INTRODUCTION

In 2004, the genome of the chicken was published in Nature (International Chicken Genome Sequencing Consortium., 2004). It was truly an international effort with more than 170 authors and contributors to the sequence of Gallus gallus. The first 11 authors were the overall coordinators, and one individual who deserves recognition in this effort is Jerry B. Dodgson. Dodgson spearheaded the coordinated task to write the White Paper to initiate federal funding for the project. Once funded, Dodgson served to distribute molecular tools that enabled researchers to refine the sequence as well as identify genes and genomic regions associated with phenotypic traits. It is important to note that the chicken was the first avian species and first domestic animal species to have its genome sequenced. It will continue to be a most useful resource that will be updated and available online to scientists around the world.

Key words: pallium, subpallium, basal ganglia, septum, central and medial extended amygdala

KEYNOTE LECTURE

Research advances made in the avian brain and their relevance to poultry scientists

Wayne J. Kuenzel

Department of Poultry Science, University of Arkansas, Fayetteville 72701

ABSTRACT The year 2014 marked the tenth anniversary since the sequence of the chicken genome was published. Two other publications occurred during that time frame in different disciplines, and all 3 have affected poultry scientists. The purpose of this paper is to briefly review 2 publications that are better known to those in animal agriculture. The third paper will be addressed in more detail because it is one that many in poultry science probably have not read. The subject matter involves the avian brain and its future impact and is related to an announcement made by the president of the United States in April 2013. Due to the recent, rapid advances in the understanding of the vertebrate brain and behavior, a national goal was announced by President Obama to map the human brain in more detail than ever before to accelerate the understanding of brain function in health and disease. The main objective is to review the third paper published a decade ago to show that it laid the foundation for the chicken and other avian species to serve as relevant animal models to advance the understanding of the human brain. Emphasis will be placed on the forebrain. The overall goal is to show that the brain of birds is not that different from the mammalian brain and therefore can serve as an excellent comparative biomodel to understand fundamental principles of brain structure and function.

Key words: pallium, subpallium, basal ganglia, septum, central and medial extended amygdala

Received August 19, 2014.
Accepted September 29, 2014.

1 Presented as the World’s Poultry Science Association (WPSA) Keynote Lecture scheduled during the Poultry Science Association’s annual meeting in Corpus Christi, Texas, July 14 to 17, 2014. This lecture was recorded, and images referred to in the text can be viewed by accessing the Poultry Science Association website: http://poultry-science.org/psa14/recordings.asp?vid=m.
2 Corresponding author: wkuenzel@uark.edu

© 2014 Poultry Science Association Inc.

http://dx.doi.org/10.3382/ps.2014-04408

2014 Poultry Science 93:2945–2952
journal Poultry Science. The paper is truly a Citation Classic.

A third paper, the subject of this review, is probably unfamiliar to many in the field of poultry science. It is related to a future national goal that was announced in April 2013 by President Obama. He called it the BRAIN (Brain Research through Advancing Innovative Neurotechnologies) Initiative to accelerate development and application of new technologies to map the human brain in more detail than ever before to explore and understand how the brain functions. The BRAIN Research Advisory Committee worked on formulating the goals and costs of the proposed decade-long national project and on June 5, 2014, published the “BRAIN Initiative: A Scientific Vision.” One of the needs was to identify model organisms that would be essential to accomplish its goals. Does the third publication relate to the proposed human BRAIN Initiative? Indeed, the objective of this paper is to hopefully convince the reader that the chicken and other avian species are competitive, biological models for this initiative to accelerate the understanding of human brain function. The paper (Reiner et al., 2004) had contributions from 28 authors and was published in the Journal of Comparative Neurology. Its title is “Revised nomenclature for avian telencephalon and some related brainstem nuclei.” One might ask, what is so important about changing names in the avian forebrain?

An issue that remained unresolved in the avian literature was the persistent use of inappropriate terms for avian brain structures, particularly in the forebrain, due to outdated assumptions of homology to mammalian structures (Reiner et al., 2004; Jarvis et al., 2005). The following sections will highlight the issue, review recent breakthroughs in the literature related to avian brain structures and functions, and convince the reader that the avian brain is not that different from the mammalian brain and could prove an exceptional biomodel for the BRAIN Initiative.

MAMMALIAN 6-LAYERED CORTEX
COMPARABLE WITH THE AVIAN PALLIUM

Importance of Names for Identifying Anatomical Structures

Names are critical not only for giving us the ability to recognize individuals and research our family genealogy, but also to communicate effectively on a daily basis. In science in particular, names have specific meanings and in the past great efforts were taken to standardize scientific terms. Names were encouraged to be composed of Greek and Latin parts to improve international communication. Additionally, when structures from different phylogenetic groups were shown to share a common ancestry or homology, then particular structures in those animal groups would eventually share similar names. The process gave scientists the ability to not only give structures unique terms, but also to provide the option to use similar names for structures when common ancestry or homology was shown to exist among various groups of organisms.

It was apparent for decades that names describing anatomical structures in the avian brain, particularly its forebrain or cerebrum, were inappropriate and negatively affected communication among comparative neuroanatomists. The heart of the issue was the use of the term striatum throughout major anatomical regions of the avian cerebrum. The names suggested that a typical avian forebrain was composed of a huge basal ganglia (BG) with only a few areas having cortical-like structures (e.g., the hippocampus and piriform cortex). Over the past few decades, data strongly suggested that mammals were not unique among vertebrates in possessing cortical-like tissue. Methods applicable to neurobiology became available particularly for the use of specific antibodies in the technique of immunohistochemistry, which helps identify the phenotype or function of neuronal groups. Tract-tracing procedures confirmed the connectivity of neurons in many vertebrate species. Importantly, the combination of neuroanatomical and careful behavioral observations in several avian species supported the view that a significant proportion of the avian forebrain was cortical like. Various behavioral studies demonstrated that birds possessed the ability to learn and to retain experiences and therefore neural structures had to be present to enable cognitive functions to be expressed by avian species. Clearly the nomenclature of the avian forebrain that persisted for decades needed to be changed and streamlined.

Tony Reiner and the Thinktank; Erich Jarvis and the Nomenclature Forum

In 1997, Tony Reiner initiated the “Thinktank” (other members: A. Csillag, W. Kuenzel, L. Medina, L. Puelles, G. Striedter, M. Wild) to begin the process of collecting data from the literature. This would be used to determine which forebrain structures need to be renamed to reflect better the comparative, multidisciplinary data currently available. Erich Jarvis joined the group in 2000 and presented the idea of organizing an Avian Nomenclature Forum composed of comparative neurobiologists who would meet face-to-face and vote on particular name changes to structures throughout the entire avian forebrain. He, among others, wrote a proposal and obtained supporting letters from willing participants to support a 3-D meeting at Duke University during the late summer of 2002. Federal funding was obtained from the National Institutes of Health and National Science Foundation (NSF) to support what turned out to be a structured, cordial, but intense meeting. During this time, all current names within the cerebrum were discussed, data were presented, and a specific name change was voted upon for each one. At the end of the forum it was agreed to spend the next
Evidence that Birds Possess a Cortical-Like Structure

Immunohistochemical evidence showed the location of dopaminergic terminal fields in the forebrain and that their cell bodies resided in midbrain structures (Figure 2A-D, Figure 4F in Reiner et al., 2004). Data clearly demonstrated that, similar to mammals, the BG of birds only occupied the mediobasal, core region of the forebrain, labeled as the striatal part of the BG. An antibody to tyrosine hydroxylase served as a marker of the major terminal field of dopaminergic neurons in the dorsal region of the BG. The substantia nigra, previously termed the nucleus tegmenti pedunculopontinus pars compacta, was shown previously to be the source of dopamine neurons in the avian midbrain that projected to the striatal region of BG (Kitt and Brauth, 1986). The straightforward evidence was quite convincing that neural structures dorsal and lateral to the BG must be avian cortical-like brain regions. From that image (Figure 2B, Reiner et al., 2004) and using sagittal sections from the pigeon and zebra finch, the brain region above and lateral to the BG was estimated to be 75% of the telencephalon, quite comparable with the estimated forebrain volume occupied by a mammalian cortex (Jarvis et al., 2005). The brain region could not be called cortex since, by definition, the mammalian cortex is composed of 6 cell layers and the avian outer telencephalic regions show no evidence of multiple layers, but rather have neurons arranged in groups or nuclei. A change voted upon by members of the Nomenclature Forum was to use the term pallium for this brain region. The term pallium means an outside covering. Thereafter, the descriptor striatum was eliminated from any structures in this region of the telencephalon.

Seminal neuroanatomical and behavioral research strongly supported the presence of extensive pallial regions of the avian forebrain. Neurogenesis was documented in the adult songbird forebrain (Goldman and Nottebohm, 1983), particularly the higher vocal center (HVC) of males that periodically learn new songs used in courtship behavior (Alvarez-Buylla et al., 1988). Subsequent research with molecular probes and the use of tract-tracing techniques revealed the well-known song acquisition and production pathways (Nottebohm, 2005). The HVC and components of the song pathways occupied pallial regions of the telencephalon. Other behavioral evidence similarly supported cortical-like brain regions. Migratory birds in north temperate zones migrate north during the spring to breed and reproduce the next generation. Hatched young will migrate south before the onset of winter. Sometime during the following spring, the first-year birds and others will migrate north and remember the approximate location of their birth place, and defend and use that space for reproductive purposes. A special term called ortstreue (place faithfulness) is in the ornithological literature based on banding records documenting birds not only returning to their original breeding grounds, but also wintering quarters (Moreau, 1969). Cortical-like structures are necessary to maintain and retrieve that spatial memory. Other examples of learning have been shown in chickens, some showing an ability to play and beat a human opponent in a game of tic-tac-toe, although there have been issues of birds being “coached” with discretely shown lights throughout some games at county fairs. Toolmaking, a skill thought to be restricted to mammals, has been demonstrated in Caledonian crows (Weir et al., 2002). African gray parrots, with considerable training, were shown capable of learning human words and using the learned words to communicate with a person (Pepperberg, 1999).

The Avian Pallium Contains Functional Columns Similar to Those in Mammalian Cortex

Another characteristic of the mammalian cortex was the finding of functional columns (Mountcastle, 1998). Regardless of whether a somatosensory, visual, or auditory pathway was identified in a particular cortical region of a mammal, a consistent organization revealed was functional columns of cells oriented perpendicular to the 6-layered cortex. Two recent, exciting findings have documented functional columns in the pallium of an avian auditory and visual pathway. Briefly, an earlier paper, using tract-tracing techniques, described for the first time the auditory pathway of a bird beginning with the cochlea, projecting to a caudal brainstem nucleus, midbrain region, thalamic nucleus, and ending in a pallial region of the forebrain called Field L (Boord, 1969). Examination of Field L in detail showed that columns of cells were shown having connectivity, providing strong evidence that functional columns exist in the auditory pallium comparable to functional columns of cells characteristic of the mammalian neocortex (Figure 2 in Wang et al., 2010). Similarly, tract tracing procedures revealed visual pathways in the bird consisted of 2 major parallel systems: the tectofugal and
the Avian Subpallium

To Examine Structures and Functions

Request To Continue the Effort

Five Neural Systems Comprise the Avian Subpallium

A schematic diagram (Figure 1 in Kuenzel et al., 2011) showed that 5 neural systems comprise the avian subpallium including the dorsal somatomotor BG, ventral viscerolimbic BG, extended amygdala and bed nuclei of the stria terminalis, basal telencephalic corticopetal system, and septum and septal neuroendocrine system. Developmental, structural, and functional data associated with each system are detailed in that publication. The 5 neural systems are summarized below.

(1) Dorsal Somatomotor BG. The dorsal somatomotor BG is involved in the control of voluntary motor movements. A terminal field consisting of dopaminergic input into the lateral and medial striatum of the dorsal BG originates from dopamine neurons in the substantia nigra (Figure 5C; Figure 6A,C in Kuenzel et al., 2011). Extensive tract-tracing and immunohistochemical studies showed a high conservation of BG structure among vertebrates (Reiner et al., 1998). Importantly, the comparative data of structures, neurotransmitters, and neuropeptides produced and function of the BG revealed that both mammals and birds have a direct pathway that facilitates body movement and an indirect pathway that functions to inhibit unwanted body movements. Data demonstrate a marked similarity of this neural system in the 2 groups of vertebrates (Reiner et al., 1998). Degeneration of dopaminergic neurons in the substantia nigra has been shown to lead to Parkinson’s disease in humans. The high similarity of dopaminergic inputs into the dorsal somatomotor BG of birds and the same 2 pathways identified regulating body movement continue to make avian species very useful models for investigating Parkinson’s disease.

(2) Ventral Viscerolimbic BG. The main function of the ventral viscerolimbic BG in birds, similar to mammals, appears to be involved in reward and reward-motivated learning. Similar to the dorsal somatomotor BG, a major source of neurotransmitter that enters the ventral viscerolimbic BG is dopamine, which is produced in a midbrain nucleus called the ventral tegmental nucleus (VTA, Figure 6A,B in Kuenzel et al., 2011). Another major neuropeptide that is released into structures identified with the ventral viscerolimbic BG is substance P (Figure 8 in Kuenzel et al., 2011). A key structure in this neural system is the nucleus accumbens (nAc). It can be distinguished in birds using antibodies to substance P, tyrosine hydroxylase, dopamine, and cyclic adenosine monophosphate-regulated phosphoprotein and neuropeptide Y (Bálint and Csillag, 2007; Figure 9 in Kuenzel et al., 2011). In mammals there is evidence that food intake results in extracellular release of dopamine in the nucleus accumbens, measured by microdialysis. Similarly, a microcannula directed to the nucleus accumbens in rats where amphetamine or cocaine could be self-administered also resulted in significant increases in extracellular dopamine. This suggests that the nAc and dopamine are associated with reward and reward-seeking behavior (Hernandez and Hoebel, 1988). In birds, estrogen-primed female sparrows showed significant gene activation in the ventral tegmental area and nAc, by quantifying an immediate early gene product (Egr-1), following the hearing of a conspecific male courtship song compared with hearing noncontextual tones (Earp and Maney, 2012). Additional studies are needed to determine whether the increased gene expression shown in the ventral tegmental nucleus and nAc of the sparrows was dopamine or one of the major neuropeptides found in the 2 structures.

(3) Extended Amygdala: Central Extended Amygdala and Medial Extended Amygdala. Heimer and coworkers were instrumental in recognizing that 2 major nuclei of the amygdaloid complex in mammals, the central and medial nuclei, each had neuronal corridors that extended from them in a medial direction toward midline (Alheid and Heimer, 1988; Alheid et al., 1995). The central and medial amygdaloid nuclei, directly or by way of the bed nuclei of the stria terminalis, are the major output nuclei of the amygdala (Paré et al., 2004; Swanson, 2000). In birds, both corridors...
can be found ventral to the globus pallidus (Figure 9.7 in Kuenzel, 2014).

**a. Central Extended Amygdala.** The central extended amygdala (CEA) includes the central amygdaloid nucleus and its corridor to the lateral bed nucleus of the stria terminalis (Figure 9.7 in Kuenzel, 2014). Currently the actual location of the subpalial central amygdaloid nucleus in birds is unresolved. [Note: After this manuscript was submitted, a paper was published locating the avian subpalial central amygdaloid nucleus (Vicario et al., 2014).] The CEA of mammals has been shown to project to several structures in the brainstem known to regulate the autonomic nervous system. Hence, the mammalian CEA has consistently been associated with the regulation of ingestion, fear, and stress/anxiety (Alheid and Heimer, 1988; Alheid et al., 1995; Swanson, 2000; de Olmos et al., 2004). A neural system proposed before the CEA was established in mammals was the visceral forebrain system (van der Kooy et al., 1984). Of interest, neural structures identified with the visceral forebrain system (VFS) are almost the same as those of the CEA and its downstream structures that regulate the autonomic nervous system. The function of the VFS was hypothesized to interfere with or override brainstem homeostatic mechanisms during periods of stress or emotional activity (van der Kooy et al., 1984), hence sharing functions identified with the CEA. In birds, it was hypothesized that they possess a VFS similar to mammals, due to the same group of neural structures displaying immunoreactivity for vasoactive intestinal polypeptide (VIP) following immunohistochemistry (Table 1 in Kuenzel and Blählser, 1993). Because VIP has several functions related to energy metabolism, metabolic aspects of circadian rhythms, and affects behavioral changes associated with the care of young, it suggests that VIP or a VIP-like neuropeptide serves in a neural network such as the VFS or CEA and is a useful marker of these neural systems (Kuenzel and Blählser, 1993, 1994; Kuenzel et al., 1997).

**b. Medial Extended Amygdala.** In mammals the medial extended amygdala (MEA) includes the subpalial medial amygdala and a corridor of neurons ending in the medial bed nucleus of the stria terminalis. Functionally it is involved in reproductive, defensive, and aggressive behavior (Alheid et al., 1994, 1995; Swanson, 2000; Choi et al., 2005). The MEA lies directly ventral to the central extended amygdala. In birds, the term nucleus taeniae amygdala (Reiner et al., 2004) was recently changed to the medial amygdala (MeA; Abellán and Medina, 2009). Therefore, the MEA comprises a neuronal corridor that continues medially from the MeA to 2 subnuclei of the medial bed nucleus of the stria terminalis (BSTM1, BSTM2; Figure 9.7 in Kuenzel, 2014). The avian BSTM is sexually dimorphic (Jurkevich et al., 1997, 1999; Aste et al., 1998). In the chicken, the BSTM is clearly identified in the embryo and posthatching birds using the neuropeptide marker, arginine vasotocin. By 35 d of age, the BSTM of females, however, is no longer visible with respect to showing immunoreactivity to arginine vasotocin (Jurkevich et al., 1999). Studies have shown that the avian BSTM is involved in similar roles as mammals (Panzica et al., 1998; Thompson et al., 1998; Absil et al., 2002), and specifically the BSTM2, located close to the third ventricle near midline, is involved in appetitive sexual behavior in males (Xie et al., 2010, 2011).

**4. Basal Telencephalic Cholinergic and Noncholinergic Corticopetal System.** The basal forebrain corticopetal system in mammals is also referred to as the region containing the substantia innominata. A major nucleus of the region is the nucleus basalis of Meynert, having large cholinergic neurons (Gritti et al., 1993, 2003). Cholinergic neurons partly overlap the globus pallidus and ventral pallidal region of the BG. The corticopetal cholinergic neurons play an important role in modulation of cortical activity and in attentional and arousal processes (Záborszky et al., 1999) that affect learning and memory (Metherate et al., 1988, 1992; Cape et al., 2000; Cape and Jones, 2000). Alzheimer’s disease is known to negatively affect neurons in this system. In birds, cholinergic neurons in and about the nucleus basalis magnocellularis (Figure 12 in Kuenzel et al., 2011) is thought to be homologous to the mammalian nucleus basalis of Meynert (Medina and Reiner, 1994; Reiner et al., 2004). A procedure developed in mammals that is known to damage the basal forebrain cholinergic system has been shown to impair memory in chicks (Gibbs et al., 2010).

**5. Septum and Septal Neuroendocrine Systems.** The septum of mammals lies between the ventral horns of the lateral ventricles of the brain that contain cerebrospinal fluid. The mammalian septum has been parcellated into numerous zones and nuclei that can be grouped into 4 major divisions (Jakab and Leranth, 1993). Functions attributed to this brain region in mammals include ingestive, defensive, and reproductive behaviors (Swanson, 2000). Using a variety of songbird species and antibodies against 10 neuropeptides and enzymes, the avian septum has likewise been divided into 4 major divisions (Goodson et al., 2004). Functionally, studies with black-capped chickadees show that the septum and hippocampus are associated with food caching (Shiflett et al., 2002) as well as plays a major role in courtship and aggressive behavior (Ramirez et al., 1988; Goodson, 1998; Goodson and Adkins-Regan, 1999; Panzica et al., 2001).

A neuropeptide system that influences septal structure and function involves gonadotropin-releasing hormone, type 1 (GnRH-1) neurons. The GnRH-1 neurons, responsible for developing and regulating the reproductive system of vertebrates, have been shown not to originate in the brain; rather, they first develop outside the brain in the olfactory placode/epithelium of amphibians (Muske and Moore, 1988), chicks (Murakami et al., 1991; Norgren and Lehman, 1991), and mammals (Wray et al., 1989; Schwanzel-Fukuda and Pfaff, 1989). The neurons migrate into the brain near
the olfactory bulbs (Figure 9.8 in Kuenzel, 2014) and then move caudally until they arrive in the septal-pre-optic-hypothalamic region (Kuenzel and Golden, 2006). An inherited human disease called Kallman’s syndrome defined by anosmia and hypogonadism (Soules and Hammond, 1980) may be related to a failure of GnRH-1 neurons from migrating from the olfactory placode to the appropriate regions of the septum and hypothalamus (Schwanzel-Fukuda et al., 1989).

**SUBSUMMARY**

The nomenclature changes made to the pallial and subpallial regions of the avian forebrain (Reiner et al., 2004; Jarvis et al., 2005) as well as updated data addressing subpallial (Kuenzel et al., 2011) and pallial structures and regions (Jarvis et al., 2013) have contributed to a set of anatomical terms that have and will continue to enhance communication among comparative neurobiologists. Additionally, this review has provided evidence that the avian pallium has fundamental cell-to-cell organization of auditory and visual systems displaying columns of neurons that resemble the columns of neurons forming functional modules in the mammalian cortex. Importantly, in reviewing the 5 basic neural systems found in the subpallium of birds, their resemblance to mammalian systems is striking. Avian species display behaviors and pathological changes quite similar to those observed in mammals including humans. Hence, the chicken as well as other avian species are excellent biological models for brain and behavioral investigations that are essential for contributing meaningful data to the BRAIN Initiative that was announced and will be initiated in fiscal year 2016.

**THE BRAIN INITIATIVE AND FUTURE FUNDING**

The BRAIN Initiative announced by the president in the spring of 2013 was followed up by a document published a year later by a BRAIN Working Group Advisory Committee. The document established goals and milestones, developed priority research areas, detailed deliverables of the initiative and its cost. On June 5, 2014, the Advisory Committee’s report, “BRAIN 2025: A Scientific Vision” called for a decade long investment of $4.5 billion that would begin in fiscal year 2016 and end in fiscal year 2025. The first 5 years (fiscal years 2016 to 2020) would provide $400 million/yr to focus on technology development, and during fiscal year 2021 to 2025, provide $500 million/yr to address application of those technologies. The major contributors for the initiative include the National Institutes of Health, the NSF, and the Department of Defense. An effort will be made to obtain contributions/funding opportunities from private sources, foundations, and companies.

Of relevance to members of the poultry science community as well as scientists working with avian species is the call for biomodels. Specifically, a range of biological organisms from simple to complex is sought for use to advance the brain initiative.

**MODEL ORGANISMS NEEDED: SHOULDN’T THE CHICKEN AND OTHER AVIAN SPECIES BE MODEL SPECIES FOR THIS INITIATIVE?**

From data presented in this review, it appears that avian species have excellent characteristics and an extensive database from past studies to be excellent biomodels to accelerate the understanding of brain function. Nonetheless, competition will be keen. Table 1 shows the organisms used most often in research on the nervous system and the number of publications produced that used each organism for the most recent, completed year, 2013. Research papers utilizing the chicken as a biomodel are also included, and *Gallus gallus* is underlined to show how it stands with other laboratory species currently used.

**ACKNOWLEDGMENTS**

Supported in part by NSF Grant IOS-082937. The author thanks Susan Pollock (Federation of Animal Science Societies, Champaign, IL) for performing the Thomas Reuters Citation Index search and Tony Stankus (University of Arkansas, Fayetteville) for providing information shown in Table 1.

**REFERENCES**


