Brain Maturation in Chickens: Biochemical, Behavioural and Electrophysiological Investigations

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BSc(Psych)(Hons)

A thesis submitted for the degree of Doctor of Philosophy

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I hereby certify that with the exception of some assistance with data collection as specified in the Acknowledgements all work contained within this thesis was performed by me.

.................................................. .................. Date:......................

Rebbekah Atkinson
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<td>auditory brainstem response</td>
</tr>
<tr>
<td>ACEC</td>
<td>animal care and ethics committee</td>
</tr>
<tr>
<td>AERP</td>
<td>auditory event related potential</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>analysis of variance</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>BCA</td>
<td>bicinchonic acid</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>CaMKII</td>
<td>calcium/calmodulin stimulated protein kinase</td>
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<td>cianonitroquinoxaline</td>
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<td>EMG</td>
<td>electromyograph</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular-signal regulated kinase</td>
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<tr>
<td>ERP</td>
<td>event related potential</td>
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fT3  free serum T3
fT4  free serum T4
GluR1 glutamate receptor 1
pS831 GluR1 GluR1 AMPA subunit phosphorylated at Ser 831
IMM intermediate medial mesopallium
ISI inter stimulus interval
ITI inter trial interval
ITM intermediate term memory
LPO lobus parolfactorius
LTD long-term depression
LTM long-term memory
LTP long-term potentiation
m mean
MAPK mitogen-activated protein kinase
MeA methylantranilate
MK801 (+)-methyl-10,11-dihydro-5H dibenzo [a,d]cyclo hepten-5,10-imine malate
MMI methimazole
NaF sodium fluoride
PAL passive avoidance learning
PFT pebble floor task
PKA cAMP-dependent protein kinase
PKC protein kinase C
PPI prepulse inhibition
PSD post synaptic density
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PTU</td>
<td>propylthiouracil</td>
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<tr>
<td>RSPI</td>
<td>relative stoichiometry of phosphorylation index</td>
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<tr>
<td>SB</td>
<td>sample buffer</td>
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<tr>
<td>SD</td>
<td>standard deviation</td>
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<tr>
<td>SDS</td>
<td>sodium dodecyl sulphate</td>
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<td>SDS-PAGE</td>
<td>SDS-polyacrylamide gel electrophoresis</td>
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<td>SEM</td>
<td>standard error of the mean</td>
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<td>STM</td>
<td>short term memory</td>
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<tr>
<td>SW-R</td>
<td>standard working reagent</td>
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<tr>
<td>T3</td>
<td>triiodothyronine</td>
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<tr>
<td>T4</td>
<td>thyroxine</td>
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<tr>
<td>TBS</td>
<td>tris buffered saline</td>
</tr>
<tr>
<td>TBST</td>
<td>TBS containing 0.1% Tween-20</td>
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<tr>
<td>TH</td>
<td>thyroid hormone</td>
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Abstract

This thesis investigates mechanisms of brain maturation by utilising the special advantages offered by the protracted maturation of neural circuits in chicken forebrain. Biochemical, behavioural and electrophysiological techniques are used in behaving animals to investigate the functional consequences of maturation changes at the molecular, behavioural and physiological levels.

Two issues are addressed: (1) do immature (2 week) and mature (8 week) chickens employ different molecular mechanisms to produce changes in neuronal function after learning a behavioural task; and (2) can quantitative non-invasive measures of neuronal function be used to monitor maturation changes in chicken forebrain?

Biochemical investigation of subcellular fractions using antibodies and western blots of chicken forebrain and intermediate medial mesopallium (IMM) revealed regional differences in expression levels of a number of components of the glutamatergic neurotransmitter system.

The discriminative taste aversion learning (DTAL) task was used to assess whether an animal learns the same task at different ages using different intracellular signalling pathways. The patterns of biochemical change seen in the IMM after DTAL training was very different at 2 weeks and 8 weeks. Two major differences were observed. Firstly, the same type of training induced changes occurred at both ages in GluR1 and CaMKII but they occurred faster at 8 weeks. Secondly the difference in ERK and CREB responses is consistent with a change in the relative contribution made by the ERK signalling pathway and CREB requirement to learning at these two ages. These results imply that the molecular changes induced by learning a behavioural task
are faster in mature than immature brain and may involve a different balance of intracellular signalling pathways.

In order to be able to investigate biological mechanisms controlling maturation and to use the chicken as an animal model in which pharmacological and/or environmental agents can be screened for potentially harmful effects on brain maturation two non-invasive measures of neuronal function were investigated. One was behavioural (prepulse inhibition: PPI) and the other was electrophysiological (auditory evoked related potentials: AERP).

PPI in the chicken was examined electromyographically and via whole body movement with a stabilimeter apparatus. In two strains of chicken (a meat breed and a laying breed) PPI was identified but shown to be small and variable compared to that in the rat. The results indicate that the phenomenon of PPI in the chicken is too small and variable to be used as a quantitative measure of neural circuit maturation.

Quantitative analysis of the chicken AERP revealed a significant decrease in amplitude of the positive AERP component and a decrease in latency of the negative AERP component with maturation. These maturation changes were comparable to developmental changes seen in human and other mammal AERPs. Such changes may reflect changes in the intracortical synaptic organisation of the auditory cortex. This technique allowed for repeated measures to be undertaken on the same animal over a number of weeks and enabled developmental changes to be monitored.

This technique was extended to investigate perturbed maturation via the induction of chemically induced hypothyroidism. Results from this study showed that the induction of late onset hypothyroidism produces measurable effects on the chicken AERP consistent with perturbation in maturation of neuronal circuits and synapses. This suggests that AERPs may be useful non-invasive functional measures of brain
maturation that can be used to study the effects of endogenous or exogenous factors on brain maturation in the chicken.

Since human brain also exhibits a protracted maturation period the availability of a well characterised animal model for protracted brain maturation provides an opportunity to identify molecules, genes and environmental factors that are important in the regulation of maturation. Such a model may provide the basis for developing rational therapies or prevention strategies for some neurodevelopmental disorders. The protracted maturation of neuronal circuits observed in chicken forebrain offers such a model.