THE INFLUENCE OF 5, 7-DIHYDROXYTRYPTAMINE LESIONS OF THE RAT FORNIX-FIMBRIA AND CINGULUM BUNDLES ON SPONTANEOUS ACTIVITY IN AN AVERSIVE MAZE

D.A.F. Robertson[1], J.E. Beattie, I.C. Reid[2], D.J.K.Balfour

Section of Psychiatry, Division of Pathology and Neuroscience,

University of Dundee Medical School

Ninewells Hospital, Dundee, DD1 9SY, Scotland UK

[1]Corresponding author: Dr Debbie Robertson

School of Health and Social Care

University of Chester

Parkgate Road, Chester CH1 4BJ

Email: d.robertson@chester.ac.uk

[2]Present address: Department of Mental Health, University Medical School, Foresterhill, Aberdeen, AB25 2ZD, Scotland UK.

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Abstract

Exposure to aversive environmental stimuli stimulates the serotonergic neurones that project to the forebrain and inhibit spontaneous activity when studied in a simple maze. This study explored the putative role of the principal 5-hydroxytryptamine (5-HT) neurones that project to the hippocampus from the median raphe nucleus in this response to an aversive environment by lesioning the 5-HT fibres that project through the fornix / fimbria and cingulum bundles. The effects of the lesions were investigated in independent groups of animals tested in an enclosed 4-arm maze and a more aversive elevated maze of the same dimensions composed entirely of 4 open arms. The rats were significantly less active in the open maze, the principal effect of maze design being observed during the first 5 minute subtrial of a 15 minute trial. This response to the more aversive environment was totally abolished by the lesion. It is concluded that exposure to an explicitly aversive environment elicits a brief stimulation of the 5-HT neurones that project to the hippocampus from the median raphe nucleus and that this stimulation inhibits the initial burst of exploratory activity that is observed in animals placed in a less aversive novel environment.

Introduction

The exploratory locomotor activity of rats exposed to an explicitly aversive stimulus, an elevated open maze, is significantly reduced when compared with the activity of rats tested in a maze of similar dimensions made less aversive by enclosing the arms of the maze with walls (Copland and Balfour 1987). This reduced activity may reflect a psychobiological mechanism which attenuates the initial exploratory burst of activity exhibited by animals when tested in a novel environment which could prove hazardous. Exposure to an aversive environmental stimulus has been shown to increase the release and turnover of 5-HT in the hippocampus (Linthorst *et al*, 2002, Pei *et al,* 1990, Wright *et al*, 1992). Other studies have demonstrated that the microinjection of 5-HT into the hippocampus decreases the spontaneous activity of a rat when tested in an open field (Plaznik *et al*, 1983). Additionally, paradigms such as post weaning isolation that diminish presynaptic serotonergic function in the hippocampus, increase spontaneous activity in the behavioural responses to novelty (Lapiz *et al*, 2003). This study explored the possibility that the inhibition of exploratory activity, evoked by exposure to an explicitly aversive test environment, is mediated by the serotonergic projections that project to the hippocampus from the median raphe through the fornix-fimbria and cingulum bundles (Azmitia and Segal 1978).

Methods

Subjects

All procedures involving animals and their care and housing were conducted in accordance with UK Home Office regulations; "*The Animals (Scientific Procedures) Act 1986*" under the auspices of Project Licence PIL 60/2845. All the experiments were sanctioned by the University of Dundee Ethical Review Process.

Adult male Sprague-Dawley rats, supplied by Harlan UK, were used throughout this study. They weighed 250 – 280g at the beginning of the experiment. They were housed in pairs and maintained in a 12 hour light/12 hour dark cycle (holding room lights on at 07.00h; off at 19.00h) and were given free access to standard laboratory chow and water. The rooms where the animals were housed and where they were stressed were maintained under a strictly controlled temperature (21^{o}C).

The 5-HT fibres, projecting to the hippocampus through the fornix/fimbria and cingulum bundles were lesioned using the procedure described by Balfour *et al* (1986). Briefly, each animal was given an intraperitoneal (i.p.) injection of desmethylimipramine (10mg.kg^{-1} Sigma UK) approximately 15 minutes prior to commencement of the surgery to protect noradrenergic fibres. Anaesthesia was induced using 5% isofluorane and maintained using 2.5% Isofluorane during the surgical procedure. The rat was positioned in a stereotaxic frame, the skin incised and burr holes were drilled. The position of bregma was determined stereotaxically and all subsequent measurements and coordinates were made with reference to this coordinate. Injections into the cingulum bundles were made using the following coordinates; in the anterior-posterior plane –1.2mm, in the lateral plane +/–1.3mm, in the vertical plane –3.2mm at angles of 14 ^{o} left and right. For the injections into the projections which pass through the fornix/fimbria, the coordinates were in the anterior-posterior plane –1.2mm, in the lateral plane +/–1.3mm and in the vertical plane –5.3mm at an angle of 14 ^{o} left. The locations of the injection sites are shown diagrammatically in figure 1. Injections were of either saline vehicle (500nl) containing 0.25mg/ml ascorbic acid (sham surgery) or 5, 7-dihydroxytryptamine (Sigma UK; 5µg in 500nl of vehicle) delivered over 2 minutes. Following each injection, the cannula was left in place for a further 2 minutes to allow the toxin or vehicle to diffuse into the brain.

Fourteen days were allowed to elapse for the lesion to develop fully before groups of lesioned and sham-lesioned animals (N = 6 per group) were placed on individually into the centre of one of two elevated mazes composed of 4 arms 45cm long x 15cm wide (Copland and Balfour, 1987). In one

maze, all 4 arms were enclosed with 30cm high sides; the arms of the other maze were open. The activity of the animals was recorded on videotape for 15 minutes before the animals were killed by stunning followed by cervical dislocation, and brain and trunk blood samples taken for the assay of 5-hydroxindole and plasma corticosterone concentrations respectively. Additional control groups of lesioned and non-lesioned animals (N = 4 per group) remained in their home cages throughout. The whole brain was removed and immediately dissected over ice following a procedure similar to that described by Glowinski and Iversen (1966). Each hippocampus was placed flat on a cooled surface and divided equally into the dorsal and ventral parts of the structure and stored at -70^{o}C for analysis. The tapes were scored for the number of entries that the animals made into the arms of the maze during each of 3 x 5 minute sub-trials.

The plasma corticosterone concentrations in the samples of trunk blood, were determined using a commercially available radioimmunoassay kit (DPC Ltd, Gwynedd, Wales, UK), which utilised [^{125}I]iodine. The blood samples were centrifuged at 10000g for 5 min and a 0.5ml sample of plasma collected. The plasma samples were diluted 1 in 2 using the buffer supplied by the manufacturer. Samples were then assayed in duplicate.

The brain samples were analysed using a procedure similar to that described by Reinhard *et al* (1980). On the day of analysis, the samples were thawed over ice, weighed and homogenized in 1ml of ice-cold 0.3 M perchloric acid. They were centrifuged at 13000g for 5 minutes to yield a pellet and a supernatant fraction. Samples (200µl) of the supernatant were ultra-filtered through a Whatman filter (0.2µm pore size) at 13000g for 15 minutes. A sample of each ultra-filtrate was analysed for 5-HT and its major metabolite, 5-HIAA by HPLC with electrochemical detection.

Statistical Analyses

Statistical analyses of the data were performed using SPSS 11.1 for Windows. All data are presented as means ± SEM. The data were analysed statistically using a two-way analysis of

variance for repeated measures. For the behavioural experiments, the lesion and the maze were used as between group factors analysed and the activity for the individual sub-trials as the within group factor. For the analysis of the brain 5-hydroxindole concentrations, brain region was used as the within group factor analysed. The plasma corticosterone concentrations were analysed using a two-way analysis of variance with the lesion and the maze as the independent factors analysed. *Post hoc* analyses were performed using the Student Neuman Keul's test.

Results

A global analysis of the behavioural data showed than the rats tested in the open maze were significantly less active ($F(1,20)=15.84$; $P<0.001$) than the rats tested in the enclosed maze (figure 2). This effect, however, interacted significantly with both time in the maze and the lesion (maze x sub-trial x lesion $F(2,40) = 11.43$; $P<0.001$). *Post hoc* analysis showed that the non-lesioned rats were more active in the enclosed maze than the open maze during the first 5 minute sub-trial ($P<0.001$), but not during subsequent sub-trials. In the rats tested the enclosed maze, the lesion evoked a modest overall increase in activity that did not interact significantly with time in the maze ($F(1,10)=5.55$; $P<0.05$). By contrast, in the rats tested in the open maze, the activity was higher in the lesioned rats during the first two sub-trials ($P<0.001$ and $P<0.05$ respectively), but not during the final sub-trial. As a result, during the first sub-trial, the activity of the lesioned rats tested in the open maze was not significantly different to that of the lesioned or non-lesioned rats tested in the enclosed maze.

The post-mortem measurements on the hippocampus showed that the lesion evoked substantial decreases in the concentrations of 5-HT ($F(1,26)=270.45$; $P<0.001$) and 5-HIAA ($F(1,26)=38.84$; $P<0.001$) which were independent of the brain area sampled. The concentration of 5-HT, however, was also influenced by the maze to which the animals had been exposed (lesion x maze $F(2,26) = 16.52$; $P<0.001$). In the non-lesioned animals, when compared with unstressed controls, exposure to

the mazes was associated with reduced concentrations of 5-HT in both the dorsal and ventral hippocampus (figure 3) which were not observed in the lesioned rats. The effects of the maze on hippocampal 5-HT depended upon the nature of the maze in the animals were tested, exposure to the open maze evoking a significantly greater reduction in hippocampal 5-HT than that evoked by exposure to the enclosed maze ($P<0.05$). Exposure to the mazes had no significant effects on the concentrations of 5-HIAA in either of the brain areas studied. However, both the lesion and exposure to the mazes independently increased 5-HT turnover (5-HIAA:5-HT ratio) in the hippocampus ($F(1,26)=6.65$; $P<0.05$ and $F(2,26)=8.61$; $P<0.001$ respectively). Statistically, the both effects were independent of the brain area investigated and did not interact together. Post hoc analysis showed that the apparent increase in turnover evoked by exposure to the enclosed maze did not approach statistical significance whereas 5-HT turnover in the animals exposed to the open maze was higher than the turnover measured in unstressed controls ($P<0.01$) and rats tested in the enclosed maze ($P<0.05$). Regression analysis, however, revealed no significant correlations between activity in the mazes and the 5-hydroxyindole concentrations or 5-HT turnover in either part of the hippocampus.

Exposure to the mazes increased ($F(2,26)=36.51$; $P<0.001$) the plasma corticosterone concentration, the increase evoked by exposure to the open maze being significantly greater ($P<0.05$) than that evoked by exposure to the enclosed maze (table 1). The lesion had no significant effects on the plasma corticosterone concentration. Regression analysis for the results as a whole showed that the plasma corticosterone concentration did not correlate significantly with any of measures of activity in the mazes, but did correlate significantly ($F(2,29)=5.50$; $P<0.01$) with 5-HT turnover in the dorsal hippocampus ($r=0.42$; $P<0.05$). The correlation with 5-HT turnover in the ventral hippocampus approached statistical significance ($r=0.32$; $P=0.055$). However, an additional analysis for the non-lesioned animals alone showed that plasma corticosterone correlated best ($F(1,14)=6.5$; $P<0.05$) with 5-HT turnover in the ventral hippocampus ($r=0.56$; $P<0.05$) whereas in the lesioned

rats, plasma corticosterone only correlated significantly ($F(1,14)=5.71$; $P<0.05$) with 5-HT turnover in the dorsal hippocampus ($r=0.55$; $P<0.05$).

Discussion

The behavioural results presented here have confirmed those reported previously from this laboratory (Copland and Balfour 1987) which showed that the rats tested an elevated open maze, composed entirely of open arms, are significantly less active than those tested in a maze of equivalent dimensions but composed of enclosed arms. The study has extended the earlier observations by demonstrating that the principal effects of the maze design were observed during the first 5 minutes of the trial, when the rats in the enclosed maze were most active, and waned as the animals habituated to the apparatus. The plasma corticosterone measurements imply that the rats found the open maze more aversive than the enclosed version of the apparatus since the mean plasma corticosterone concentration in the rats tested in the open maze was significantly higher than that measured in the rats tested in the enclosed maze. Thus, it seems reasonable to suggest that the increased aversive properties of the open maze caused this initial suppression of activity.

The major finding of the study was that lesions of the principal 5-HT projections from the median raphe to the hippocampus abolished the effect of the aversive stimulus on exploration of the open maze, but only had a modest effect in the rats tested in the enclosed maze. As a result, the pattern and level of activity of the lesioned rats tested in the open maze closely resembled that of the rats tested in the enclosed maze. Previous studies have demonstrated that microinjections of 5-HT into the hippocampus decrease exploration of an open field (Plaznik *et al*, 1983). Other studies suggest that depletion of brain 5-HT or lesions of the 5-HT projections to the hippocampus attenuate behavioural habituation to an open field (Bidzinski *et al*, 1998; Williams *et al*, 1990). It seems reasonable to conclude, therefore, that the suppression of activity, evoked by exposure to the more

aversive environment, is related to increased 5-HT release from the neurones that project to the hippocampus.

Microdialysis studies have shown that acute exposure to a stressful stimulus preferentially increases 5-HT release from neurones that project to areas of the brain innervated from the dorsal raphe nuclei (Pei *et a*l, 1990, Wright *et al,* 1992). By contrast, increased overflow in the dorsal hippocampus, which is innervated preferentially from the median raphe nucleus, is only observed following repeated daily exposure to the same inescapable stressor (Storey *et al,* 2006). The findings reported here suggest that the suppression of activity, evoked by acute exposure to the more stressful maze, occurs predominantly during the first 5 minute sub-trial and does not persist for the full 15 minute trial. Furthermore, Adell and colleagues (1997) have reported that a 5 minute exposure to a forced swimming paradigm elicits an increase in 5-HT overflow in the median raphe that causes a reduction in 5-HT release in the hippocampus that persists beyond completion of the stress. This observation seems consistent with the results of this study in which the concentrations of 5-HT in the dorsal and ventral hippocampus were reduced at the end of the trial in the animals tested in the mazes in a way that seemed dependent upon the stress of the procedure to which the animals have been exposed. Thus, although sufficient to influence the initial behavioural response of the animals, the stimulation of the 5-HT projections from the median raphe evoked by acute exposure to the stressful stimulus may be of a short duration that does not lead to an increase of 5-HT overflow which is sufficiently persistent to be measured using microdialysis.

Although the results support the conclusion that the suppression of spontaneous activity, evoked by exposure to an explicitly aversive environment, depends upon 5-HT release from the neurones that project to the hippocampus from the median raphe, regression analysis failed to reveal any clear correlations between spontaneous behavioural activity and the post-mortem measures of serotonergic function. Thus, the mechanism by which the pathway influences spontaneous activity remains to be determined. Nevertheless, the results suggest that stimulation of the 5-HT projections

from the median raphe to the hippocampus may play a role in the neural mechanisms by which aversive environmental stimuli inhibit the initial burst of exploratory activity evoked by exposure to a novel environment. The behavioural value of this response may lie in its ability to inhibit high levels of exploratory activity that could put the organism at risk.

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Table 1

The Effects of the Lesion and Maze Exposure on Plasma Corticosterone

		Plasma Corticosterone (ng/ml)		
Experimental Group	N	Non-Lesioned	Lesioned	Significance
Control	4	21 ± 7	10 ± 2	
Enclosed Maze	6	160 ± 37	216 ± 34	***
Open Maze	6	266 ± 28	274 ± 15	***/††

The data are expressed as means ± sem of 4-6 observations as indicated in the table. The lesion had no significant effects on plasma corticosterone. Significantly different from the control groups: ***; $P<0.001$; significantly different from rats tested in the enclosed maze: ††; $P<0.01$

Figure Legends

Figure 1: Neurotoxin injection sites

The diagram depicts the sites at which the neurotoxin, 5, 7-dihydroxtryptamine, was microinjected into the 5-HT fibres that project to the hippocampus through the cingulum bundles and fornix / fimbria. The section is equivalent to figure 22 in Paxinos and Watson (1986). Each rat received 3 injections of the toxin at the sites numbered 1, 2 and 3 in the diagram. The solid lines indicate the cannula placements for the injections into the cingulum bundles. The cannula inserted into the right side of the brain was used for microinjections 1 and 2, the cannula being advanced into the fornix/fimbria following the microinjection into the cingulum bundle. This is shown by the dotted line. Each injection was delivered in 500nl of vehicle (saline containing 0.25mg/ml ascorbic acid) over 2 minutes; non-lesioned control animals received microinjections of the vehicle. cc = corpus callosum; Fi = fimbria; LV = lateral ventricle

Figure 2: Maze entries

Entries into the arms of the mazes were recorded in 5 subtrials and are presented as means ± sem of 6 observations. The open columns represent the data for the non-lesioned rats; the filled columns represent the data for the lesioned rats. The rats tested in the open maze were significantly less active ($F(1,20)=15.84$; $P<0.001$) than the rats tested in the enclosed maze. The increase in activity evoked by the lesion in the rats tested in the enclosed maze was significant ($F(1,10)=5.55$; $P<0.05$) and did not interact with time in the maze. The increases in activity evoked by the lesion in the rats tested in the open maze were significant for the first two sub-trials ***; $P<0.001$ and *$P<0.05$ respectively.

Figure 3: Hippocampal 5-hydroxyindole concentrations and turnover

The concentrations of 5-HT and 5-HIAA and measures of 5-HT turnover (5-HIAA:5-HT ratio) were measure in dorsal and ventral hippocampus and are presented as means ± sem (N = 6 for the rats tested in the mazes; N = 4 for the control rats). The unfilled columns represent the data for the non-lesioned rats; the filled columns represent the data for the lesioned rats. The decreases in the concentrations of 5-HT and 5-HIAA evoked by the lesions were significant ($F(1,26) = 270.45$; $P<0.001$ and $F(1,26) = 38.84$; $P<0.001$ respectively). The increases in 5-HT turnover evoked by the lesion and exposure to the mazes were also significant ($F(1,26)=6.65$; $P<0.05$ and $F(2,26)=8.61$; $P<0.001$ respectively). Both effects were independent of the part of the hippocampus investigated and did not interact significantly. Significantly different from control; *$P<0.05$; **$P<0.01$; significantly different from rats tested in the enclosed maze; +$P<0.05$

Figure 1

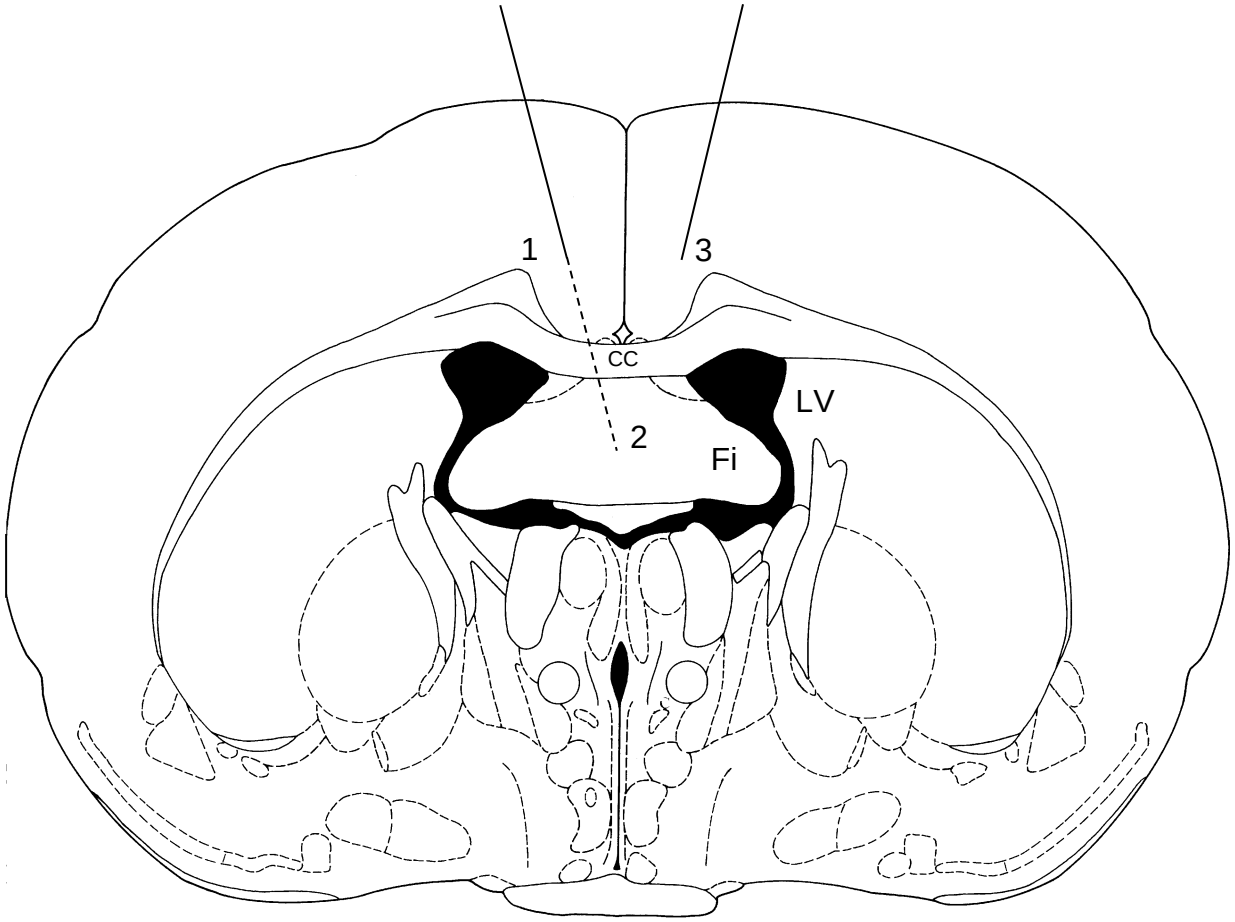


Figure 2

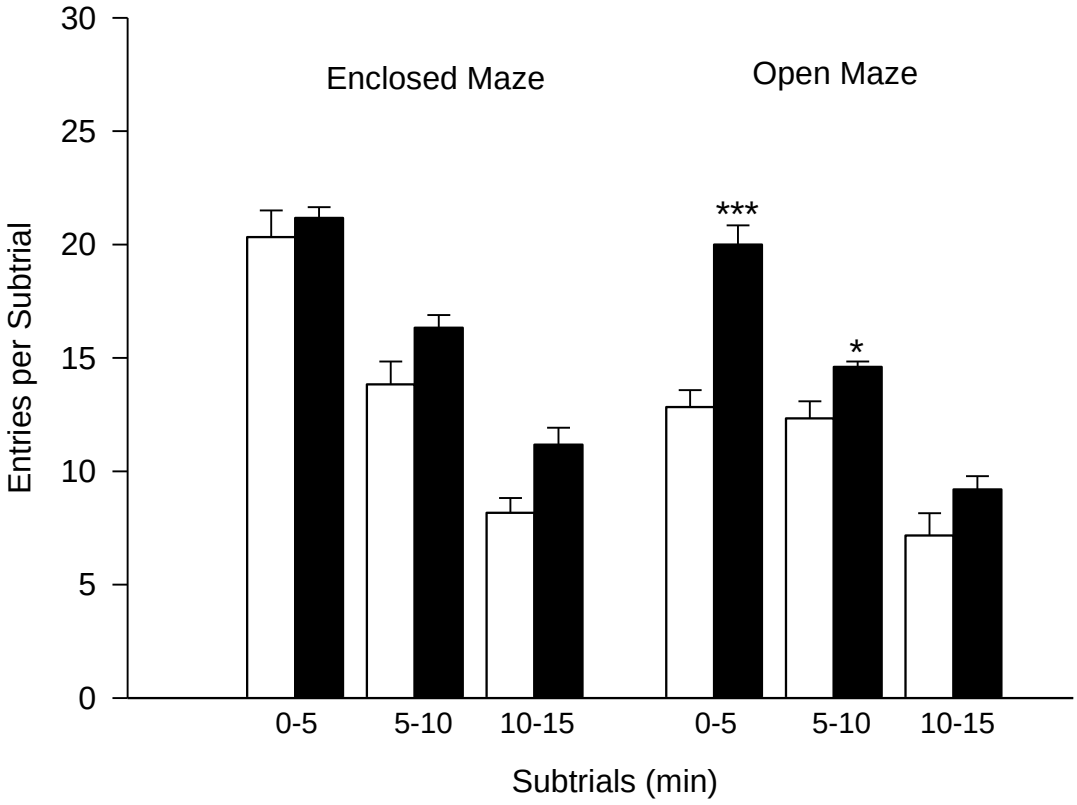
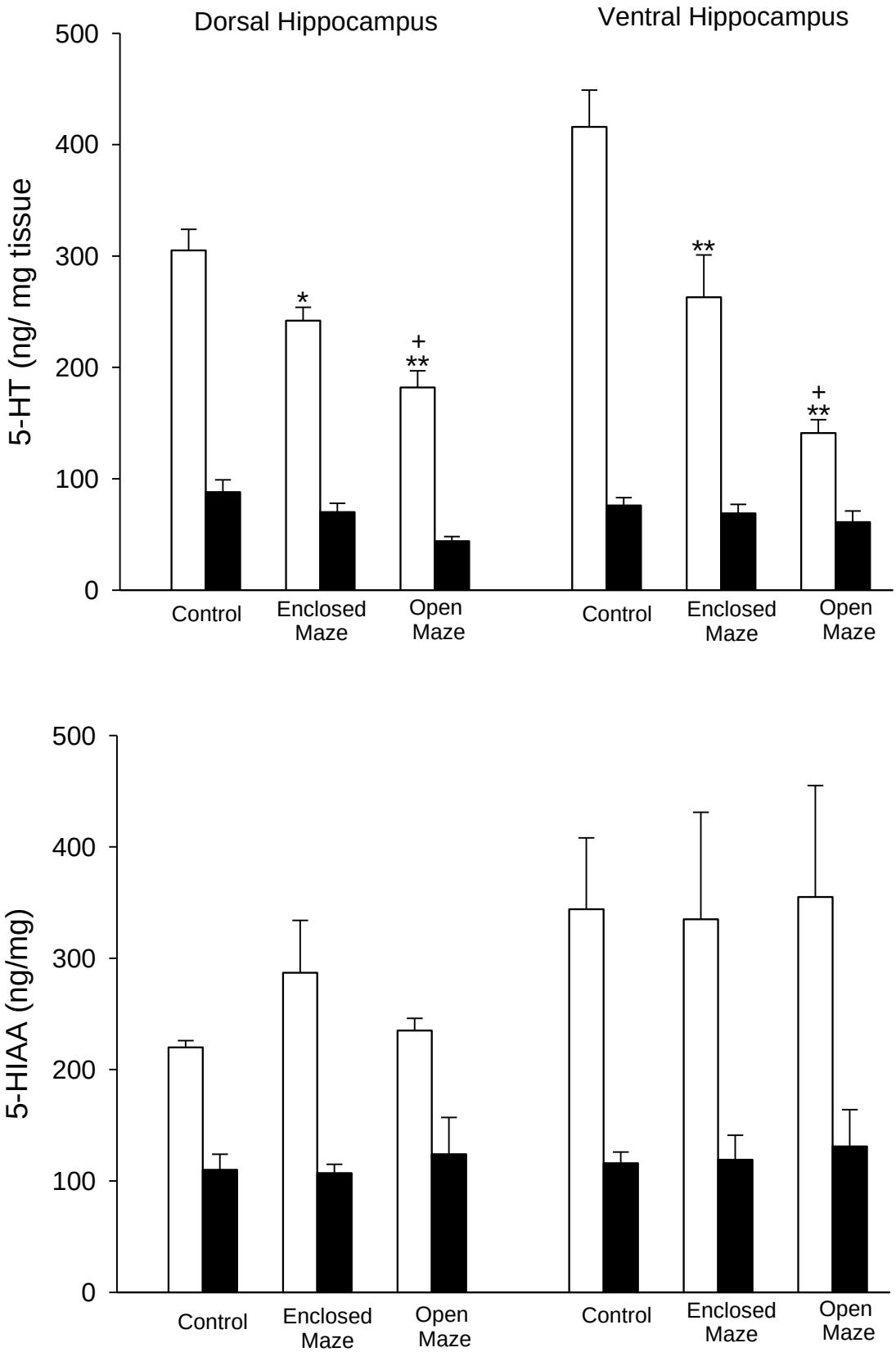

30
25
20
15
10
5
0
Entries per Subtrial
Enclosed Maze
Open Maze
***
*
0-5
5-10
10-15
0-5
5-10
10-15
Subtrials (min)

Figure 3


Dorsal Hippocampus
Ventral Hippocampus
5-HT (ng/ mg tissue
500
400
300
200
100
0
*
+
**
**
+
**
Control
Enclosed Maze
Open Maze
Control
Enclosed Maze
Open Maze
5-HIAA (ng/mg)
500
400
300
200
100
0
Control
Enclosed Maze
Open Maze
Control
Enclosed Maze
Open Maze