

**Neurochemical and Behavioural Factors
Affecting the Sensitive Period for Imprinting**

by

Carl Harold Parsons, B.Sc. (Hons) (UNE)

A thesis submitted for the degree of Doctor of
Philosophy of the University of New England.

March, 1994

Acknowledgments

I am indebted to my supervisor, Professor Lesley Rogers. Lesley's guidance has been invaluable, as has her support. For this I am very grateful.

I would also like to acknowledge Dr. Tim O'Shea who acted as temporary supervisor for six months while Professor Rogers was on study leave.

Part of this work could not have been performed without the generous assistance of Dr. Peter Dodd at the Royal Brisbane Hospital Foundation, who provided the facilities and materials for the radioligand binding assay.

Members of the Brain and Behaviour Laboratory, at the University of New England, Armidale, provided stimulating discussion, friendship and helpful advice. In particular, I would like to thank Amy Johnston, Michelle Hodgkinson and Tom Burne.

Technical assistance from within the department of physiology was available when needed and I am grateful for that. Special mention must go to Allan Rummery and David Creed of the physiology workshop who manufactured the imprinting apparatus.

Saving one of the most important persons for last, I would very much like to thank my special friend, my wife Abi. Her support, encouragement and assistance throughout this period was very important to me.

Abstract

An investigation of the neurochemical properties underlying the sensitive period for imprinting was undertaken by exploring the action of a mixture of an N-methyl-D-aspartate (NMDA) receptor antagonist ketamine (55 mg/kg) and an α_2 -adrenergic agonist, xylazine (6 mg/kg) (KX) on the ability of dark-reared chicks to imprint on day 8 post-hatching. Chicks treated with this mixture at 10, 20 or 40 h after hatching and which were reared in the dark were able to imprint on day 8. Controls treated with saline were unable to imprint on day 8. Similarly, chicks treated with the KX mixture or with saline on day 4 or day 7 were not able to imprint on day 8. Two stimuli were used to imprint the chicks; a rotating stuffed hen, and a rotating red and black box. While day 2 chicks were able to imprint on the hen or the box, groups of day 8 chicks that had been treated with KX at 10, 20 or 40 h after hatching only imprinted if they had been exposed to the hen. Box-exposed groups did not imprint. This is not a predisposition-like effect because KX-treated chicks showed a preference for the hen only if they had previously been exposed to it.

Although most of the experiments used dark-reared chicks, the extension of the sensitive period by KX treatment is not restricted to dark-reared chicks. Light-reared KX-treated chicks were also able to imprint on day 8. However, in contrast to dark-reared KX-treated chicks, light-reared KX-treated chicks could imprint on the box or the hen.

There is good evidence that the NMDA receptor system is involved in producing the extended sensitive period. If chicks were given two injections of ketamine (55 mg/kg at 10 h and again at 12 h post-hatching) they could imprint on a hen on day 8 post-hatching. Similarly, chicks treated with MK-801 (5 mg/kg at 10 h post-hatching), a more potent NMDA receptor antagonist, were also able to imprint on a hen on day 8 post-hatching. Chicks given a single dose of ketamine (55 mg/kg), a single dose of xylazine (6 mg/kg) or two doses of xylazine (6 mg/kg at 10 h and again at 12 h post-hatching) were unable to imprint on a box or a hen on day 8 post-hatching. However, there does

appear to be a synergistic action between ketamine and xylazine because the mixture of KX (a single 55 mg/kg dose of ketamine + a single 6 mg/kg dose of xylazine) did extend the sensitive period.

[³H]-MK-801 binding was used to measure the density of NMDA receptors in the intermediate medial portion of the hyperstriatum ventrale (IMHV) of day 8 saline-treated and KX-treated chicks. The density of NMDA receptors in dark-reared KX-treated chicks that received no visual stimulation was significantly lower than the density of NMDA receptors in similarly reared saline-treated chicks. Eight hours after exposure to an imprinting stimulus on day 8, there was an increase in the density of NMDA receptors in the left and right IMHV's of KX-treated chicks. The increase in density of NMDA receptors was significant in the left IMHV of KX-treated chicks, but was not significant in the right IMHV. Thus, on day 8 the underlying neurochemical mechanisms of an imprinting memory in KX-treated chicks appear to share some similarities with imprinting in chicks aged 1-2 days.

It is suggested that treatment with KX at 10, 20 or 40 h after hatching, or treatment with the two doses of ketamine or MK-801, delays a developmental change in NMDA receptors which forms at least part of the system that is responsible for the ability to form an imprinting memory.

Contents

	Acknowledgments	iii
	Abstract.....	iv
	Contents	vi
	List of Figures	ix
	List of Tables.....	xi
	List of Abbreviations.....	xii
Chapter 1	Introduction.....	1
	1.1 A Pilot Experiment Showing the Effect of Ketamine- Xylazine on the Sensitive Period for Imprinting	2
	1.2 Imprinting and its Sensitive Period.....	6
	1.3 Plasticity in the Visual Cortex.....	23
	1.4 The Intermediate Medial Portion of the Hyperstriatum Ventrale - an Area Known to be Involved in Imprinting	29
	1.5 An Overview of the Present Study.....	40
Chapter 2	General Methods	41
	2.1 Introduction	41
	2.2 Subjects.....	41
	2.3 Injecting the chicks.....	42
	2.4 Dark rearing.....	43
	2.6 Imprinting Methods.....	46
	2.7 Statistical Analysis.....	54
Chapter 3	The Sensitive Period for Imprinting.....	57
	3.1 Introduction	57
	3.2 Methods.....	58
	3.3 Results	58
	3.4 Discussion.....	64
	3.5 Conclusion	69
Chapter 4	An Investigation into the N-methyl-D-aspartate Binding Changes in the Intermediate Medial Portion of the Hyperstriatum Ventrale Following Treatment with Ketamine-Xylazine and Exposure to an Imprinting Stimulus on Day 8.....	70
	4.1 Introduction	70
	4.2 Methods.....	72
	4.3 Results	76
	4.4 Discussion.....	81
	4.5 Conclusion	85

Chapter 5	A Sensitive Period for the Action of Ketamine-Xylazine.....	86
	5.1 Introduction	86
	5.2 Methods.....	86
	5.3 Results	87
	5.4 Discussion.....	96
	5.5 Conclusion	100
Chapter 6	Determining the Neurochemical Factors Involved in the Extension of the Sensitive Period by Ketamine-Xylazine Treatment.....	101
	6.1 Introduction	101
	Experiment 6-1	101
	6-1.1 Methods.....	102
	6-1.2 Results.....	103
	6-1.4 Discussion.....	108
	Experiment 6-2	110
	6-2.1 Methods.....	110
	6-2.2 Results.....	111
	6-2.3 Discussion.....	116
	Experiment 6-3	118
	6-3.1 Methods.....	119
	6-3.2 Results.....	120
	6-3.3 Discussion.....	123
	6.5 Chapter Discussion	124
	6.6 Conclusion.....	128
Chapter 7	Does Ketamine-Xylazine Treatment Induce a Predisposition?.....	129
	7.1 Introduction	129
	7.2 Methods.....	130
	7.3 Results	132
	7.3 Discussion.....	135
	7.4 Conclusion	138
Chapter 8	Extension of the Sensitive Period in Chicks Reared in the Light	139
	8.1 Methods.....	141
	8.2 Results	142
	8.3 Discussion.....	146
	8.4 Conclusion	149

Chapter 9	Effects of Ketamine-Xylazine treatment on behaviours other than imprinting	150
	Experiment 9-1 An investigation into the effect of Ketamine-Xylazine treatment on pebble floor visual discrimination learning	150
	9-1.1 Methods.....	151
	9-1.2 Results	153
	9-1.3 Discussion.....	154
	Experiment 9-2 Open Field Ambulation	158
	9-2.1 Methods.....	158
	9-2.2 Results	159
	9-2.3 Discussion.....	162
	Experiment 9-3 A bias in the turning direction in the imprinting wheels	163
	9-3.1 Methods.....	163
	9-3.2 Results	164
	9-3.3 Discussion.....	165
	9-4 Conclusion	166
Chapter 10	General Discussion	167
References	179

List of Figures

Figure 1-1. Mean \pm SEM percent preference scores of untreated and ketamine-xylazine-treated chicks.	4
Figure 2-1. Diagram showing the timing of events in the imprinting experiments.	41
Figure 2-2. Layout of the dark-rearing room.	43
Figure 2-3. Rearing cage that was used in the dark-room.	44
Figure 2-4. Layout of training and testing room.	47
Figure 2-5. One of the six imprinting wheels that was used in training and testing.	49
Figure 2-6. Day 8 chicks in imprinting wheels approaching an imprinting stimulus during a training session.	50
Figure 2-7. Chick in an imprinting wheel during a testing session.	52
Figure 3-1. Mean \pm SEM percent preference scores of the groups trained on days 2, 4 or 6 post-hatching.	63
Figure 4-1. Schematic diagram of the NMDA receptor.	71
Figure 4-2. Mean \pm SEM percent preference scores of the ketamine-xylazine-treated and the saline-treated groups from which IMHV samples were obtained.	78
Figure 4-3. Mean \pm SEM density of NMDA receptors in the left and right IMHV of saline treated or KX-treated chicks.	80
Figure 5-1. Mean \pm SEM percent preference scores for the groups in the test 1 h after training.	92
Figure 5-2. Mean \pm SEM percent preference scores for the groups in the test 24 h after training.	94
Figure 5-3. Percent preference scores plotted separately for the box and hen-trained chicks in the test 24 h after training.	95
Figure 6-1. Mean \pm SEM percent preference scores in the imprinting test 1 h after training.	106
Figure 6-2. Mean \pm SEM percent preference scores in the imprinting test 24 h after training.	107

Figure 6-3. The mean \pm SEM percent preference scores in the test 24 h after training.	115
Figure 6-4. Percent preference scores of MK-801 and saline-treated chicks tested 1 h and 24 h after training.	122
Figure 7-1. Mean \pm SEM percent preference scores of untrained chicks treated with KX or saline.	134
Figure 8-1. Presents the mean \pm SEM percent preference scores of light-reared chicks imprinted on day 8 and tested 1 h after training.	144
Figure 8-2. Presents the mean \pm SEM percent preference scores of light-reared chicks imprinted on day 8 and tested 24 h after training.....	145
Figure 9-1. Pebble floor test of groups treated with saline, KX, ketamine alone or xylazine alone.	154
Figure 9-2. Mean \pm SEM activity of the chicks in the open field arena.	160
Figure 9-3. Ranked activity in preference tests is plotted against the ranked activity in the open field.	161
Figure 9-4. Mean \pm SEM number of left and right turns in imprinting wheels.	164

List of Abbreviations

[³ H]	tritium
6-OHDA	6-hydroxydopamine
AP5, APV	2-amino-5-phosphonopentanoic acid amino phosphonovalarate
CPP	4-(3(-phosphonoprop-1-yl)piperazine-2-carboxylic acid
dpm	disintegrations per minute
DSP4	N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride
EAA	excitatory amino acid
EPSC	excitatory postsynaptic currents
GABA	gamma amino-butyric acid
h	hour
Hz	hertz
IMHV	intermediate medial portion of the hyperstriatum ventrale
KX	ketamine and xylazine
LSD	least squares difference
LTP	long term potentiation
MK-801	5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine
NA	noradrenaline
NMDA	N-methyl-D-aspartic acid
PCP	phencyclidine
PPR	persistent potentiation of the response
PTP	post tetanic potentiation