Brain Maturation in Chickens: Biochemical, Behavioural and Electrophysiological Investigations

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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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Abstracts

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Abbreviations

ABR	auditory brainstem response
ACEC	animal care and ethics committee
AERP	auditory event related potential
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid
ANOVA	analysis of variance
ATP	adenosine triphosphate
BCA	bicinchonic acid
BSA	bovine serum albumin
CaMKII	calcium/calmodulin stimulated protein kinase
pT286 CaMKII	CaMKII phosphorylated at Thr 286
cAMP	cyclic 3',5'-adenosine monophosphate
CNQX	cianonitroquinoxaline
CNS	central nervous system
CREB	cAMP response element binding protein
DNQX	6,7-dinitroquinoxaline-2,3-dione
DTAL	discriminative taste avoidance learning
ECL	enhanced chemiluminescence
EDTA	ethylene diamine tetraacetic acid
EEG	electroencephalogram
EGTA	ethylene glycol tetraacetic acid
EMG	electromyograph
ERK	extracellular-signal regulated kinase
ERP	event related potential

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	methylanthranilate
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

PTU	propylthiouracil
RSPI	relative stoichiometry of phosphorylation index
SB	sample buffer
SD	standard deviation
SDS	sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SEM	standard error of the mean
STM	short term memory
SW-R	standard working reagent
T3	triiodothyronine
T4	thyroxine
TBS	tris buffered saline
TBST	TBS containing 0.1% Tween-20
TH	thyroid hormone

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.