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14. ABSTRACT Nerve agent-induced seizures cause neuronal damage in brain limbic and cortical circuits leading to persistent behavioral and cognitive deficits. Without aggressive anticholinergic and benzodiazepine therapy, seizures can be prolonged and neuronal damage progresses for extended periods of time. The objective of this study was to determine the effects of the nerve agent soman on expression of cyclooxygenase-2 (COX-2), the initial enzyme in the biosynthetic pathway of the proinflammatory prostaglandins and a factor that has been implicated in seizure					
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Report Title

Microglia as primary mediators of nerve agent neuropathy

ABSTRACT

Nerve agent-induced seizures cause neuronal damage in brain limbic and cortical circuits leading to persistent behavioral and cognitive deficits. Without aggressive anticholinergic and benzodiazepine therapy, seizures can be prolonged and neuronal damage progresses for extended periods of time. The objective of this study was to determine the effects of the nerve agent soman on expression of cyclooxygenase-2 (COX-2), the initial enzyme in the biosynthetic pathway of the proinflammatory prostaglandins and a factor that has been implicated in seizure initiation and propagation. Rats were exposed to a toxic dose of soman and scored behaviorally for seizure intensity. Expression of COX-2 was determined throughout brain from 4 hr to 7 days after exposure by immunohistochemistry and immunoblotting. Microglial activation and astrogliosis were assessed microscopically over the same time-course. Soman increased COX-2 expression in brain regions known to be damaged by nerve agents (e.g., hippocampus, amygdala, piriform cortex and thalamus). COX-2 expression was induced in neurons, and not in microglia or astrocytes, and remained elevated through 7 days. The magnitude of COX-2 induction was correlated with seizure intensity. COX-1 expression was not changed by soman. Increased expression of neuronal COX-2 by soman is a late-developing response relative to other signs of acute physiological distress caused by nerve agents. COX-2-mediated production of prostaglandins is a consequence of the seizure-induced neuronal damage, even after survival of the initial cholinergic crisis is assured. COX-2 inhibitors should be considered as adjunct therapy in nerve agent poisoning to minimize nerve agent-induced seizure activity.

List of papers submitted or published that acknowledge ARO support during this reporting period. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Angoa-Perez, M., Kreipke, C.W., Thomas, D.M., Van Shura, K.E., Lyman, M., McDonough, J.H. and Kuhn, D.M. Soman increases neuronal COX-2 levels: Possible link between seizures and protracted neuronal damage. *Neurotoxicology*, in press, 2010. PMID: 20600289.

Number of Papers published in peer-reviewed journals: 1.00

(b) Papers published in non-peer-reviewed journals or in conference proceedings (N/A for none)

Number of Papers published in non peer-reviewed journals: 0.00

(c) Presentations

Angoa-Perez, M., Verbeem, D.M., Thomas, D.M., Van Shura, K., Lyman, J.H., McDonough, J.H., and Kuhn, D.M. The nerve agent sarin causes widespread microglial activation in brain. *Soc. Neurosci.*, 154.7, 2008.

Number of Presentations: 1.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts): 0

(d) Manuscripts

Number of Manuscripts: 0.00

Patents Submitted

Patents Awarded

Graduate Students

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
Mariana Angoa-Perez	0.25
FTE Equivalent:	0.25
Total Number:	1

Names of Faculty Supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>	National Academy Member
Donald M. Kuhn	0.10	No
FTE Equivalent:	0.10	
Total Number:	1	

Names of Under Graduate students supported

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
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The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

<u>NAME</u>
Total Number:

Names of personnel receiving PHDs

<u>NAME</u>
Total Number:

Names of other research staff

<u>NAME</u>	<u>PERCENT SUPPORTED</u>
FTE Equivalent:	
Total Number:	

Sub Contractors (DD882)

Inventions (DD882)

Statement of the problem studied:

Nerve agents can cause seizures after acute intoxication and these seizures can lead eventually to neuronal damage. The purpose of the present study was to assess the extent to which seizure development after exposure of rats to soman resulted in the up-regulation of cyclooxygenase 2 (COX-2) in brain areas known to be damaged by soman.

Summary of the results:

Soman causes a time-dependent increase in COX-2 expression

The effects of soman on COX-2 protein expression were examined by immunohistochemical analysis over a broad time course (i.e., 4 hr to 7 days after soman). Counts of COX-2 positive cells revealed a somewhat delayed response to soman (Fig 1). Very few cells expressing COX-2 were seen from 4-12 hr after soman treatment (data not shown). By 24-48 hr, large numbers of COX-2 positive cells were seen in hippocampal CA3 regions and especially the dentate gyrus (Fig. 1A and 1B) as well as in the amygdala (Fig. 1C) and piriform cortex (Fig. 1D). By 7 days COX-2 levels declined slightly below those seen at 48 hr in each brain region, but remained significantly elevated over control. Increases in COX-2 immunoreactivity were observed in cingulate cortex and ventral thalamus as well (data not shown).

Soman increases COX-2 expression at the cellular level in a highly circumscribed manner as revealed by immunohistochemistry

Immunohistochemical analyses revealed the highly circumscribed effect of soman on COX-2 expression at the cellular level. After treatment with soman (48 hr), COX-2 positive cells essentially define the anatomical facets of the dentate gyrus, CA3 and CA1 regions of the hippocampus (Fig 2). Cells expressing COX-2 immunoreactivity were small and uniformly round. The piriform cortex and amygdala also showed substantial increases in the number of COX-2 immunoreactive cells after soman (Fig 3). These cells were somewhat more diffuse in the piriform cortex and many displayed an extensive axonal network. COX-2 positive cells were more densely packed in the amygdala of soman-treated animals by comparison to the piriform cortex (see Fig 3). Soman did not change the expression of COX-1 at any time (4 hr to 7 days) in any brain region examined (Fig 4). COX-1 immunoreactivity was very weak in hippocampus of controls (Fig. 4A) and soman treated rats (Fig. 4B). COX-1 containing cells were also seen throughout the amygdala with no apparent alteration by soman in their number or in the intensity of their staining for COX-1 (Fig. 4C and 4D).

Soman-induced increases COX-2 protein levels are correlated with seizure intensity

Immunoblot analysis provided independent confirmation of soman effects on COX-2 expression. Soman caused increases in hippocampal COX-2 that varied considerably (Fig 5A). Because all rats were injected with the same soman dose (i.e., 1.2 X LD₅₀), these results suggest that the changes in COX-2 were not linked to soman per se. All rats were scored for seizures as described in Materials and Methods and behavioral scores were plotted versus the fold-increase in COX-2 immunoreactivity on western blots. This analysis indicated that COX-2

expression was positively correlated with seizure intensity (Fig 5B). Soman-treated rats showing no fasciculations, tremors or seizure activity (behavioral score of 0) showed slight increases in COX-2 (~1.5-2 fold over controls). Animals showing mild fasciculations (behavioral score of 1) and tremor (behavioral score of 2) showed increases in COX-2 expression that increased by 4-10 fold. By far, the largest increase in COX-2 expression (7-15-fold) was seen in rats showing the most intense seizures (behavioral score of 3). Similar results for COX-2 expression were seen in other brain regions (data not shown). Immunoblot analyses also confirmed that hippocampal COX-1 protein levels were not altered by soman at any seizure intensity (see Figs 5A and 5B).

Soman increases COX-2 expression in neurons and not in microglia or astrocytes

COX-2 can be expressed in neurons and by activated microglia and astrocytes (Minghetti and Levi, 1998) so efforts were made to identify the cell-type in which COX-2 expression was increased by soman. First, hippocampus was examined 48 hr after soman exposure for changes in astrocyte and microglial reactivity using GFAP and Isolectin B₄, respectively. The density and staining intensity of astrocytes were increased substantially in hippocampus after soman (Fig. 6B) by comparison to controls (Fig. 6A). Microglial activation in hippocampus was also increased dramatically by soman (Fig. 6D) by comparison to controls (Fig. 6C). In light of this soman-induced gliosis in hippocampus, brain sections were labeled with COX-2 antibodies followed by co-labeling with antibodies against either NeuN to identify neurons, antibodies against GFAP to identify astrocytes, or ILB₄ to identify activated microglia. Patterns of COX-2 (Fig. 7A) and NeuN (Fig. 7B) fluorescence in hippocampus were very similar and when merged, a near-total overlap of cells that are immuno-positive for both COX-2 and NeuN was evident (Fig 7C). The soman-induced microglial activation is evident throughout the hippocampus (Fig. 7E) and it is clear from the merged image (Fig 7F) that the pattern of COX-2 fluorescence staining shows no overlap with that of microglia. Finally, intense GFAP reactivity is evident after soman treatment in the area between CA3 and the dentate gyrus of the hippocampus (Fig 7H), and the lack of overlap of COX-2 containing cells with astrocytes is apparent in the merged image (Fig 7I). The morphology of COX-2 containing cells in the hippocampus of soman treated rats also gives a clear indication of their identity as neurons.

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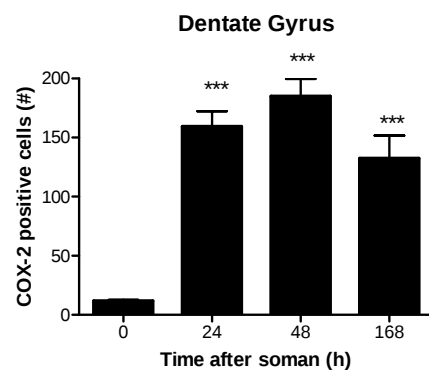
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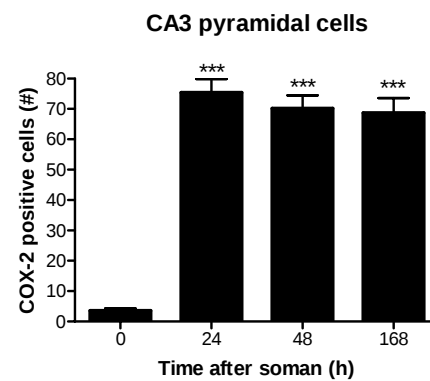
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Fig. 1

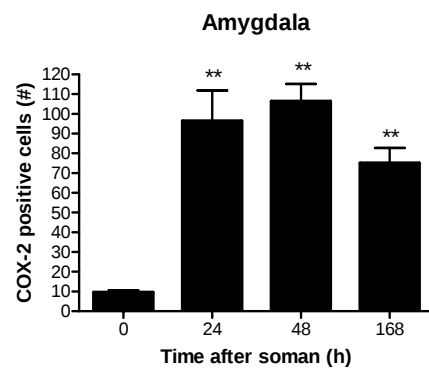
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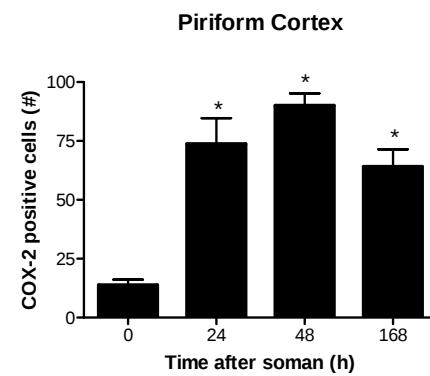
B



C



D



* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Fig.2

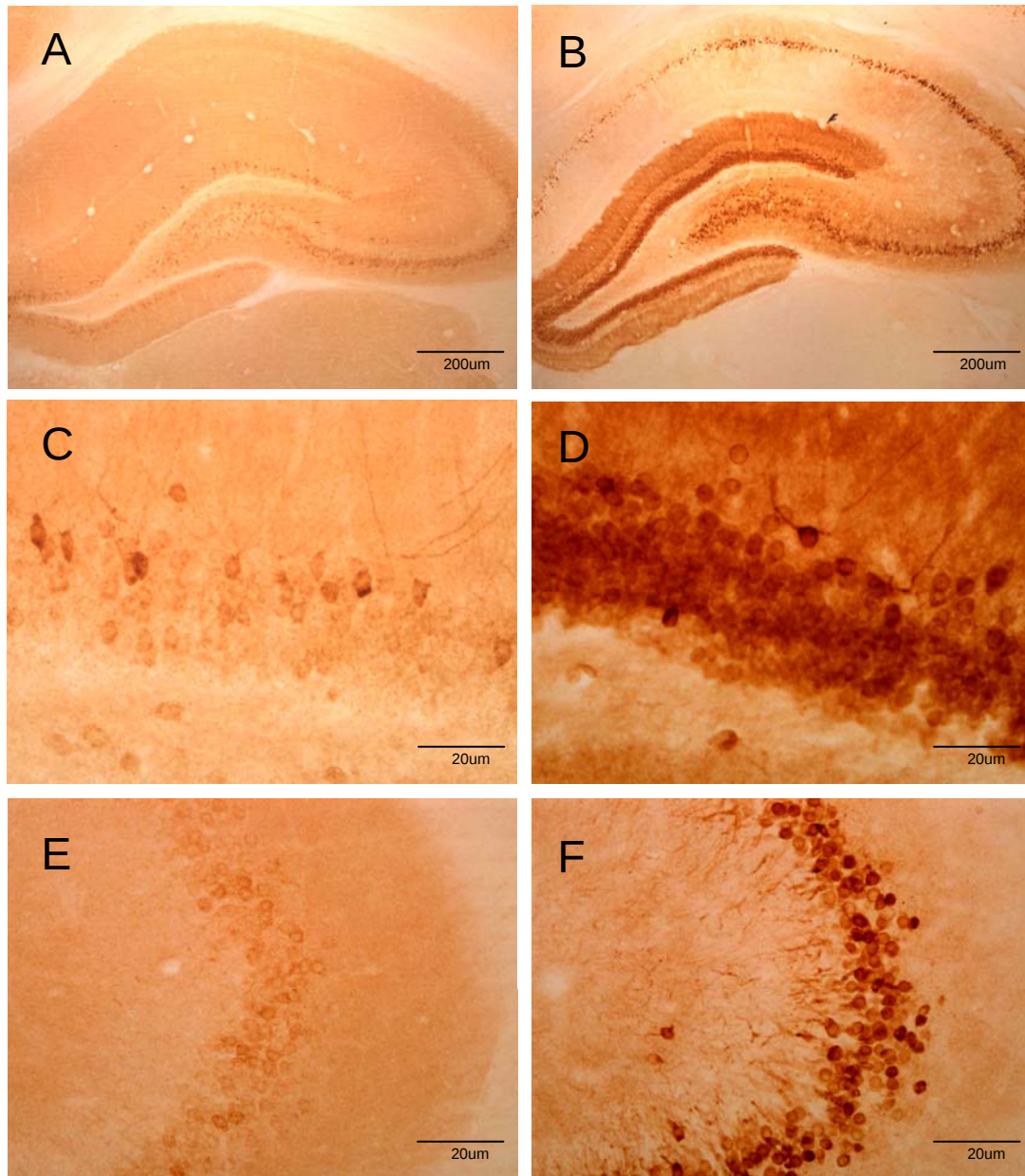


Fig.3

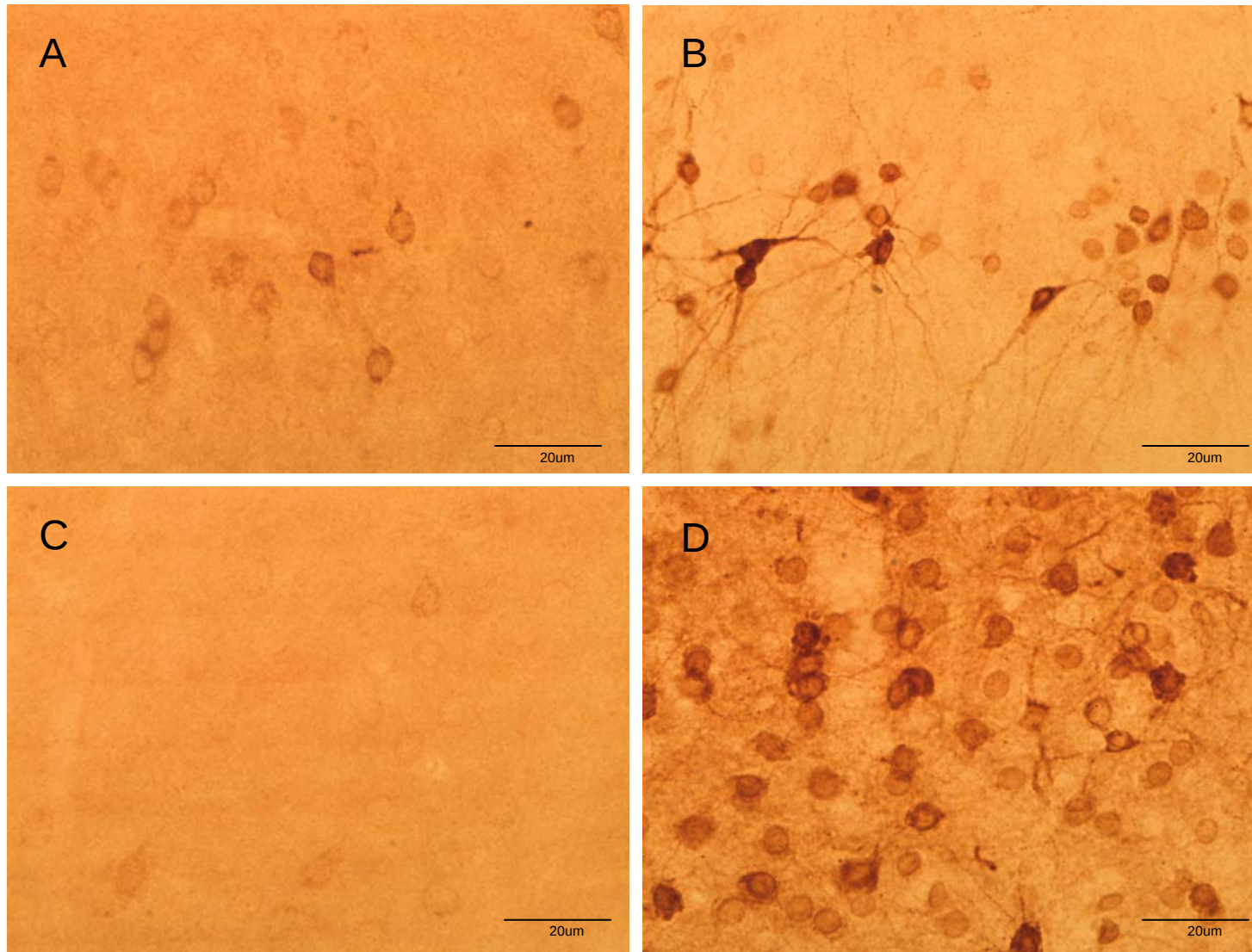


Fig. 4.

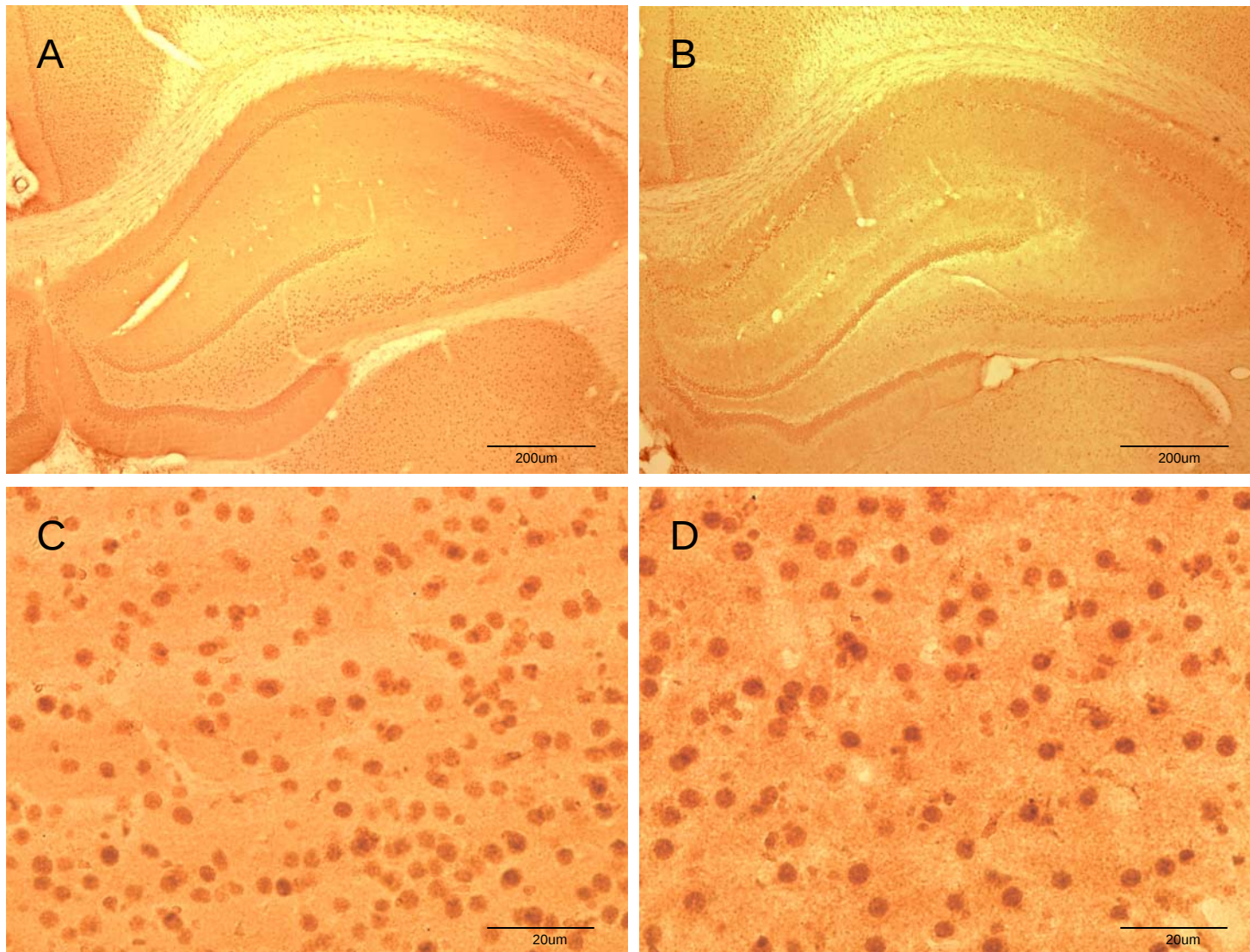
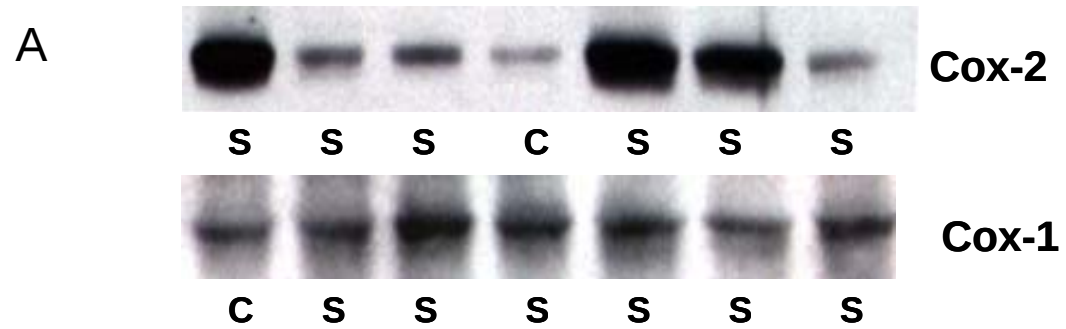


Fig. 5



B

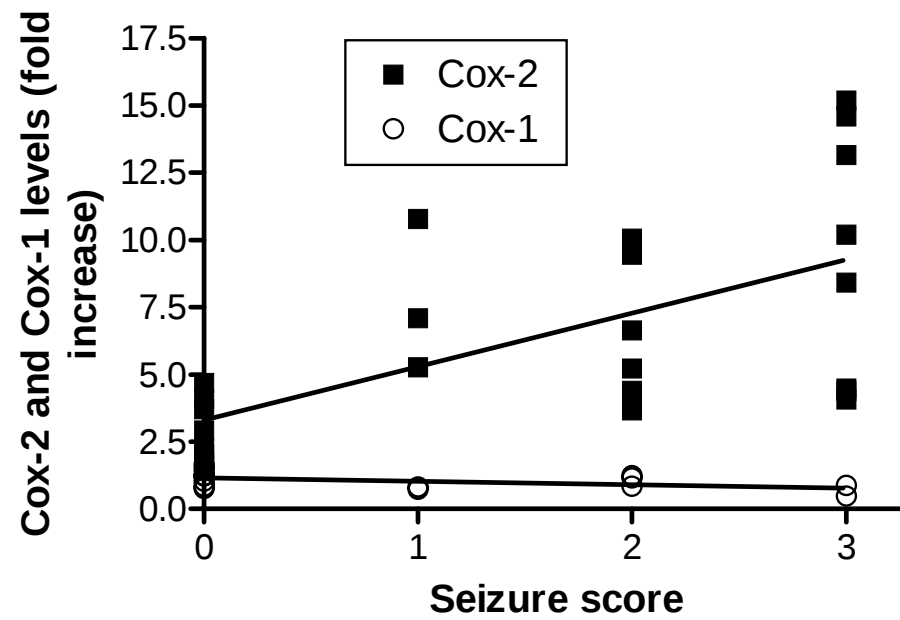


Fig 6.

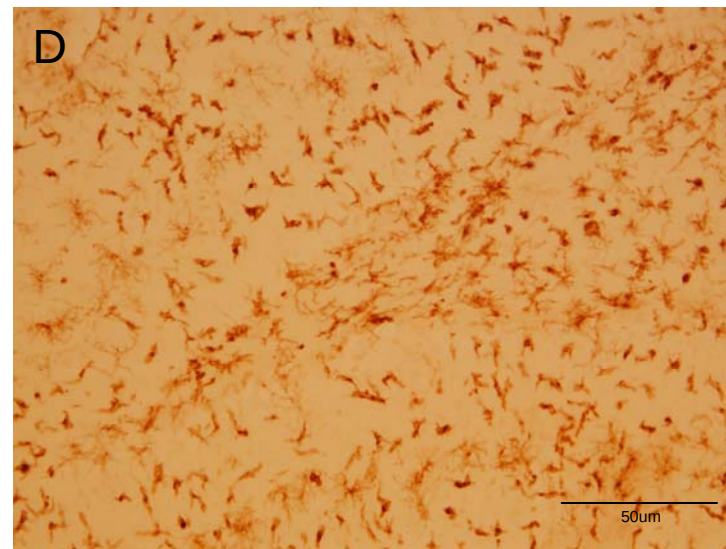
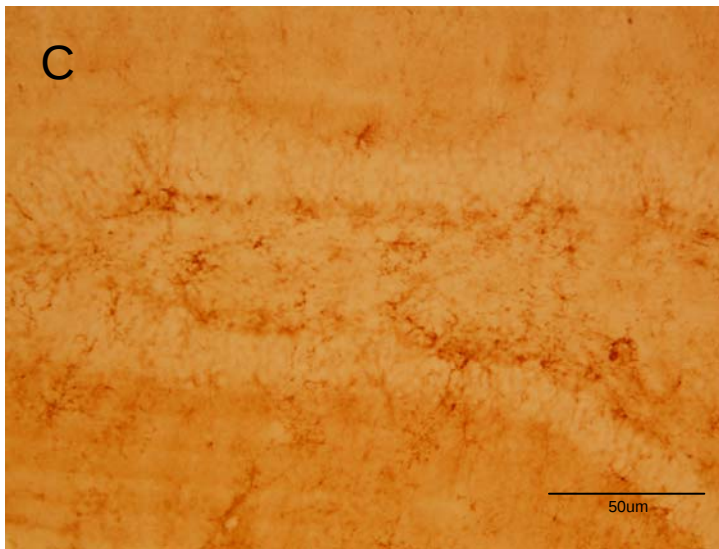
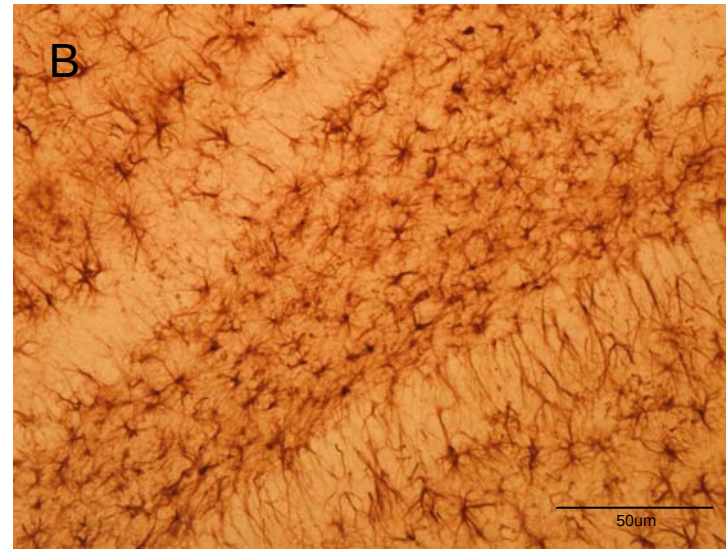
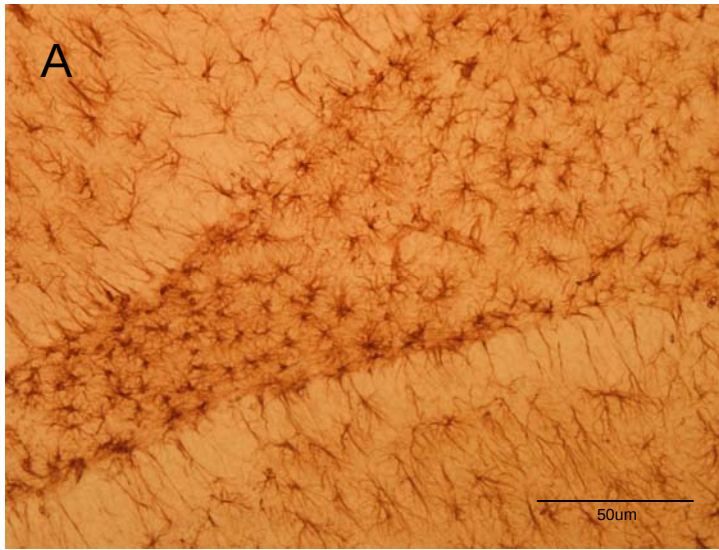


Fig 7.

