

**LMA1****FOS EXPRESSION IN THE HYPERSTRIATUM VENTRALE AND NEOSTRIATUM OF THE CHICK FOREBRAIN AFTER VISUAL IMPRINTING***H.J.E. Jones, R.M. Marsden, and B.J. McCabe\***Department of Zoology, Sub-Department of Animal Behaviour, Madingley, Cambridge CB3 8AA, UK*

The intermediate and medial part of the hyperstriatum ventrale (IMHV) is a restricted region in the chick forebrain that is crucial for the learning process of imprinting. Taken together, the evidence indicates that the IMHV is a site of storage of information about the imprinting stimulus (2). One hour after an hour's training with an imprinting stimulus, there is an increase, related to the strength of visual imprinting, in the number of neurons in the IMHV that are immunopositive for the immediate early gene product Fos. A learning-related change in Fos-like immunoreactivity was also detected in the hyperstriatum accessorium but not the other forebrain regions examined (lateral neostriatum, hippocampus, lobus parolfactorius and hyperstriatal component of the medial neostriatum/hyperstriatum ventrale [MNH]) (3). The MNH has been implicated in auditory imprinting (1). We have enquired whether the change in Fos expression induced in the IMHV by imprinting extends laterally within the hyperstriatum ventrale and whether similar learning-related changes occur in the neostriatal component of the MNH. Measurements were made on sections that contributed to the previous study of Fos expression (McCabe and Horn 1994). Chicks were trained by exposure for 1 h to an imprinting stimulus (a rotating, red box) and the maternal call of a hen. Control chicks remained in darkness. After training, the chicks were given a preference test in which they were exposed, with no maternal call, alternately to the training stimulus and to an alternative stimulus (a rotating, blue box). A preference score (percentage of approach during the test that was directed towards the training stimulus) was assigned to each trained chick as an index of the strength of imprinting to the visual stimulus. Trained chicks were classified as 'good' and 'poor' learners according to whether their preference scores were > or < 65 respectively. The chicks were killed by decapitation 1 h after the end of training and brain sections stained for Fos. Fos-positive nuclei in the lateral hyperstriatum ventrale and neostriatal component of the MNH were counted by computer. Immunocytochemistry and counting were performed blind. In each region examined, there were no significant differences between numbers of Fos-positive nuclei in good learners, poor learners and untrained chicks. The learning-related change in the IMHV does not therefore extend to the adjacent lateral part of the hyperstriatum ventrale. The fact that no effect was detected in the MNH at this time may indicate that changes in the MNH occur after those in the IMHV.

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(1) Braun et al. (1999) *Behav. Brain Res.* 98:211; (2) Horn (1998) *Trends Neurosci.* 21:300; (3) McCabe & Horn, G. (1994) *Proc. Nat. Acad. Sci. USA* 91:11417.

**LMA2****VOCALISATION AND IMPRINTING IN DOMESTIC CHICKS***R. Suge\* and B.J. McCabe**Dept. Zoology, Sub-Dept. Animal Behaviour, Madingley, Cambridge, CB3 8AA, UK*

In the course of experiments investigating the biochemical consequences of imprinting in the domestic chick, we have measured the frequency of distress calls and total vocalisations (Collias, 1952; see Andrew, 1991) during and after imprinting training. We have enquired whether this vocal behaviour, which provides information about an animal's emotional state, is correlated with a measure of learning. Sixty-four chicks were exposed to an imprinting stimulus (rotating red box or rotating blue cylinder) for 15 min. Ninety minutes after the end of the training, the chicks were given a preference test, in which they were exposed alternately to the two stimuli. The strength of imprinting was measured by means of a preference score (the percentage of total approach activity during the test that was directed towards the training stimulus). We counted number of distress calls, total number of calls and ratio of distress calls to total number of calls during training and in three successive 15 min periods after this. Imprinting training for 15 min gave rise to a mean preference score ( $71.63 \pm 4.53$  s.e.m.) that was significantly greater than the 'no preference' value of 50 ( $t_{45} = 4.78$ ,  $P < 0.001$ ), indicating that chicks had learned characteristics of the training stimulus. Counts of vocalisations were subjected to an analysis of variance with factors Training Stimulus (red box, blue cylinder) and Time (0-15 min (training period), 15-30 min, 30-45 min, 45-60 min). Regression analysis was employed to determine whether the measures of vocalisation were related to preference score. There were significant effects of Time on all three measures of vocalisation (distress calls:  $F_{3,182} = 65.35$ ,  $P < 0.001$ , total calls:  $F_{3,182} = 106.20$ ,  $P < 0.001$ , ratio:  $F_{3,179} = 27.79$ ,  $P < 0.001$ ). The time courses of these three measures were different. For the number of distress calls and ratio of distress calls to total number of calls, there was a peak during the 15 min after the end of training followed by a decrease. The total number of calls showed a peak during training and declined thereafter. None of the three measurements showed a significant regression on preference score. No significant interaction of the regression with Training Stimulus or Time was observed. We conclude that, under these experimental conditions, there is no evidence for an association between level of stress and strength of imprinting.

Collias, N. E. (1952) *Auk*, 69, 127; Andrew, R. J. (1991) *Neural and Behavioural Plasticity*, Andrew, R. J. ed., Oxford University Press, Oxford, p8.

**LMA3****THE DEVELOPMENT OF A LEARNING-RELATED BIAS IN VISUAL RESPONSIVENESS IN A RECOGNITION SYSTEM**

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Imprinting is a learning process whereby visually naive domestic chicks when trained by exposure (1-2h) to an object come to recognise that object and subsequently approach it in preference to others. The intermediate and medial part of the hyperstriatum ventrale (IMHV) in the forebrain has been implicated as a memory store for imprinting (1). The responsiveness of IMHV neurons is selectively biased by imprinting:  $\geq 20$ h after training the proportion of neurons responsive to a stimulus is greater in chicks imprinted with that stimulus than in chicks imprinted with another stimulus or in untrained chicks. Here we demonstrate that this bias in responsiveness develops non-monotonically. Chicks were hatched and maintained in a dark incubator to  $\sim 8$ h old. Each chick was anaesthetised (0.12ml Equithesin, i.p.) and two miniature microdrives fitted to the skull to enable a pair of microelectrodes to be positioned within each IMHV (left and right). Next day a chick ( $\sim 24$ h old) was placed in a running wheel and exposed to either a red box ( $n=7$  chicks) or a blue cylinder ( $n=7$  chicks) during two 1h training sessions separated by  $1\frac{1}{2}$ h. Spikes were software-sorted from multiunit activity sampled at each electrode. Single neurons were tested for responsiveness to each stimulus during five tests: T1 (pre-training), T2 (after 1h training), T3 (after 2h training), T4 (3-6h post-training) and T5 (20-23h post-training). Seven untrained chicks received tests T1-T5 but no training. In each chick recordings were from the same neurons throughout T1-T3. After both T3 and T4 chicks rested in the incubator and electrodes were moved  $\sim 100\mu\text{m}$  deeper along their tracks. In trained chicks the proportion of neurons responsive to the training stimulus increased ( $P<0.001$ ) over the period of training: 26/230 (11%) at T1, 57/230 (25%) at T2 and 50/230 (22%) at T3. At T4 this proportion (74/451, 16%) was no longer significantly greater than that at T1. However, at T5 this proportion (90/251, 36%) was again above that at T1 ( $P<0.001$ ). The proportion of neurons responsive to the alternative stimulus or to either stimulus in untrained chicks showed no systematic changes. Of the neurons responsive to the training stimulus at either T1 or T3, the strength of the response increased from T1-T3 (15/71, 21%) for a higher ( $P<0.01$ ) proportion than decreased (4/71, 6%). Thus IMHV responsiveness to the training stimulus changes during training in complex and non-linear ways that cannot be predicted by interpolation from start and end measures.

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(1) Horn (1998) *Trends Neurosci.*, 21:300.

**LMA4****SUSCEPTIBILITY OF PREFERENCE TO MODIFICATION FOLLOWING FILIAL IMPRINTING IN THE DOMESTIC CHICK***J.M. Henderson, R.M. Meredith\*, and B.J. McCabe**Department of Zoology, Sub-Department of Animal Behaviour, Madingley, Cambridge, CB3 8AA, UK*

Filial imprinting is a process by which the young of precocial species learn the characteristics of an object to which they are exposed and subsequently restrict their filial behaviour towards it. After domestic chicks have been trained with an imprinting stimulus, retraining with a second stimulus can reduce or reverse the chicks' acquired preferences (4, 1). This effect is predicted by a neural network model of imprinting (2) and has been studied by Griffiths (3) in the light of the model. We investigated the effect on preference modification of (i) time of introduction of the second stimulus and (ii) visual and auditory components of the second stimulus. In Experiment 1, 24 chicks were given imprinting training for 1 h with a red box or blue cylinder and the maternal call of a hen. The chicks received a preference test in which they were exposed alternately to each stimulus (no maternal call) < 4 h or 10 h or 24 h after training. Strength of imprinting was measured by a preference score (approach to training stimulus as a percentage of total approach during the test). In Experiment 2, 144 chicks were trained for 1 h using the red box and maternal call; < 4 h or 22 h after training, they were exposed for 1 h to the blue cylinder (with either familiar or novel maternal call) or received no further training. The chicks received a preference test 24 h after training. Experiment 1: Curvilinear regression showed a significant effect of time of testing [ $F(1,18) = 5.58, p = 0.030$ , quadratic term]. Mean preference scores at < 4 h and 24 h did not differ significantly from each other and together were significantly greater than the chance level of 50 [ $t(15) = 4.10, p < 0.001$ ]. Combined values at < 4 h and 24 h were significantly lower than the level at 10 h [ $t(18) = 2.53, p = 0.02$ ]. Experiment 2: Exposure to the novel stimulus significantly reduced mean preference score from 76.5 to 57.6 [ $F(1,132) = 35.38, p < 0.001$ ]. Reduction in preference score was similar at both time points [training x time interaction,  $F(1,132) = 0.34, p = 0.56$ ]. The blue cylinder was slightly more effective at reducing preference when accompanied by the novel call than when accompanied by the familiar call [ $F(1,132) = 2.92, p = 0.045$ , 1-tailed comparison]. The results indicate that chicks' preferences are equally vulnerable to modification < 4 h and 24 h after imprinting training.

*Supported by the BBSRC*

(1) Bateson & Bolhuis (1990) *Anim. Behav.* 40:472; (2) Bateson & Horn (1994) *Anim. Behav.* 48:695; (3) Griffiths, D. G. (1998) *PhD dissertation*, University of Cambridge; (4) Salzen, E. A. & Meyer (1967) *Brain Res.*, 205:361.

**LMA5****LATERALISATION OF A DECLARATIVE MEMORY IN THE CHICK AS REVEALED BY AN INCENTIVE-DEVALUATION PROCEDURE***C. Cozzutti<sup>1</sup>\* and G. Vallortigara<sup>2</sup>**<sup>1</sup>Dipartimento di Psicologia Generale, Università degli Studi di Padova, Via Venezia 8, 35131 Padova, Italy and <sup>2</sup>Dipartimento di Psicologia, Università degli Studi di Trieste, Via dell'Università 7, 34123 Trieste, Italy*

Once acquired, information can be stored either as a procedural or as a declarative representation. Recently, evidence that hens form declarative representations has been obtained using a devaluation technique (Forkman, 1997, Abstracts from St. Andrews Meeting "Biological Aspects of Learning"). We tested the ability of young chicks to store information in declarative terms and investigated whether the two sides of the birds' brain play similar roles in encoding/retrieval of such memories. Chicks underwent a learning session of ten minutes, twice a day, consecutively for three days, from day 2 to day 4. During the learning trials, chicks were placed into a large experimental enclosure with two identical food-plates, filled with different type of seeds. The food-plates were positioned in two adjacent corners on the same side of the arena. The location of the plates remained fixed throughout the learning trials, so that a certain kind of seeds was found always in the same position. On day 5 chicks underwent a procedure of devaluation in their home cage : for thirty minutes food-deprived animals were fed ad libitum exclusively one type of seeds and in this way they selectively satiated for that kind of seeds. Chicks were tested in binocular conditions or with one eye temporally closed by an eye patch (due to a complete decussation at the optic chiasma in birds information entering each eye is largely, though not completely, processed by neural structures in the contralateral hemisphere). Both binocular and right-eyed (left eye patched) chicks preferentially approach the food-plates containing the seeds that had not been devaluated. In contrast, chicks using the left eye chose at random. In order to check whether monocular chicks could differ in their motivation to approach the food-plates, the experiment was repeated omitting any devaluation procedure : both right-eyed and left-eyed chicks showed interest and approached both food-plates. These findings demonstrate that 5-day-old chicks are able to encode and store memory information concerning the particular kind of food located in a certain place. Such a memory seems to reside in (or perhaps can be retrieved only from) the left hemisphere.

**LMA6****THE ROLE OF FEAR IN ONE-TRIAL PASSIVE AVOIDANCE LEARNING IN JAPANESE QUAIL CHICKS***S. Richard<sup>1,2\*</sup>, D.C. Davies<sup>2\*</sup>, and J.M. Faure**<sup>1</sup>Station de Recherches Avicoles, INRA, Nouzilly, France and <sup>2</sup>Department of Anatomy and Developmental Biology, St. George's Hospital Medical School, London, UK*

Emotionality has been shown to affect learning in mammals, but little is known about the relationship between fear and learning in birds. Bilateral lesions of the archistriatum, a telencephalic structure involved in fear/escape responses, impair the acquisition of a one-trial passive avoidance learning (PAL) task in domestic chicks. This task relies on the fact that young chicks will spontaneously peck at small, visually conspicuous objects, *e.g.* a bead. If the bead has been coated with a distasteful substance, chicks learn in a single trial not to subsequently peck a similar bead. Therefore, acquisition of the task involves the learned association between the bead and the noxious taste, followed by expression of an avoidance response. Thus, the effect of archistriatal lesions could reflect a reduction in avoidance behaviour rather than an impairment of learning *per se*. In order to investigate this possibility, the performances of quail chicks (*Coturnix japonica*) from lines divergently selected for high or low levels of underlying fearfulness, were compared in a one-trial PAL task. Day-old chicks were housed in pairs of either line and one chick from each pair was presented with a silver-coloured bead, coated randomly with either water or the taste aversant, methyl anthranilate (MeA). If a chick did not peck this training bead within 30 s, or did not exhibit a disgust response after pecking the MeA-coated bead, it was not investigated further. Two hours later, the chicks were presented with a bead similar to the training bead, except that it was dry. If a chick did not peck within 30 s it was recorded as having avoided the bead. Irrespective of the stimulus, significantly more ( $P < 0.001$ ) chicks from the low fear line (77%,  $N=108$ ) pecked the training bead compared to those from the high fear line (53%,  $N=142$ ). However, there was no significant difference in avoidance of the test bead between chicks of the low (61%) and high (75%) fear lines trained on the MeA-coated bead, and thus, they learned the task equally well. Since the selection criteria for the two lines precludes any overlap in levels of underlying fearfulness, the similar performance of the two lines in the test is unlikely to be due to their homogenisation in respect of fearfulness, resulting from the lower training rate of high fear line chicks. These results suggest that the archistriatum plays a direct role in avoidance learning in birds, strengthening the suggestion of a functional homology between the avian archistriatum and the mammalian amygdala.

**LMA7****EPISODIC-LIKE MEMORY IN ANIMALS: THE WHAT, WHERE AND WHEN OF FOOD STORING AND RECOVERY***D.P. Griffiths<sup>1</sup>\*, A. Dickinson<sup>2</sup>, and N.S. Clayton<sup>1</sup>**<sup>1</sup>Section of Neurobiology, Physiology and Behaviour, Briggs Hall, One Shields Avenue, University of California, Davis, California, USA 95616 and <sup>2</sup>Department of Experimental Psychology, University of Cambridge, Cambridge, UK*

Episodic Memory, the encoding and storage of memories concerning unique, personal experience, has long thought to be unique to humans. It provides information about the what, where and when of specific, individual events. The aim of the experiments described here is to provide a working model for the study of episodic memory in animals. In the initial experiments, adult scrub jays were allowed to cache two food types, peanuts and worms, in a trial-unique, compartmentalised tray and were permitted to recover the items either 4 or 124 hours later. Jays preferentially eat worms over peanuts. In the group “Degrade” the worms had perished and become inedible at 124 hours, but in the group “Replenish” the worms were replaced with fresh worms after 124 hours, making edible worms available at both times. When tested with all food items removed to control for olfactory cues, the birds in the “Degrade” group searched preferentially for worms after 4 hours, but reversed this preference and searched in peanut sites after 124 hours. “Replenish” birds, however, preferentially searched in worm sites after both time intervals. These results suggested that the birds could recall a) what they had stored, b) where they had stored it, c) how long ago the item was stored, all on the basis of a one-trial experience. The jays therefore seemed to meet the behavioural criteria for displaying episodic memory. The experiments were controlled so that the result could not be explained in terms of recency or relative familiarity with the trays or food items. Another feature of human episodic memory is that it is the last type of memory capability to develop in childhood. In a second set of experiments, juvenile scrub jays were given the same task as the adults to test whether this episodic-like ability follows a similar developmental time path to that observed in humans. Preliminary results suggest that while the juvenile jays are capable of recalling what is stored where, they cannot initially integrate the temporal component as quickly as the adults. This suggests that there is a difference between juvenile and adult jays in rate acquisition of this episodic-like memory task, suggesting a similarity between the development of episodic memory in birds and humans.

**LMA8****MEMORY FOR “WHAT” AND “WHERE” IN ZEBRA FINCHES***K.H. Sanford\* and N. S. Clayton**Section of Neurobiology, Physiology and Behaviour, Briggs Hall, One Shields Avenue, University of California, Davis, CA 95616, USA*

Many species of animals can remember combinations of information about “what and where”, “when and

where”, and some can even recall “what, where and when”. In mammals, the hippocampus is thought to play an important role in these memories. We have developed a behavioural model in birds for investigating the role of the hippocampus in learning and memory. We have shown that zebra finches can remember where food is located in an experimental tray, irrespective of whether it is cued by hippocampally-dependent spatial stimuli or hippocampally-independent colour stimuli. There is no difference between space-trained birds in either the mean number of looks or in the mean latency to find the hidden food reward. To test the hypothesis that zebra finches can remember “what and where”, we trained zebra finches on a one-trial learning task in which the birds searched for two types of food (egg and millet seed). Each food was hidden under one of seven flaps in one of two different trays. On the spatial version of the task, these two food types were hidden in different spatial locations on the two trays, whereas in the colour version of the task the two foods were hidden under different coloured flaps. Half the birds were trained on the spatial version of the task and half the birds were trained on the colour version of the task. The colour and spatial cues varied from trial to trial to ensure that the combination of “what and where” was unique to each trial. Each trial consisted of two phases, separated by a retention interval of 10 minutes. During phase I the birds searched at random for the hidden food in each tray, and could eat a portion of both food types for 30 seconds. During phase II of the same trial the bird was presented with the same two trays to test for its ability to remember “where” each food was located. Zebra finches prefer egg over millet seed. During phase II of a trial they should preferentially search in the tray in which egg had been hidden if they remember “what” as well as “where”. To ensure that the birds were using memory rather than odour cues, each bird also received probe trials in which no food reward was present during phase II. These behavioural tests of memory may be a useful model with which to test questions about the role of the hippocampus in learning and memory.

## LMA9

### LOCALISED IMMEDIATE EARLY GENE EXPRESSION IN THE ZEBRA FINCH BRAIN IS RELATED TO THE STRENGTH OF SONG LEARNING

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Recent evidence suggests that song perception in songbirds involves neural activation in brain regions that have not traditionally been implicated in the control of song production or song learning, notably the caudal part of the neostriatum (NCM) and of the hyperstriatum ventrale (CHV). We studied the expression of the protein products of the immediate early genes (IEGs) *egr-1* (ZENK) and *c-fos* in the forebrain of zebra finch males (*Taeniopygia guttata*) that were exposed to the song with which they had been tape-tutored previously. Zebra finch males were reared without their fathers. During the sensitive period for song learning (day 35-65 after hatching) they were exposed to a tape recorded song. They were