

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

Investigating the electrical response of the brain of the domestic chicken (*Gallus gallus domesticus*) to nociception through the use of depth electroencephalography (dEEG)

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Physiology

at Massey University, Manawatū, New Zealand.

Peter Dennis Trebilcock

2015

Abstract

Nociception is an unavoidable side effect of many routine management and clinical procedures in animals. Electroencephalography (EEG) has previously been used to investigate the effect of nociception on mammalian brain activity. This study aimed to develop a method of assessing the avian response to nociception through depth electroencephalography (dEEG) of brain regions believed to be involved in central pain processing. Two groups of chickens were used in this study to investigate two brain regions, the rostral hyperpallium apicale (HA) and the caudomedial nidopallium (NCM). These regions were chosen due to the afferent and efferent projections they receive from the sensory thalamus and their previous implication in pain processing. Subjects were anaesthetised, and a concentric needle electrode was inserted into the brain to record the electrical activity in response to a number of stimuli. These stimuli included one non-painful, somatosensory stimulus, and four nociceptive stimuli (mechanical, thermal, feather removal and electrical). The dEEG data was then run through a spectral analyser which generated the median frequency (F50), spectral edge frequency (F95) and total power (P_{TOT}). Inspection of these variables determined that within the HA there were two populations of birds, therefore these birds were treated as separate groups in the analysis (hHA and lHA).

It was seen that spectral characteristics of the three groups investigated differed significantly, indicating differences in activity and function. The response to stimulation was seen to be significantly different between these brain regions. Following stimulation, the hHA was seen to have a significantly lower percentage of baseline spectral edge frequency and median frequency compared to the NCM and lHA. In response to stimulation the activity of the NCM and lHA remained constant and showed no distinguishable response, while the hHA was more variable. The hHA was much more variable. Although there was no consistent response to stimulation, there was a significant decrease in total power following electrical stimulation in the hHA.

This study presents a number of interesting findings and demonstrates that different regions of the brain respond in differing ways to stimulation. The findings suggest that the hyperpallium apicale may respond to nociceptive stimulation, however further work is required to distinguish this. The presence of two populations within the HA group suggests that recordings were taken from two distinct brain regions, one of which displayed comparatively higher sensitivity to nociceptive stimulation. Elucidation of this brain region and further research into the response to nociception is required to further understand the

response of the avian brain to pain. For future studies, the development of more precise methods will be required to enable more accurate recording of the activity occurring throughout the avian brain.

Acknowledgements

I would firstly like to thank my supervisors, Craig Johnson, Ngaio Beausoleil, Preet Singh and Kavitha Kongara, for their expertise and guidance in all aspects.

To Nikki Kells for providing me with a great deal of advice, guidance, proofreading and for being around for a chat.

I am grateful to a number of people who helped me during the course of my study. Matt Perrot and Evelyn Lupton for help in preparing for my study, Paul Chambers for assisting me with anaesthesia and Ty Mirko for being around the lab to help out. Also, a shout out to the guys at the Massey Poultry Unit who were always up for a chat when I visited.

I would also like to thank the IVABS Avian Research Fund for providing the funding to complete this research and to Massey University for my Masterate Scholarship which was a great help during my studies.

A huge thanks to Abigail Sharrock, who proofread countless assignments and to Jono Oh for providing my technology inept self with both a phone and a laptop when was in need of them, that was hugely helpful.

To my football team, thanks for providing me with some stress relief over the weekend and for all the good times. MTIG.

I would also like to thank the internet, you are a great source of information but a huge distraction. I have completed this masterate because of, and in spite of you.

Finally, to my friends and family for your encouragement and support, for chats about the Warriors, for encouraging me to take a trip to the Cricket world cup final despite all logic, and for keeping me sane. You guys are all right.

Table of Contents

Abstract.....	ii
Acknowledgements.....	iv
Table of Contents.....	v
List of Figures.....	viii
List of Tables.....	ix
Abbreviations.....	x
1. Literature review.....	11
1.1. Introduction.....	11
1.2. Animal welfare.....	11
1.3. Pain.....	12
1.3.1. Transmission and integration of nociceptive information.....	12
1.3.2. Brain regions involved in pain.....	13
1.4. Avian brain structure.....	13
1.4.1. Avian pain centres.....	14
1.4.2. Hyperpallium.....	16
1.4.3. Nidopallium.....	17
1.5. Brain response to pain.....	17
1.5.1. Electrophysiological recording.....	17
1.5.2. Electrical changes in response to nociception.....	18
1.5.3. Mammalian response to nociception.....	19
1.5.4. Avian response to nociception.....	20
1.6. Study objectives.....	21
2. Material and Methods.....	22
2.1. Animals/General care.....	22
2.2. Preparation of study.....	22
2.2.1. Determination of brain regions.....	22
2.2.2. Stereotaxic positioning.....	23
2.2.3. Determination of correct electrode placement.....	23
2.3. Experimental procedure.....	23
2.3.1. Anaesthesia.....	23
2.3.2. Electrode placement.....	24
2.3.3. Recording electrical brain activity.....	25
2.3.4. Treatments.....	25

2.4.	Analysis	26
2.4.1.	Analysis of electrical activity	26
2.4.2.	Populations of the HA	28
2.4.3.	Unstandardised data analysis	28
2.4.4.	Statistical analysis	29
3.	Results.....	31
3.1.	Analyses of unstandardised baseline data.....	31
3.2.	Overall repeated measures model of standardised data	33
3.3.	Analyses of individual time points	36
3.3.1.	Pre-treatment	37
3.3.2.	10–40 seconds following stimulation	37
3.3.3.	40–70 seconds following stimulation	37
3.3.4.	70–100 second following stimulation	37
3.3.5.	100–130 seconds following stimulation	38
3.4.	Analyses of individual stimuli.....	40
3.4.1.	Non-painful stimulation	41
3.4.2.	Mechanical stimulation.....	41
3.4.3.	Thermal stimulation.....	41
3.4.4.	Feather removal.....	41
3.4.5.	Electrical stimulation.....	41
4.	Discussion.....	43
4.1.	Aims of the study	43
4.2.	Findings	43
4.2.1.	Analysis of unstandardised data	43
4.2.2.	Analyses of individual time points	44
4.2.3.	Analyses of individual stimuli.....	45
4.3.	Implications of these findings	46
4.3.1.	Variation in activity throughout the brain	46
4.3.2.	Differing response of the brain to stimulation	48
4.3.3.	Response of the brain to nociceptive stimulation	48
4.3.4.	The effect of noxious stimulation on the mammalian EEG	50
4.4.	Possible reasons for the differences seen	51
4.4.1.	Brain anatomy.....	52
4.4.2.	Depth electroencephalography (dEEG)	53

4.4.3.	Intensity of stimulation	54
4.4.4.	Anaesthesia	56
4.4.5.	Accuracy of electrodes	58
4.4.6.	Pain in birds.....	58
4.5.	Limitations to the Study	59
4.5.1.	Variation in subject size	59
4.5.2.	Accuracy of electrode placement	59
4.5.3.	Sample size.....	60
4.5.4.	Determination of correct electrode position.....	60
4.5.5.	Age of the stereotaxic atlas	61
4.5.6.	Use of alternative avian models	62
4.5.7.	Brain regions targeted	63
4.5.8.	Minimal anaesthesia model	64
4.5.9.	Contralateral control of the vertebrate brain.....	64
4.5.10.	EEG Analysis	65
4.5.11.	Alternative methods of assessing brain activity	66
4.5.12.	External warming protocol of birds	67
5.	Conclusions and future work	69
6.	References	71
7.	Appendix A.....	78

List of Figures

Figure 1. Comparative view of the avian (Zebra Finch, A) and mammalian (Human, B) brain to illustrate the differences in the structure and organisation of the brain (From Jarvis <i>et al.</i> , 2005).....	14
Figure 2. Sagittal section of the avian brain showing sensory inputs from the body throughout the brain (red). Sensory inputs from the body enter through the dorsal root ganglia (DRG), through the thalamic nuclei (DIVA and cDLP) to the hyperpallium apicale (HA) and the caudomedial nidopallium (NCM). (Adapted from Kuenzel (2007).	15
Figure 3. Representation of the power spectrum produced by Fast Fourier Transformation (FFT). The dashed line represents median frequency (F50), the solid line represents spectral edge frequency (F95) and the area under the curve gives the total power (From Murrell & Johnson, 2006)	19
Figure 4. The correct positioning of the head in the stereotaxic apparatus. The ear bars are inserted into the ventro-posterior aspect of the auditory canals, the head is centralised in the apparatus and the plane of the skull is parallel to the surface of the apparatus.....	23
Figure 5. An example of a brain slice showing ink injected into the correct area for the hyperpallium apicale (HA).	26
Figure 6. A diagram representing the time periods used to generate the percent changes prior to and following stimulation.	27
Figure 7. Unstandardised total power of the chick dEEG (least squared means \pm SE) for each pre-treatment baseline period by order. All brain regions were significantly different at all times ($p < 0.05$)......	32
Figure 8. Unstandardised F50 of the chick dEEG (least squared means \pm SE) for each pre-treatment baseline period by order. All brain regions were significantly different at all times ($p < 0.05$)......	32
Figure 9. Unstandardised F95 of the chick dEEG (least squared means \pm SE) for each pre-treatment baseline period by order. All brain regions were significantly different at all times ($p < 0.05$)......	33
Figure 10. Standardised F95 of the chick dEEG (least squared means \pm SE) for all brain regions investigated in response to all stimuli, calculated for each time period. Different letters indicate significant differences between brain regions within the same time period ($p < 0.05$)......	38

Figure 11. Standardised F50 of the chick dEEG (least squared means \pm SE) for all brain regions investigated in response to all stimuli, calculated for each time period. Different letters indicate significant differences between brain regions within the same time period ($p < 0.05$).	39
Figure 12. Standardised P _{TOT} of the chick dEEG (least squared means \pm SE) of the hHA from the brain region by time interaction of the electrical stimulus. The * represents a significant change from the pre-treatment value prior to stimulation ($p < 0.05$).	42
Figure 13. The position of the HA within the brain as indicated by the stereotaxic atlas (From Kuenzel & Masson, 1988)	78
Figure 14. Photographs of the brain slices for birds in the HA group showing the position of the ink injection.	79
Figure 15. The location of the NCM (N) in the brain, as indicated by the stereotaxic atlas (From Kuenzel & Masson, 1988).	80
Figure 16. Photographs of the brain slices for birds in the NCM group showing the position of the ink injection.	81

List of Tables

Table 1. The co-ordinates determined for the location of the brain regions to be targeted. Distances given are relative to a specified zero point.	22
Table 2. Results of ANOVA of unstandardised dEEG data looking at the effects of brain region, order and their interaction on the changes in F50, F95 and P _{TOT} of the chick dEEG... ..	31
Table 3. Results of ANOVA of standardised dEEG data looking at the effects of brain region, stimulus, time and their interactions on the changes in F50, F95 and P _{TOT} of the chick dEEG	35
Table 4. Results of ANOVA of standardised dEEG data looking at the effects of brain region, stimulus and their interaction on the changes in F50, F95 and P _{TOT} of the chick dEEG at each time point	36
Table 5. Results of ANOVA of standardised dEEG data looking at the effects of brain region, time and their interaction on the changes in F50, F95 and P _{TOT} of the chick dEEG for all stimuli	40

Abbreviations

ACC – Anterior cingulate cortex

EEG – Electroencephalogram

dEEG – Depth electroencephalogram

FFT – Fast Fourier transformation

F50 – Median frequency

F95 – Spectral edge frequency

hHA – ‘High’ Hyperpallium apicale

lHA – ‘Low’ Hyperpallium apicale

NCM – Caudomedial nidopallium

P_{TOT} – Total Power

S1 – Primary somatosensory cortex

S2 – Secondary somatosensory cortex