmed from 0-6 wk and in the group trimmed from 6-12 wk (Figs. 1 and 3). The mean decreases for these groups were similar (7.46%, $p<0.001$, and 7.65%, $p<0.001$, respectively, Table 1). In addition, small decrease was observable in most of the reversibility animals, which had whiskers trimmed from 0-6 wk, followed by a 10-wk recovery period (3.29%, $p<0.001$; Figs. 2 and 3). The ratios for the reversibility group were significantly smaller than those for the rats trimmed from P0-6 wk ($p<0.001$), indicating a partial reversal of the deprivation effect. Although autoradiographs from rats trimmed from P0-P10 showed was no obvious deprivation effect (Fig. 2), densitometry revealed a statistically significant decrease (2.26%; $p<0.05$). The percent decrease between adjacent and non-adjacent non-deprived rows was negligible and not statistically significant (0.18%; $p<0.5$).

In sections stained for cytochrome oxidase (CO) activity (Fig. 2), the deprivation effects were not obvious upon visual inspection. In many cases, the staining appeared somewhat uneven, perhaps due to changes in the tissue during the months of film exposure. However, the decrease in staining was significant in three deprivation groups (2.33% in P0-6wk, $p<0.001$; 3.15% in 6-12 wk, $p<0.001$; and 1.82% in the reversibility group, $p<0.001$; Table 1). In the rats deprived from P0-10 there was a small decrease (1.00%, $p<0.1$). There were no significant differences among the groups' CO ratios.
Table 1. Percent decrease in the deprived barrel rows relative to intact rows (mean ± S.E.M.) for each group.

<table>
<thead>
<tr>
<th>Deprivation period</th>
<th>[3H]Muscimol binding (% decrease)</th>
<th>p-value</th>
<th>Cytochrome oxidase density (% decrease)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-6 wk</td>
<td>7.47 ± 0.79</td>
<td>&lt;0.001</td>
<td>2.23 ± 0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6-12 wk</td>
<td>7.65 ± 0.71</td>
<td>&lt;0.001</td>
<td>3.15 ± 0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0-6 wk + 10 wk recovery</td>
<td>3.29 ± 0.44</td>
<td>&lt;0.001</td>
<td>1.83 ± 0.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>P0-P10</td>
<td>2.26 ± 0.93</td>
<td>&lt;0.05</td>
<td>1.00 ± 0.45</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
Fig. 1. [3H]Muscimol binding decreased in deprived whisker barrel cortex. When rows of whiskers were chronically trimmed from P0 - 6 wk, [3H]muscimol binding decreased in the deprived barrel rows. Compare autoradiographs from a C-trimmed (upper left) and an ABDE-trimmed rat (lower left). In adult deprived rats, the effects were similar, as is shown when row C whiskers were trimmed (upper right) and when rows ABDE were trimmed (lower right).
Fig 2. Deprived whisker barrel cortex: $[^3H]$muscimol binding and cytochrome oxidase staining. Trimming row C from P0 to P10 did not result in obvious decreases (upper left), though the group average (2.26%) was statistically significant. Small decreases remained in rats trimmed from P0 - 6 wk, after additional 10 wk without trimming for recovery (lower left). After preparation of autoradiographs (upper right) the same sections were stained for cytochrome oxidase activity (lower right).
Fig 3. Effects of whisker trimming on [$^3$H]muscimol binding. Ligand binding in deprived relative to intact rows declined in rats with whiskers trimmed for 6 wk, beginning at birth (***p<0.001) or in adulthood (***p<0.001). The deprivation effect persisted after the whiskers had regrown (**p<0.001), though it was reduced relative to the P0 - 6wk group (**p<0.001). The group trimmed from P0 and examined on P10 also showed a small decrease (*p<0.05). Each value represents the mean ± S.E.M.
Fig. 4. Effects of whisker trimming on cytochrome oxidase (CO) activity. After 6 wk of whisker trimming, deprived barrels showed a decrease in the density in CO staining, either when deprivation began at birth (*p<0.001) or at 6 wk (*p<0.001). Ten wk of recovery did not completely reverse the deprivation effects (*p<0.001). Deprivation from birth to P10 produced no significant change (p<0.1). Each value represents the mean ± S.E.M.
DISCUSSION

Effects of whisker trimming on GABA_A binding: Comparison among groups

The similarity in magnitude of the effect of whisker trimming on [3H]muscimol binding from P0 - 6 wk and from 6 -12 wk suggests that there is no obvious critical period for the effect of trimming on GABA_A receptors. The observation that effects of deprivation from P0 - 6 wk persist for additional months after the whiskers have regrown, is compatible with the report of Simons and Land ('87) that three additional months without clipping were not enough to reverse neurophysiological signs of increased disinhibition. Whisker stimulation does not appear to be necessary for the transient developmental peak in GABA_A receptor binding observed around P10 (Fuchs, '93), based on the observation that P0 - P10 deprived barrels showed only small effects of trimming relative to the magnitude of the developmental peak. The developmental onset of whisking behavior only after P10-P12 (Fox, '92), and the short deprivation period, could contribute to the observation that P10 rats showed the smallest deprivation effect.

Effects of sensory deprivation on GABAergic systems.

This study suggests that decreases in GABA_A receptors may contribute to the enduring disinhibition in deprived barrel neurons, such as increased spontaneous activity and less preference for responding to specific angles of whisker deflection (Simons and Land, '87). This increased disinhibition appears to arise in cortex (Simons and Land, '94).
The present results show that GABA\(_A\) receptor binding decreases not only in the adult-deprived rows, but also in the ones deprived from birth to 6 wk. Moreover, the deprivation effects in both GABA\(_A\) receptors and physiological characteristics were long-lasting. Thus, these observations corroborate and extend earlier reports from this lab (Fuchs, '93; Salazar et al., '94) suggesting that a decrease in GABA\(_A\) receptors may underlie the physiological changes observed by Simons and Land ('87).

A decline in inhibitory neurotransmission mediated by GABA\(_A\) receptors could contribute to the increased disinhibition in deprived barrel neurons. However, because the present study is based on ratios between deprived and adjacent rows, the alternative possibility remains that there is instead an increase in GABA\(_A\) receptors in adjacent non-deprived rows. Arguing against this alternative is the finding that there was no difference between \(^3\text{H}\)muscimol levels in rows adjacent versus non-adjacent to a deprived row. The observations that whisker trimming for the first 6 wk does not affect GAD levels (Akhtar and Land, '87) but does affect GABA\(_A\) receptor binding are not contradictory. GAD immunoreactivity and GABA\(_A\) receptors are likely to be present in somewhat different populations of neurons. The distribution of GABA\(_A\) receptors, being largely post-synaptic, should not be expected to match the distribution of GABA or GAD (Fuchs, '95).

Cytochrome oxidase activity

This study shows that chronic whisker trimming of neonatal and adult rats results in decreased level of cytochrome oxidase (CO) staining in the corresponding barrels.
Similarly, a reduction in CO activity has been reported after follicle cauterization in adult deprived barrels, as well as in neonatally deprived mice, although in the latter group, the barrels are severely reduced in size (Wong-Riley and Welt, '80). Whisker trimming results in a distinct reduction in CO staining in both neonatally and adult trimmed rats (Land and Simons, '85; Akhtar and Land, '91), although these changes were not analyzed statistically.

In the present study, the 6 wk of whisker trimming resulted in a similar decrease in CO staining in neonatally and adult deprived barrels, suggesting the absence of a critical period. Second, the decrease in CO staining after 6 wk of deprivation from birth remained without significant change after 10 wk of recovery, suggesting a possible involvement of a long-lasting decrease in metabolic activity in the persistent changes in barrel neurons deprived during the same developmental period (Simons and Land, '87). However, the physiological changes reported by Simons and Land remained for up to 3 months, and it is not known whether the present changes should persist that long. Trimming from birth to P10 did not produce a significant change in CO staining, probably due to the short deprivation period and late onset of whisking behavior, as mentioned before.

Possible functional implications.

Is the reduction in GABA<sub>A</sub> receptor binding merely a consequence of reduced metabolic activity, or does it represent a more specific effect? Although the percent changes in CO density were smaller that those for [³H]muscimol binding, the relation between optical density and CO activity is not known, so the magnitudes of the changes in
[³H]muscimol and CO cannot be compared quantitatively. The observation that not all neurotransmitter systems seem to be affected by sensory deprivation suggests that the involvement of GABA systems may be fairly specific. For example, whisker trimming for the first 6 postnatal wk results in no apparent change in NMDA receptor binding (Fuchs, '93). Additional support for specific involvement of the GABA system comes from the observation that in deprived monkey visual cortex, GABA_A receptors decrease while several others do not (Fuchs and Hendry, unpublished). Perhaps the reduction in GABA_A receptor binding serves to compensate by disinhibiting the reduced excitatory sensory input. It may be that excitatory and inhibitory actions are maintained in balance and that changes in levels of sensory stimulation are compensated for changes in inhibitory neurotransmission.
REFERENCES


Hand, P.J., Plasticity of the rat cortical barrel system. In A.R. Morrison and P.L. Strick


Pidoux, B., Diebler, M.F., Savy, C.L., Farkas, E., and Verly, R., Cortical organizations of
the postero-medial barrel subfield in mice and its reorganization after destruction of

Roberts, E., Chase, T.N. and Tower, D.B., (Eds.), *GABA in Nervous System Function*, 

Salazar, E., Carpenter, R.L., Miller, R.L., Austin, T.A. and Fuchs, J.L., Effects of whisker
trimming on GABA_A receptor binding in developing rat whisker barrel cortex, *Soc.

Simons, D.J., Response properties of vibrissa units in the rat SI somatosensory neocortex,

Simons, D.J. and Land, P.W., Early experience of tactile stimulation influences organization

Simons, D.J. and Land, P.W., Neonatal whisker trimming produces greater effects in
nondeprived than deprived thalamic barreloids, *J. Neurophysiol.*, 72 (1994) 1434-
1437.

Simons, D.J. and Woolsey, T.A., Functional organization of mouse barrel cortex, *Brain

Van der Loos, H. and Woolsey, T.A., Somatosensory cortex: structural alterations

Van Eden, C.G., Mrzljak, L. Voorn, P. and Uylings, H.B.M., Prenatal development of
213-227.

Warren, R., Tremblay, N., and Dykes, R.W., Quantitative study of glutamic acid


Weller, W.I. and Johnson, J.I., Barrels in cerebral cortex altered by receptor disruption in new-born but not in five-day-old mice (Cricetidae and Muridae), *Brain Research*, 83 (1975) 504-508.

