

Research report

Context-dependent prey avoidance in chicks persists following complete telencephalectomy

Gergely Zachar^{a,*}, Aniko Schrott^{b,1}, Péter Kabai^{b,2}^a Department of Anatomy, Semmelweis University, 58 Tűzoltó u., Budapest 1094, Hungary^b Department of Ecology, Szent Istvan University, 50 Rottenbiller u., Budapest 1078, Hungary

ARTICLE INFO

Article history:

Received 15 August 2007

Received in revised form

23 November 2007

Accepted 11 February 2008

Available online 6 March 2008

Keywords:

Aposematic

Shape

Visual

Unconditional

Colour

Preference

ABSTRACT

Young naïve domestic chicks readily attack green insects and avoid insects painted red but show no discrimination of the same colours when applied to fruit-like objects, a discrimination that has been depicted as context-dependent preference. To study the neural representation of such preference we performed bilateral telencephalectomy on 1-day-old domestic chicks and tested them on an unlearned prey discrimination paradigm. Here we show that following complete decerebration, young domestic chicks preferentially peck at red fruit versus red insects and tend to choose green insects over green fruit indistinguishably from unoperated chicks. The present study provides the first direct evidence that sophisticated context-dependent, unlearned colour preference is processed by subtelencephalic areas of an amniote species.

© 2008 Elsevier Inc. All rights reserved.

1. Introduction

Nutrients of the yolk sac provide newly hatched domestic chicks a few days to learn to choose the right objects to feed themselves. Such rapid learning might be enhanced by unlearned avoidance of poisonous insects or unconditional preference for palatable fruits. As the signal for such conflicting qualities is often conveyed by the same conspicuous red colouration [10,26], colour itself cannot be a universal signal of profitability for an omnivorous bird. Indeed, Gamberale-Stille and Tullberg showed that 3-day-old naïve domestic chicks process colour information in a context-dependent manner: while they readily attack green insects and avoid insects painted red, chicks show no discrimination of the same colours when applied to fruit-like objects [9].

Coloured stimuli are applied to associate visual and taste information in the passive avoidance-learning paradigm [25], an effective model system to study memory formation. As genetically determined and acquired preferences often interact [18], it

is important to localize critical brain regions mediating context-dependent colour and shape preferences. Information on the visual processing of aposematic stimuli is also important to understand the evolution of aposematic signals [31]. It is argued that the brain evaluates high-level visual information by a slow “seeing system” of the telencephalon, whereas stereotypic responses to simple stimuli are mediated by a fast “on-line” system [24], positioned wholly or partially in subtelencephalic areas. Swards and Swards [28] suggested that innate stimulus recognition and the pathway that triggers adaptive behavioural response are located caudal to the telencephalon and homologous among tetrapods. The main areas responsible for such processing are likely the optic tectum, the pretectal areas and the brainstem reticular formation as an executive centre [28] (Fig. 1). According to the Avian Brain Circuitry Database the connectivity of the three areas in birds is similar to those of other vertebrates [27], therefore their function is also likely similar. Unfortunately, the number of studies supporting functional homology between tectal and pretectal descending pathways of avian species and other vertebrates is limited [28].

To reveal if any of the telencephalic areas play a role in unconditioned visual preferences we performed complete telencephalectomy [16] (Fig. 1) on newly hatched chicks, instead of sacrificing a large number of animals by performing serial lesions of specific telencephalic regions [5]. In pilot studies we found that that decerebration causes gradual shift in wavelength preference.

* Corresponding author. Tel.: +36 1 459 1500; fax: +36 1 215 5158.

E-mail addresses: gzachar@gmail.com (G. Zachar), aniko.schrott@gmail.com(A. Schrott), peter.kabai@gmail.com (P. Kabai).¹ Tel.: +36 1 478 42 32; fax: +36 1 478 42 32.² Tel.: +36 1 478 42 34; fax: +36 1 478 42 32.

Table 1
Number of pecking and non-pecking individuals and the latency of the first peck on stimuli according to the colour of presentation (red and green), surgery (TEL-X and UNOP) and the choice of the chicks

	Red		Green	
	TEL-X	UNOP	TEL-X	UNOP
Pecking chicks (<i>n</i>)	15	14	11	16
Non pecking chicks (<i>n</i>)	9	2	14	1
Latency of the insect choice (<i>s</i> , mean \pm S.E.M.)	11.3 \pm 4.8 (<i>n</i> = 3)	81.5 \pm 78.5 (<i>n</i> = 2)	86.7 \pm 23.1 (<i>n</i> = 7)	88.7 \pm 22.3 (<i>n</i> = 10)
Latency of the fruit choice (<i>s</i> , mean \pm S.E.M.)	91.4 \pm 22.6 (<i>n</i> = 12)	208.3 \pm 33.9 (<i>n</i> = 12)	115.8 \pm 27.0 (<i>n</i> = 4)	75.7 \pm 19.6 (<i>n</i> = 6)

To eliminate the effect of such changes we presented objects of the same colouration but different shape simultaneously.

2. Materials and methods

Male domestic chicks (*Gallus gallus domesticus*) purchased from a commercial hatchery (Kamaras KFT, Mogyorod, Hungary) were transferred into the laboratory 3 h following hatching. All experiments were approved by the ethical committee of the Semmelweis University and the procedures were in harmony with the EC Council directives on laboratory animals (86/609/EEC). On hatch day chicks were either operated under deep ketamine–xylazine anaesthesia (TEL-X group, *n* = 32) by removing both hemispheres of the telencephalon by aspiration [17] or left intact as controls (UNOP, *n* = 33). Five out of 32 operated chicks died during or after the surgery. Chicks were kept in a dark incubator at 37 °C for 24 h then placed under 60 W incandescent light bulbs. To limit any effect of experience with food particles on the preference test all chicks were handfed through a plastic tube directly inserted to the crop. Pecking preference was tested with stimuli of two shapes with two colours. For fruit-like stimuli pastry spheres with a diameter of 3.8–4.5 mm were made of flour, margarine and water. For insect stimuli 6.1–7.7 mm long, 1.9–2.1 mm wide African cricket larvae (*Gryllus bimaculatus*) were used. All stimulus items were painted red or green following the methods of Gamberale-Stille and Tullberg [9] using children's paint, Gouache tempera (Color and co., Lefranc and Bourgeois, Le Mans, France). Red insect and red fruit or green insect and green fruit were glued 20 mm apart on pieces of 20 mm \times 20 mm white cardboard positioned in transparent plastic boxes (CD holders). Albeit the overall size of the insects was generally larger than that of the fruit stimuli, the area of the painted surface was approximately the same on the two presented objects. On the third or fourth day of age chicks to be tested were put in a 21 cm \times 26 cm rectangular wooden box in the company of a bird fed before testing. Following a 3 min adaptation period, pairs of green insect and green fruit or red insect and red fruit were presented. The insects and fruits were randomly presented on the left and right. If a chick did not respond within 5 min the test was repeated 2 h later. Latency of the first peck, laterality (left or right), and the object (fruit or insect) pecked at were recorded. Control chicks were tested only once. As decerebrated chicks form no memory trace of pecking, all but five operated birds were tested twice, on the third and fourth day for colours new to them so as to reduce the number of animals to be lesioned. Extent of lesion (Fig. 1) was

determined after standard Nissl staining procedure [17]. The whole telencephalon with small and irregular exceptions of parts of the septum and the caudalmost parts of the olfactory tubercle was ablated bilaterally (Fig. 1). χ^2 tests for goodness of fit were used to determine whether the stimulus choice was different from random, and χ^2 tests for homogeneity were applied to compare the TEL-X and UNOP individuals or red and green presentations. The latencies of the first pecks at the two stimuli were compared by univariate ANOVA with the colour of stimuli and the surgery as independent factors and the choice of the shapes as covariate. Birds that did not peck during the total 600 s of experiments were excluded from the analysis.

3. Results

There was no significant difference among the proportion of pecking and non-pecking individuals according to the colour of presentation either in TEL-X ($\chi^2 = 1.68$, *p* = 0.195) or in UNOP chicks ($\chi^2 = 0.44$, *p* = 0.509) (Table 1). Effects of surgery, colour of stimuli, and shape choice of chicks on the latency of the first peck at one of the stimuli were analyzed by ANOVA (*F* = 3.64, d.f. = 3, *p* = 0.019). Decerebration was the only significant main factor effecting latency to peck (*F* = 4.91, d.f. = 1, *p* = 0.031, Table 1). Neither the colour of the stimuli (*F* = 1.04, d.f. = 1, *p* = 0.31, Table 1) nor the shape chosen by chicks (*F* = 2.58, d.f. = 1, *p* = 0.12, Table 1) had significant effect on the latencies. Both the UNOP and the TEL-X chicks often pecked at the floor or the wall of the chamber before pecking at one of the stimuli. Some of the UNOP but none of the TEL-X chicks showed freezing or stepped back away from the plastic tray when presented. Such fright reaction exhibited by control birds seemed to be responsible for most of the differences in the latency. Laterality of stimulus presentation had no significant effect on the choices (Table 2).

In the red fruit–red insect discrimination test more chicks pecked at the fruit than at the insect and the difference was significant in both groups (tests for goodness of fit; TEL-X: $\chi^2 = 5.4$, *p* = 0.02; UNOP: $\chi^2 = 7.14$, *p* = 0.008, Fig. 2). In the green fruit–green insect choice situation chicks of both groups apparently pecked at the green insect more readily, however, choices were not different from random (TEL-X: $\chi^2 = 0.82$, *p* = 0.366; UNOP: $\chi^2 = 1.0$, *p* = 0.317, Fig. 2). The performance of decerebrated chicks was indistinguishable from controls in both test situations (red–red choice: $\chi^2 = 0.166$, *p* = 1; green–green choice: $\chi^2 = 0.004$, *p* = 1, Fig. 2).

4. Discussion

After bilateral telencephalotomy the chicks were capable to recognize small objects and selectively peck on them (Fig. 2). Many of the TEL-X chicks showed lower level of activity which caused them not to peck on the presented stimuli (Table 1), however those TEL-X chicks which pecked, tended to respond even quicker than UNOP animals (Table 1).

Table 2
No effect of lateral position on the choice of chicks

Choice	TEL-X		UNOP	
	<i>n</i>	Statistics	<i>n</i>	Statistics
Left	15	$\chi^2 = 0.615$	13	$\chi^2 = 0.533$
Right	11	<i>p</i> = 0.557	17	<i>p</i> = 0.465

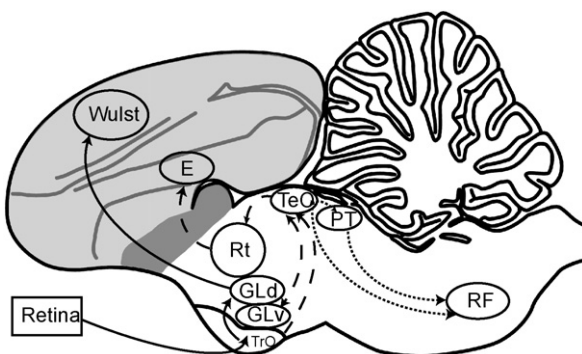


Fig. 1. Major visual areas and the extent of lesions in the brain. The telencephalic areas of the thalamofugal (solid line) and the tectofugal pathways (dashed line) were completely ablated. The lesion covered the entire telencephalon (light grey area) in all TEL-X subjects. To avoid damage to the underlying thalamic, hypothalamic areas, a thin layer (dark grey area) of the caudal part of the septum and olfactory tubercle was saved resulting in some variation of the extent of ablation. The dotted line represents the pathway responsible for the unlearned stimulus recognition as suggested by Swards and Swards [28]. E: nucleus entopallialis, GLd: nucleus geniculatus lateralis pars dorsalis principalis, GLv: nucleus geniculatus lateralis pars ventralis, Pt: pretectal area, RF: reticular formation of the mesencephalon and brainstem, TrO: nucleus rotundus, TeO: tectum opticum, and TrO: tractus opticus.

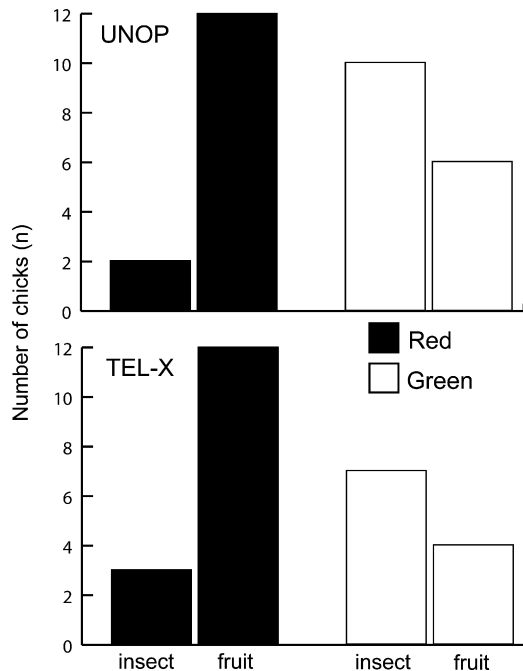


Fig. 2. Number of decerebrated (TEL-X) and control (UNOP) chicks pecking at the insect or fruit stimulus in the green–green (black) and red–red (white) shape choices.

Pecking at red fruits versus red insects might indicate either red fruit preference or red insect dispreference. However, in an earlier study chicks exhibited no preference for red fruit-shaped stimuli when presented together with green fruits [9], therefore our present results together with data of Gamberale-Stille and Tullberg [9] suggest unlearned red insect avoidance by young chicks.

Although previous studies have shown that decerebrated chicks are capable of pecking at small targets [17], the present results are the first to indicate that subtelencephalic areas suffice for sophisticated discrimination between possible food objects in birds. Neural mediation of such discrimination is not known [28]. In all amniotes studied so far visual input is transferred via two pathways, the tectofugal and thalamofugal systems in birds, or the, respectively homologous extrageniculocortical and geniculocortical pathways in mammals [29] (Fig. 1). Subtelencephalic visual areas projecting massively to telencephalic regions (nucleus geniculatus lateralis pars dorsalis, GLd, and nucleus rotundus) are unlikely candidates for coding context-dependent preference, as colour discrimination was only slightly impaired following lesions in GLd [3,4] or nucleus rotundus [4]. Furthermore, both regions suffer significant cell loss after telencephalectomy [2,17]. The existence of pathways originating from TeO to the premotor and motor centres of the medial medullary reticular formation and the ventral horn of the spinal cord (for a review, see Swards and Swards [28]), and its reciprocal connection to the GLv, an area containing colour sensitive neurons [22] make TeO a likely candidate to process complex visual information and trigger adequate motor responses (Fig. 1).

Subtelencephalic areas suffice for accurate prey stimulus recognition in Anurans, the only tetrapods in which the capacity to generate complete adaptive behaviour by the tectum opticum was studied directly [6,7,8,12,13]. However, the similarities between anurans and mammals in electrophysiological responsiveness to configurational stimuli and connectivity of the optic tectum or its mammalian homologue, the superior colliculus (SC) [21,27], suggest that the function of subtelencephalic structures in vision is evolutionarily conservative. The possibility that mammalian SC or the pulvinar play a role in processing complex visual or even

multisensory stimuli in a cognitive manner was raised recently [1,11,15,19,29,30]. It is possible that the visual responsiveness to perceptual characteristics (e.g., wavelength or shape) of visual stimuli in cortically blind humans is a result of the function of subtelencephalic visual centres such as TeO [20].

The present study is the first showing that an amniote species is able to recognize and discriminate natural food objects without telencephalon and the first study showing that a decerebrated vertebrate discriminates between colours and shapes in a context-dependent manner. In conclusion a complete system which includes (1) complex stimulus recognition, (2) decision making, and (3) the activation of adaptive behavioural responses, seems to be located caudal to the telencephalon. Our results together with evidence on high degree of homology in brain structures and intellectual capacity between birds and mammals [14] suggest that studies aiming to investigate the higher neural correlates of visual recognition and discrimination in mammals should not neglect the processing ability of the subtelencephalic visual areas [23].

Conflicts of interest

The authors claim no conflict of interest.

Acknowledgement

This work was supported by OTKA grant T-033069.

References

- [1] L.A. Benevento, D. Port, Single neurons with both form/color differential responses and saccade-related responses in the nonretinotopic pulvinar in the behaving macaque monkey, *Vis. Neurosci.* 12 (1995) 523–544.
- [2] S.M. Cerutti, S. Diaz-Cintra, L. Cintra, E.A. Ferrari, Operant discriminative learning and evidence of subtelencephalic plastic changes after long-term decerebration in pigeons, *Neural Plast.* 10 (4) (2003) 247–266.
- [3] L.M. Chaves, W. Hodos, O. Gunturkun, Color-reversal learning: effects after lesions of thalamic visual structures in pigeons, *Vis. Neurosci.* 10 (6) (1993) 1099–1107.
- [4] A. Csillag, P. Kabai, J.K. Kovach, Effects of diencephalic lesions on approach responses and color preferences in quail, *Physiol. Behav.* 58 (4) (1995) 659–667.
- [5] D.C. Davies, Lesion studies and the role of IMHV in early learning, in: R.J. Andrew (Ed.), *Neural and Behavioural Plasticity: The Use of the Domestic Chick as a Model*, Oxford University Press, Oxford, UK, 1991, pp. 329–343.
- [6] J.-P. Ewert, Der Einfluß von Zwischenhirndefekten auf die Visuomotorik im Beute- und Fluchtverhalten der Erdkröte (*Bufo bufo L.*), *Z. Vergl. Physiol.* 61 (1968) 41–70.
- [7] J.P. Ewert, H. Buxbaum-Conradi, F. Dreisvogt, M. Glasgow, C. Merkel-Harff, A. Röttgen, E. Schürg-Pfeiffer, W.W. Schwippert, Neural modulation of visuomotor functions underlying prey-catching behaviour in anurans: perception, attention, motor performance, learning, *Comp. Biochem. Physiol. A* 128 (2001) 417–461.
- [8] J.-P. Ewert, E. Schürg-Pfeiffer, W.W. Schwippert, Influence of pretectal lesions on tectal responses to visual stimulation in anurans: field potential, single neuron and behavior analysis, *Acta Biol. Hung.* 47 (1996) 89–111.
- [9] G. Gamberale-Stille, B.S. Tullberg, Fruit or aposematic insect? Context-dependent colour preferences in domestic chicks, *Proc. Biol. Sci.* 268 (1485) (2001) 2525–2529.
- [10] T. Guilford, Evolution of aposematism, in: D.L. Evans, J.O. Schmidt (Eds.), *Insect Defences*, State University of New York Press, Albany, NY, 1990, pp. 23–61.
- [11] G.D. Horwitz, A.P. Batista, W.T. Newsome, Representation of an abstract perceptual decision in macaque superior colliculus, *J. Neurophysiol.* 91 (5) (2004) 2281–2296.
- [12] D. Ingle, Two visual systems in the frog, *Science* 181 (1973) 1053–1055.
- [13] D. Ingle, Detection of stationary objects by frogs (*Rana pipiens*) after ablation of the optic tectum, *J. Comp. Physiol. Psychol.* 91 (1977) 1359–1364.
- [14] E.D. Jarvis, et al., Avian brains and a new understanding of vertebrate brain evolution, *Nat. Rev. Neurosci.* 16 (2) (2005) 151–159.
- [15] M.H. Johnson, Subcortical face processing, *Nat. Rev. Neurosci.* 6 (2005) 766–774.
- [16] P. Kabai, J.K. Kovach, Subtelencephalic visual discrimination in selected lines of Japanese quail, *Neuroreport* 4 (3) (1993) 255–258.
- [17] P. Kabai, J.K. Kovach, Persistence of approach response after decerebration in newly hatched quail chicks, *Physiol. Behav.* 53 (4) (1993) 699–707.
- [18] J.K. Kovach, P. Kabai, Effects of bilateral hemispherectomy on genetically variable stimulus preferences and imprinting in quail chicks, *Brain Res.* 629 (2) (1993) 181–188.

- [19] R.J. Krauzlis, D. Liston, C.D. Carello, Target selection and the superior colliculus: goals, choices and hypotheses, *Vis. Res.* 44 (2004) 1445–1451.
- [20] S.E. Leh, H. Johansen-Berg, A. Ptito, Unconscious vision: new insights into the neuronal correlate of blindsight using diffusion tractography, *Brain* 129 (2006) 1822–1832.
- [21] G. Manteuffel, S. Fiseifis, Configuration-sensitive visual responses in the superior colliculus of the house mouse (*Mus musculus domesticus*), *Brain Behav. Evol.* 35 (1990) 176–184.
- [22] H.R. Maturana, F.J. Varela, Color-opponent responses in the avian lateral geniculate: a study in the quail (*Coturnix coturnix japonica*), *Brain Res.* 247 (2) (1982) 227–241.
- [23] B. Merker, Consciousness without a cerebral cortex: a challenge for neuroscience and medicine, *Behav. Brain Sci.* 30 (1) (2007) 63–81.
- [24] D. Milner, M. Goodale, *The Visual Brain in Action*, Oxford University Press, Oxford, 1995.
- [25] S.P. Rose, God's organism? The chick as a model system for memory studies, *Learn. Mem.* 7 (1) (2000) 1–17.
- [26] H.M. Schaefer, V. Schmidt, Detectability and content as opposing signal characteristics in fruits, *Proc. R. Soc. Lond. B* 271 (Suppl.) (2004) S370–S373.
- [27] A. Schrott, P. Kabai, ABCD: a functional database for the avian brain, *J. Neurosci. Methods* 167 (2) (2008) 393–395.
- [28] T.V. Sowards, M.A. Sowards, Innate visual object recognition in vertebrates: some proposed pathways and mechanisms, *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 132 (4) (2002) 861–891.
- [29] T. Shimizu, H.J. Karten, The avian visual system and the evolution of the neocortex, in: H.P. Zeigler, H.-J. Bischof (Eds.), *Vision, Brain and Behavior in Birds*, MIT Press, Cambridge, MA, 1993, pp. 103–114.
- [30] B.E. Stein, Neural mechanisms for synthesizing sensory information and producing adaptive behaviors, *Exp. Brain Res.* 123 (1998) 124–135.
- [31] M. Stevens, Predator perception and the interrelation between different forms of protective coloration, *Proc. Biol. Sci.* 274 (1617) (2007) 1457–1464.