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THE NEUROETHOLOGY OF COGNITIVE MAPS: CONTRIBUTIONS FROM RESEARCH ON THE HIPPOCAMPUS AND HOMING PIGEON NAVIGATION

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INTRODUCTION

The hippocampal formation of mammals has been the subject of numerous investigations. These studies have revealed a basic importance of this brain structure in a wide spectrum of functional activities, such as learning, memory, sensory regulation, motor activity (28). One of the best investigated aspects of hippocampal function is concerned with the involvement of the hippocampal formation in the organization of spatial behavior. Indeed, several studies have demonstrated that the vertebrate hippocampal formation is critical for spatial cognition (4, 27, 46, 49). Lesioning the hippocampal formation impairs spatial performance in a wide variety of experimental contexts including eight-arm radial maze (50) and water maze (45) in rats, and seed recovery in caching birds (36, 55). Neurons in the rat hippocampal complex, so-called place cells (48), respond when an animal is in a particular environmental location. Other neurons, so-called direction-sensitive cells of the postsubiculum, respond when an animal is oriented in a particular direction with respect to some external reference (59). Finally, features of the hippocampal formation anatomy have been found to correlate with spatial cognitive ability (37) or natural spatial behavior (26, 35, 56). Together, these findings indicate that the hippocampal formation is necessary for spatial orientation based on the recognition of local environmental cues. In line with the "cognitive map" theory (49), the hippocampal complex would be involved in the formation and possible use of a map relative to the animal's environment, thus allowing goal directed orientation from anywhere within an animal's familiar environment.

One research tactic that has been exploited during the past ten years has been an attempt to perform relatively controlled experimental animal studies in a "natural world" setting. The aim of these studies has been to provide a better understanding of how hippocampal involvement in memory processes may manifest itself within the complexity of free-living conditions. In this context, research on the avian hippocampus has made its most important contribution by investigating the role of the hippocampal formation in naturally occurring spatial behavior. In particular, these studies have examined the importance of the hippocampus for memory processes in the context of spatial recognition and spatial navigation in homing pigeons. In the present paper, we provide a review of the experimental data concerning the hippocampal formation of homing pigeons. These data have been organized into two main sections: i) possible homology between mammalian and avian hippocampal formations, relying primarily on studies describing pathway connections as well as distribution and development of transmitter-identified neuronal populations. These investigations suggest that the hippocampal formation of homing pigeons is indeed homologous to the mammalian hippocampal formation and thus it is a reliable experimental model to gather information on the hippocampus that may be extended to other vertebrate classes. *ii*) Behavioral studies employing homing pigeons with ablation of the hippocampal formation in various experimental conditions. This research allows one to examine the role of the hippocampal formation in different aspects of spatial information processing.

I. *Evidence for a homology between avian and mammalian hippocampal formations.*

As reported above, the primary goal of research on the mammalian hippocampal formation is focused on understanding the neural regulation of memory processes. If bird research is to provide a novel contribution to this central issue applicable to mammals, then it is necessary to demonstrate, within reason, that avian and mammalian hippocampal formations are structurally homologous, and thus organized, at least in part, along similar anatomical and physiological schemes. Physiological investigations of the mammalian hippocampus have implicated long-term potentiation (LTP), a persistent increase in synaptic efficiency, as one important mechanism that may underly certain kinds of learning and memory (47, 58, 60). Similarly, a long-lasting facilitation of synaptic transmission that resembles LTP has been reported in the avian hippocampus (64, 65). From a structural point of view, the anatomical characteristics of the non-mammalian hippocampal formation have been investigated for many years, and at least some features that are common across amniotes have been identified. For example, the avian hippocampal complex, consisting of a medial hippocampus and a dorsomedial parahippocampus (29), shares with the mammalian hippocampus a similar topological relationship with respect to the lateral ventricle (18), its three-layered organization (21), and a large diversity of cell types (42). However, the avian and mammalian hippocampal formations are strickingly different in appearance, as the characteristic subdivisions of the mammalian hippocampus (dentate gyrus, hilar region, Ammon's horn) are not discernible in birds.

1. *Pathway connections.* - The examination of pathway connections provides an important source of information to address the question of homology, as well as being central to any question of functional similarity. The afferent and efferent connections of the avian hippocampal formation have been investigated using

different neuroanatomical tracing methods. These studies have shown some differences in the connectivity of the two major subdivisions of the avian hippocampal formation, i.e. the hippocampus proper and the parahippocampus or area parahippocampalis. More importantly, these investigations have revealed some striking similarities in the organization of the neural circuitries involving the hippocampal formation in birds and mammals (1, 16, 33; see Fig. 1). The most important findings are that, as in mammals, the avian hippocampal formation is in receipt of projections from monoaminergic brain stem nuclei (medial raphe, locus coeruleus), mammillary area of the hypothalamus, a region of the ventral medial telencephalon - the archistriatum (thought to be homologous with the mammalian amygdala [69]), and the diagonal band of the ventral septum. There is also a robust commissural system and efferent projections to the septum, mammillary region of the hypothalamus, and archistriatum. One possible source of confusion, however, is the apparent absence of an entorhinal cortex in birds and thus the absence of a perforant path homologue. Beyond the question of homology, the absence of sensory input to the avian hippocampal complex would also raise serious doubts regarding functional similarity with the mammalian hippocampus. Casini *et al.* (16), however, demonstrated a reciprocal pathway connection between the avian hippocampal formation and one layer of a multilaminated area of the anterior forebrain, the Wulst (41). The Wulst is a recipient area of fibers originating from a number of distinct thalamic nuclei and is a telencephalic target of visual (30, 57) and somatosensory (19) inputs. Indeed, the particular layer of the Wulst that connects with the hippocampal formation, the hyperstriatum dorsale, has been reported to receive projections from both visual and nonvisual thalamic nuclei (30). The avian hyperstriatum dorsale, therefore, appears similar to the entorhinal

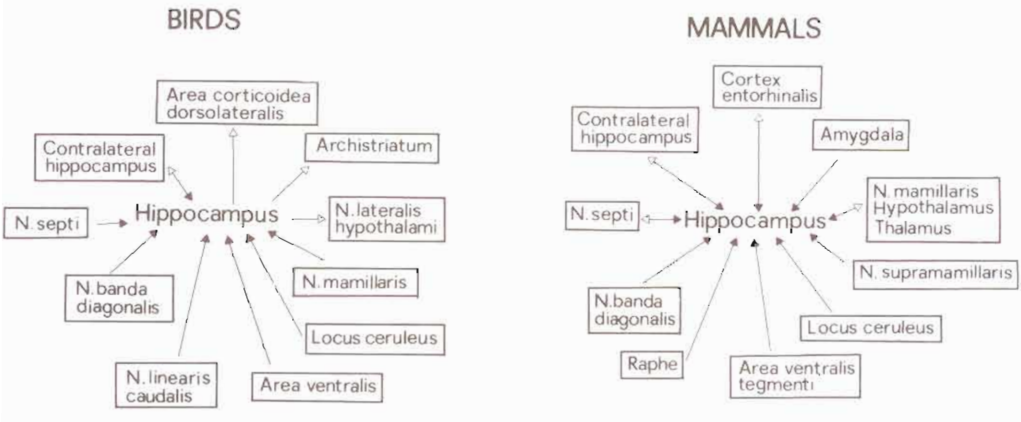

Fig. 1. - *Schematic reconstruction of the afferent and efferent connections of the avian and mammalian hippocampal formations as revealed by neuroanatomical tracing methods (1, 16, 33).*

Striking similarities in the organization of pathway connections provide evidence for a homology among the avian hippocampal complex and the hippocampal formation of mammals.

cortex of mammals, receiving sensory input that may be further processed at the level of the hippocampus via a reciprocal pathway connection. In addition to these features of hippocampal pathway connections shared by birds and mammals, many similarities have also been observed with pathway connections involving the hippocampal formation of reptiles (15, 38, 39). It appears that a correspondence between the hippocampus and the large- and small-celled areas of the mediodorsal cortex, and the parahippocampus and the dorsal cortex of birds and reptiles, can be drawn (see ref. 16 for a comprehensive review). Taken together, these findings provide evidence for homology among the avian hippocampal formation and the hippocampal formation of reptiles and mammals, suggesting that the basic organization of hippocampal connections already existed before protomammalian therapsids diverged from ancestral reptiles and remained conserved throughout the course of evolution.

2. *Transmitter-identified neuronal populations.* - Another source of information that can be brought to bear on the question of homology is an examination of the presence and distribution of neurotransmitter and transmitter-related substances. Comparative immunohistochemical studies of mammals have provided new insights concerning neuroactive substances that are conserved and remain characteristic of certain hippocampal subdivisions. Similar analyses of the avian hippocampal formation employing immunohistochemical and in *situ* hybridization techniques have provided the basis for identifying possible equivalent subdivisions in birds (22, 34, 43). Indeed, the avian hippocampal formation contains many of the same neuroactive substances that have been detected in the hippocampus of mammals. In particular, combining immunohistochemical with pathway connection observations, it appears that cholinergic and catecholaminergic afferents constitute the major ascending pathways to the hippocampal complex both in birds and in mammals (34). In addition, studies on the distribution of neuropeptides, including substance P, Leu-enkephalin, vasoactive intestinal peptide, cholecystochinin, neuropeptide Y and somatostatin, indicate that at least some of the major subdivisions of the mammalian hippocampal formation are also present in birds (22). In summary, in the pigeon hippocampal formation four major subdivisions can be compared with subdivisions of the mammalian hippocampus: *i*) an area associated with the medial fiber tract passing through the septo-hippocampal junction and extending dorsally, corresponding to the alveus of mammals, *ii*) a ventral v-shaped area of large cells, corresponding to the mammalian Ammon's horn, *iii*) a dorsomedial area rich in neuropil, corresponding to the hilar layer of the dentate gyrus of mammals, *iv*) an area containing vasoactive intestinal peptide-immunoreactive cells that may correspond to the granule layer of the dentate gyrus (22). However, there are also differences, such as the apparent absence of a mossy fiber system in birds (22). In terms of the classical subdivision of the avian hippocampal formation in a medial hippocampus and a dorsolateral area parahippocampalis (29), the v-shaped area of large cells (corresponding to the mammalian Ammon's horn) is a part of the hippocampus, while the region including the area with vasoactive intestinal peptide-

immunoreactive cells and the dorsomedial area rich in neuropil (corresponding, respectively to the granule and hilar layer of the mammalian dentate gyrus) are located in the area parahippocampalis.

Recent studies examining the developmental pattern of expression of different neuroactive substances in the pigeon hippocampal formation (17, 23) have further established the notion of a strict correspondence between the avian and the mammalian hippocampal formations. The development of cell populations expressing choline acetyltransferase, tyrosine hydroxylase, GABA, Met-enkephalin, neuropeptide

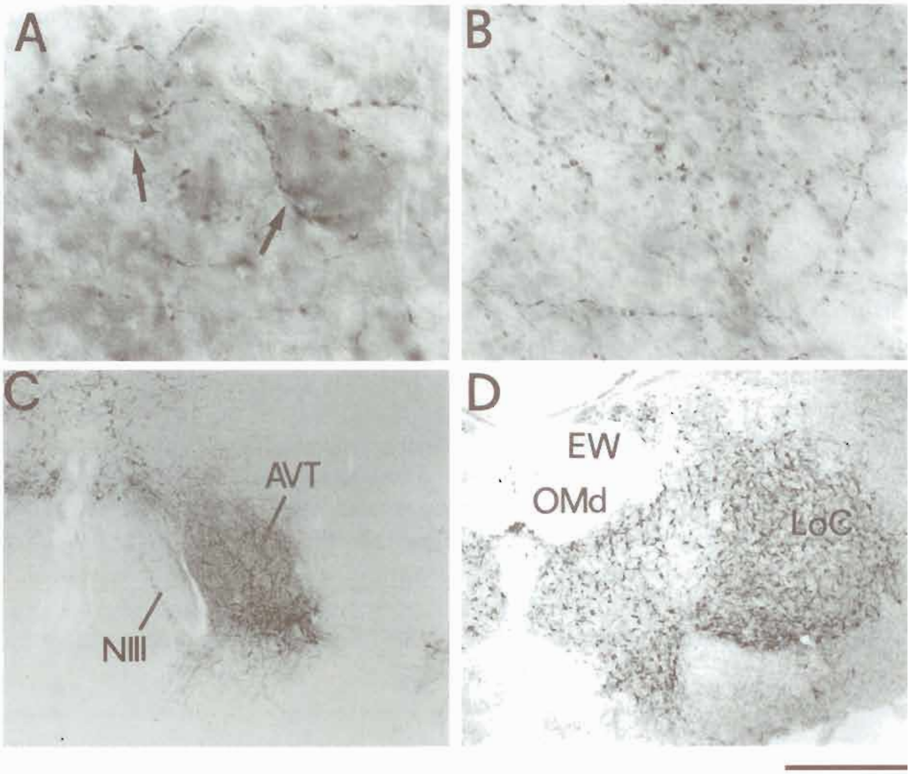

Fig. 2. - *Distribution of tyrosine hydroxylase (TH) immunoreactivity in the pigeon hippocampal formation at the adult stage (A) and at hatching (B-D).*

The adult pattern of TH immunoreactivity is characterized by the presence of TH-positive bouton-like varicosities with a basket-like appearance (arrow in A) which surround unlabeled cells. Some of these varicosities can be observed in the dorsal part of the hippocampal proper (Hp) whereas they are especially numerous in the area parahippocampalis (APH). At hatching, numerous TH-containing fibers or terminal-like processes can also be observed although diffusely distributed (B). They increase in density over the posthatching period and elaborate the basket-like structures typical of the adult stage. C and D, at hatching numerous TH-containing cells can be observed in brain stem structures known to project to the hippocampal complex (16), i.e. the area ventralis of Tsai (AVT) and the locus coeruleus (LoC). The density of labeled cells is comparable to that found in the adult. EW=nucleus of Edinger-Westphal; NIII=nervus oculomotorius; OMd=nucleus nervi oculomotorii, pars dorsalis. Scale bar=5mm for A and B, 1.1mm for C and D. (From 23).

Y, substance P or somatostatin immunoreactivities (23) as well as preproenkephalin mRNA (17) in the pigeon hippocampal formation has been recently analyzed using both morphological and quantitative approaches. The cholinergic and the catecholamineregic projection systems to the hippocampal formation show different time courses of maturation (23). In particular, the maturation of the cholinergic pathway is largely restricted to the posthatching period, with very rare cholinergic fibers observed in the hippocampal formation of newly hatched pigeons. In contrast, catecholaminergic projections are already present in the hippocampus at hatching, although their maturation, including the differentiation of characteristic varicosities and basket-like structures around unlabeled cells, is completed only during late stages of posthatching development (Fig. 2A and B). The presence of catecholaminergic fibers in the hippocampal formation at hatching is consistent with the finding at the same age of catecholaminergic cells bodies in brain stem nuclei known to originate such projections (Fig. 2C and D). The time course of developmental changes in the distribution and density of the cells expressing different neurochemical markers varies considerably. Figure 3 summarizes the development of the cells expresing neuropeptide Y immunoreactivity as an example of these investigations. The cells that are labeled by neuropeptide Y immunoreactivity in the adult hippocampal formation constitute a heterogeneous population of polymorphic cells. These cells are detected at embryonic stages, and they are characterized by immature morphology. The mature morphology is attained at 9 days posthatching. Both in the hippocampus and in the area parahippocampalis there is an increase in immunostained neuropil over the same period of development. In general, the developmental patterns of neurochemically-identified cell populations are different in the two major subdivisions of the pigeon hippocampal formation (hippocampus proper and area parahippocampalis), although the adult pattern is usually attained by the third week of age. As shown in Figure 4, quantitative analysis of the development of different neurochemically-identified cell populations in the pigeon hippocampal formation shows that for most of these populations, the number of labeled cell bodies increases after hatch-

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Fig. 3. - *Distribution of neuropeptide Y (NPY) immunoreactivity in the pigeon hippocampal formation at the adult stage (A and C) and at hatching (B and D).*

In the photomicrographs, the two major subdivisions of the hippocampal complex i.e. the hippocampal proper (Hp) and the area parahippocampalis (APH) are shown at two levels of the Karten and Hodos (29) atlas (A9.00 in A-B and A6.50 in C-D). The adult distribution of NPY immunoreactivity is characterized by the presence of a heterogeneous population of densely labeled polymorphic cells (insets in A and C). In the rostral hippocampal complex (A9.00 in A), immunostained cells are found in an elongated band that extends dorsally and laterally into the APH. More caudally (A6.50 in C), NPY-containing cells are localized largely to the ventral Hp. Immunostained cells are very rare in the dorsal Hp while they are more numerous in the ventral APH. The distribution of NPY immunoreactivity at hatching is characterized by the presence of numerous immunoreactive cells which can be observed at either rostral (A9.00 in B) or caudal (A6.50 in D) levels. NPY-containing cells exhibit long dendritic processes oriented toward the ventricle (insets in B and D). HA=hyperstriatum accessorium; LFS=lamina frontalis superior; PA=paleostriatum augmentatum; SL=nucleus septalis lateralis. Scale bar=300mm for A-D, 50mm for insets. (Modified from 23).

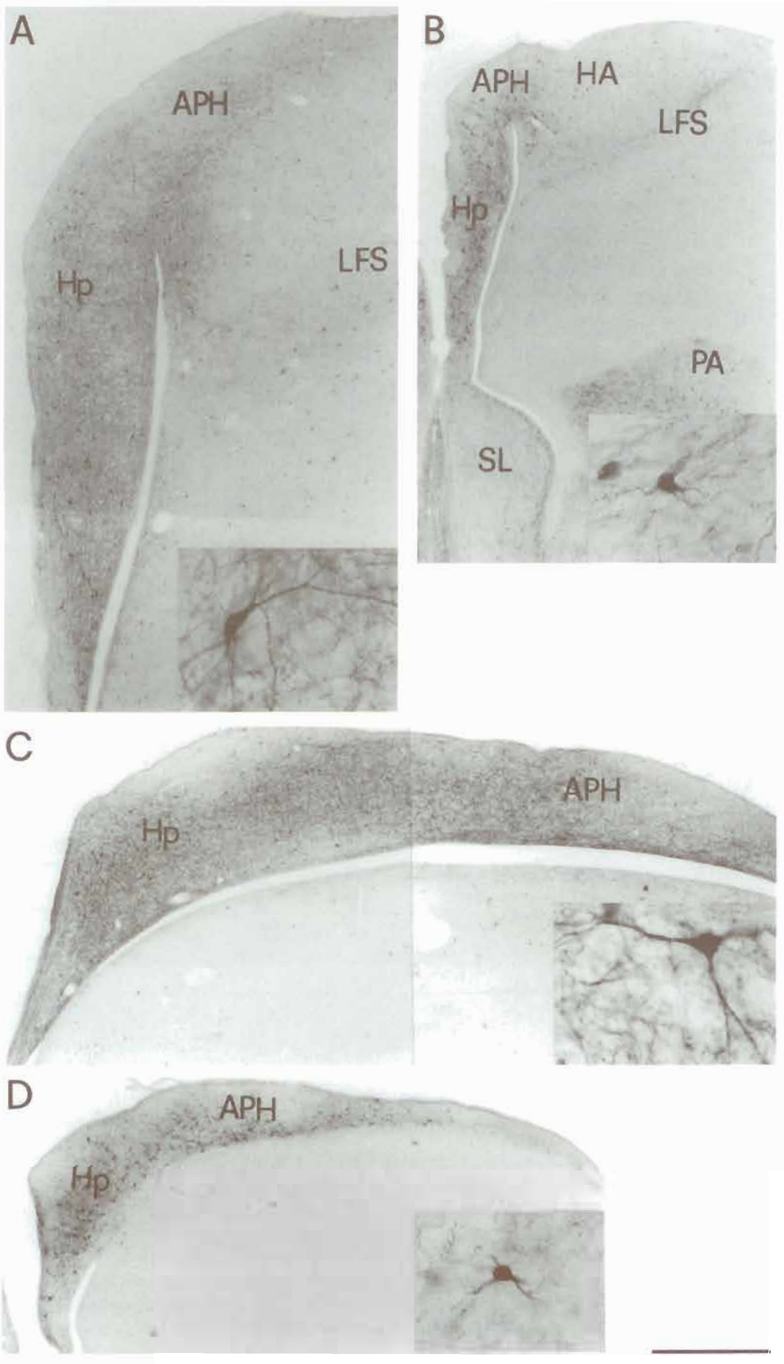

Fig. 3.

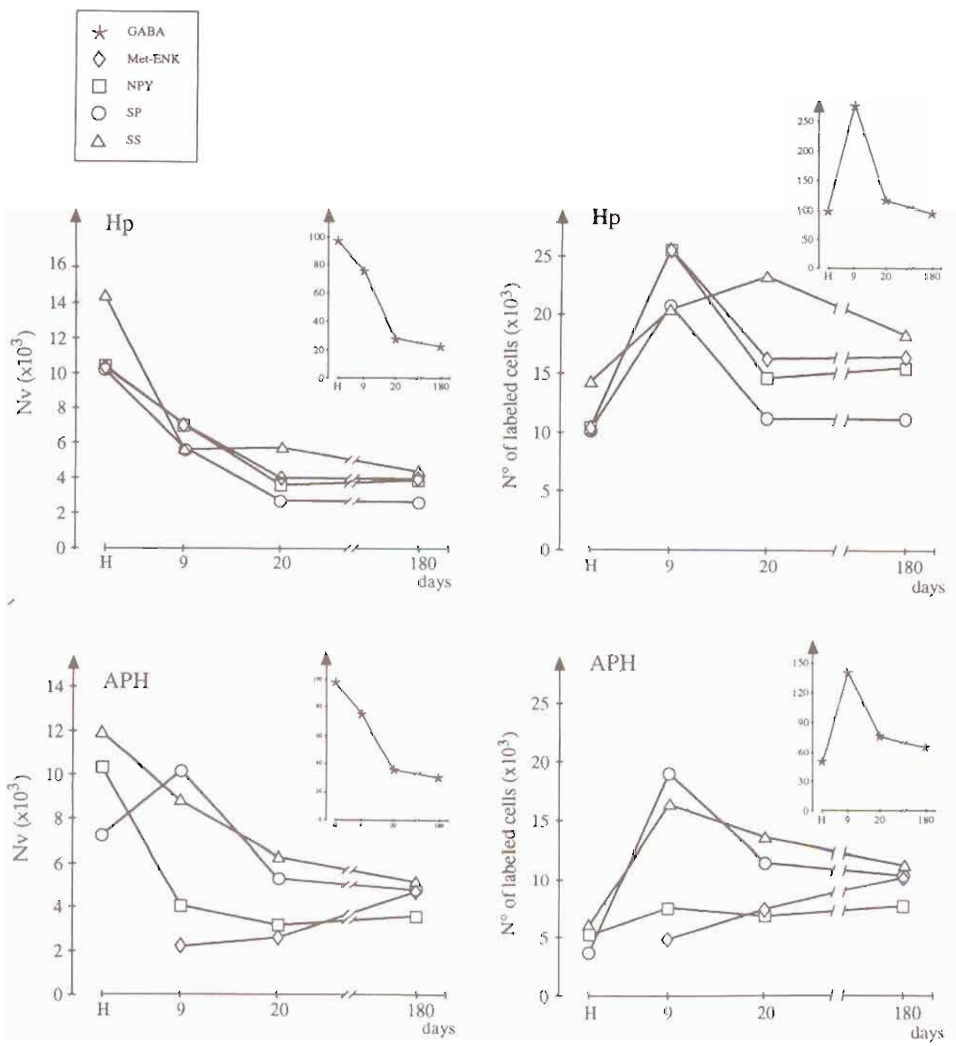

Fig. 4. - *Computer-assisted analysis of quantitative differences in the density and number of transmitter-identified cells of the pigeon hippocampal formation at different stages after hatching (H).*

On the left, the graphs show the numerical density (Nv) of immunoreactive cell bodies which refers to the number of immunostained cells per cubic millimeter of tissue. On the right, the graphs show the immunostained cell number which is obtained by multiplying Nv by the volume of the region. Values are expressed as means and are obtained from six to ten sections covering the entire hippocampal complex of four brains collected at different developmental stages. Both Nv and the number of labeled cells refer to the hippocampus proper (Hp) and the area parahippocampalis (APH). It can be noticed that both the numerical density and the number of transmitter-identified cells generally attain the adult values around the third week of age in both Hp and APH. This developmental pattern can be summarized, with a few exceptions, by a general decrease in the density of immunostained cells until the third week of age and an increase of the cell number from hatching to 9 days posthatching. The adult cell number is then attained as a result of an almost 50% loss of immunoreactive neurons between 9 days and the third week of age. GABA=g-aminobutyric acid; Met-ENK=met-enkephlin; NPY= neuropeptide Y; SP= substance P; SS=somatostatin. (Modified from 23) ing to peak at 9 days posthatching and then decreases until reaching adult levels. The developmental features of specific transmitter- and/or peptide-expressing neuronal populations in the pigeon hippocampal formation are similar to those of the corresponding cell populations in the mammalian hippocampus (see Ref. 23 for details). These studies, together with neurochemical investigations of the adult pigeon (22,34,43) and with the data on pathway connections reported above, further indicate that the avian hippocampal formation contains subdivisions that may correspond to at least some of the well-defined structures of the mammalian hippocampus, therefore establishing a consisten basis of experimental evidence for the hypothesis of a homology between avian and mammalian hippocampal formations.

II. *Pigeon homing behavior as a tool to investigate hippocampal functions.*

In the last fifty years, a large body of experimental evidence has led to an understanding of the basic processes by which homing pigeons navigate (51, 62, 66). The best model explaining how homing pigeons home from long distances involves a two step process. For the first step, pigeons would use a "navigational map" (31, 32, 62) to determine their direction of displacement with respect to home. Experiments conducted over the last twenty years have convincingly demonstrated that the sensory basis of the navigational map is primarily constituted by atmospheric odors, which represent the most important cues used by homing pigeons to determine their relative position with respect to home (25, 51, 62). This "olfactory" navigational map allows a bird to determine the direction of displacement with respect to home. The navigational map of homing pigeons may represent perhaps the best naturally occurring example (3) of a "cognitive map" in the sense of O'Keefe and Nadel (49) and Gallistel (24). Cognitive maps are generally conceptualized as representing the spatial relationships among environmental stimuli, allowing an animal to locate its position in space and efficiently move between goal locations. However, a navigational map alone would not be sufficient to actually direct the orientation response of a bird. Therefore, a second step, referred to as the "compass", is used to identify directions in space. Generally, pigeons rely on their "sun compass" (53, 68), which is a time compensated mechanism that defines directions in space independent of location. By using its internal clock, a bird is able to take into account the apparent movement of the sun during the course of the day, thus maintaining a stable directional framework (53).

The olfactory map is used when a bird has to home from distant, unfamiliar locations. However, when homing pigeons fly over a familiar terrain, they can independently rely on familiar landmarks as an additional source of map-like information to return home (63). Although the range of sensory modalities that can be used to perceive familiar landmarks remains unclear, some landmarks are certainly visual in origin (14, 54). The available evidence suggests that navigation by familiar landmarks, like the navigational map, operates together with the sun compass in allowing pigeons to home (40). In addition, there is evidence that, under some conditions, familiar landmarks can also be used to directly guide the orientation response of birds (5).

1. *Hippocampal formation and spatial cognition in homing birds.* -
In their initial work on homing pigeons, Bingman *et al.* (9, 10) performed a series of experiments to examine the effects of hippocampal formation lesions on the navigational map of pigeons known to rely on atmospheric odors. Experimental birds generally failed to return home whether they were released 50 Km or just 500 m from and in full view of their loft. Several birds released from long distances were observed to return to the vicinity of their loft, but they appeared to be unable to locate their home. However, when released from distant locations, these birds were nonetheless observed to fly off in the direction of home, indicating that they were still able to rely on their olfactory map. The most plausible explanation for these observations was that, as a result of hippocampal ablation, the pigeons were unable to recognize their loft, suggesting an important role of the hippocampal formation in the neural regulation of how animals learn and remember the distribution or location of stimuli in space associated with their familiar, landmark-based map. However, these data also indicated that the hippocampal formation plays no necessary role in the operation of an already learned olfactory map system. The results obtained in these early experiments were in general agreement with the original hypothesis of O'Keefe and Nadel (49) describing the hippocampal formation as part of a cognitive mapping system.

The observations reported above, together with results obtained in a variety of different animal models, clearly identify the hippocampal formation as a structure necessary for spatial learning. However, they do not allow an understanding of what type of processing is regulated by the hippocampus which could lead to the observed robust deficits in spatial cognition after hippocampal lesions. Following, is a discussion of experiments aimed at clarifying the involvement of the hippocampal formation in the learning and operation of navigational maps.

2. *Learning and operation of an "olfactory navigational map".* - The first observations on the homing behavior of pigeons after lesions of the hippocampal formation reported, in addition to the inhability of these birds to recognize their loft, a substantial maintenance of the olfactory map function. Indeed, when pigeons are lesioned as experienced adults, their ability to orient homeward from distant, unfamiliar locations is unimpaired (9, 11). This finding demonstrates that in experienced birds, the hippocampal formation plays no necessary role in the *operation* of their previously acquired navigational map based on atmospheric odors and associated compass mechanisms. In contrast, lesions of the hippocampal formation in young, inexperienced birds have a dramatic effect on their ability to *learn* such an olfactory map (13). As shown in Figure 5, when young pigeons with hippocampal ablations are released from a distant site for the first time, the distribution of their vanishing bearings is significantly more dispersed than that of two different groups of control pigeons. A similar impairment in navigational map

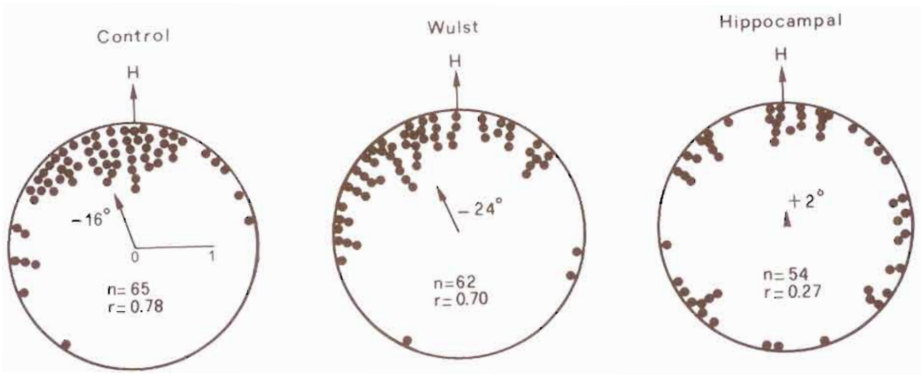

Fig. 5. - *Role of the pigeon hippocampal formation in the acquisition of navigational ability.*

Young homing pigeons were subjected to hippocampal lesion before having acquired a functional navigational map. Wulst ablations were performed in another group of animals to control for the specificity of the effects induced by hippocampal lesions. The diagrams show the initial orientation of control, Wulst-, and hippocampal-ablated pigeons across all releases. Each dot represents the vanishing bearing of one bird for one release plotted with respect to the homeward direction (H). Inner arrows represent the mean vectors, the lenghts of which (r) can be read using the scale found in the first diagram. It can be noticed that in contrast to experienced pigeons, naive young homing pigeons that underwent hippocampal ablation show a robust deficit in their ability to orient homeward when released from distant unfamiliar locations thereby suggesting the hippocampal complex as a structure critical for the acquisition of the navigational map. (From 13)

learning has been observed in hippocampal ablated adult birds that were transferred to a new loft and found to be impaired in learning a new navigational map (8). Together, these results suggest a critical role for the hippocampal formation in olfactory-based navigational map learning, but no necessary role in the operation of this navigational map once acquired.

3. *Learning and operation of a "familiar landmark map"*. - In contrast to the olfactory map, where the participation of the hippocampal formation appears to be limited to acquisition, the available evidence suggests that both the *operation* and the *acquisition* of familiar landmark navigation involves the participation of the hippocampal formation. Although adult, experienced homing pigeons subjected to hippocampal lesions are still able to orient homeward from distant locations, their ability to navigate near the loft is impaired. Indeed, the time taken by hippocampal ablated pigeons to return to their lofts after release from a distant location is significantly longer than in control birds (11). This effect is likely to be due to difficulties in maintaining a correct homeward direction during flight, therefore resulting in increased homing time. Tracking experiments performed with lesioned birds released from locations near the loft area show that indeed these birds have enormous difficulties in maintaining a direct course homeward (Fig. 6). These observations indicate a fundamental role of the hippocampal formation in the *operation* of a previously learned mechanism of familiar landmark navigation.

The evidence that hippocampal formation is involved in familiar landmark navigational *learning* can be summarized as follows. Navigational performance near the loft of pigeons given hippocampal ablation as adults never reaches control levels, even after months of postoperative training. In addition, when limited to using familiar landmarks from a novel perspective to determine the home direction, pigeons with hippocampal ablations are impaired in orienting homeward. These results indicate an impaired ability to relearn the spatial organization of familiar landmarks for navigation. In summary, the experimental evidence indicates that, as reported above, lesions of the hippocampal formation impair navigational map learning when based on olfaction. Additionally, these lesions also impair navigational learning based on familiar landmarks when novel responses to those landmarks are necessary (4, 7, 12).

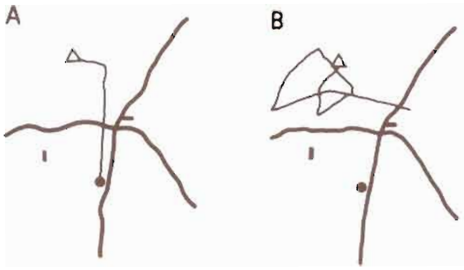

Fig. 6. - *Role of the pigeon hippocampal formation in the local navigation near the loft.*

Homeward flight paths of Wulst (A) and hippocampal (B)-ablated homing pigeons were recorded by radiotelemetry, following release from the University of Maryland peach farms. It can be noticed that hippocampal-lesioned pigeons show a local navigational impairment in the vicinity of the loft where landmark cues are thought to be important. (From 7)

Olfactory navigational map and navigation by familiar landmarks are two distinct mechanisms, yet hippocampal lesions impair learning of both. The location of stimuli (olfactory cues or familiar landmarks) in space is thought to be represented in the central nervous system within the framework of a directional reference: the sun compass. Recent work on homing pigeons has provided important clues for understanding how pigeons learn a map of space based on atmospheric olfactory information (25). The results of this research demonstrate that young pigeons acquire a representation of space based on odors during their stay at the home loft. To do so, these birds need to rely on some compass mechanism (most likely the sun compass) to associate a directional reference with the location of specific atmospheric odors. Similarly, the sun compass is likely to be of importance also in learning a navigational map based on familiar landmarks. These observations strongly indicate that the sun compass, which is used for orientation, may also serve an important function in both olfactory- and familiar landmark-based spatial learning. Therefore, it is possible to hypothesize that hippocampal lesioned birds are impaired at learning to navigate specifically because they cannot

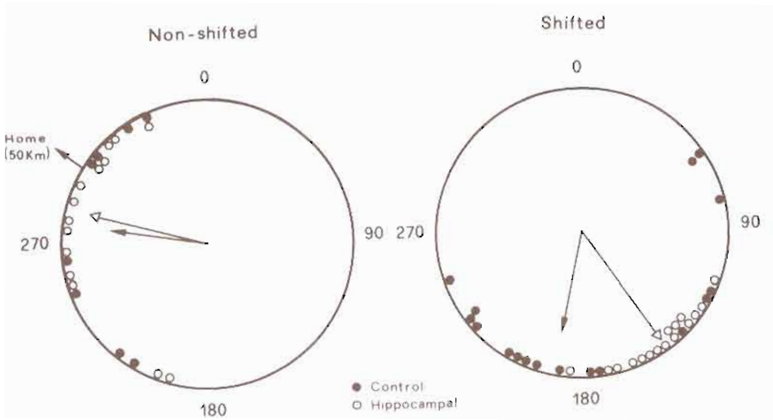

Fig. 7. - *Role of the pigeon hippocampal formation in sun compass orientation.*

Hippocampal-lesioned pigeons and untreated controls where subjected to a clock-shift procedure through shifting their internal clocks six hours fast.Their homewrd orientation from a distant, unfamiliar location was then compared to that of unshifted birds, either hippocampal-lesioned or untreated controls. The diagrams show the initial orientation of non-shifted (left) and six hour fast-shifted (right) hippocampal-lesioned (open circles) and untreated control pigeons (filled circles) from a distant, unfamiliar location. Each circle on the periphery of the circular diagram represents the vanishing bearing of one bird. The arrows inside the diagrams represent the mean vectors of the respective groups (closed arrowhead, controls; open arrowhead, hippocampal-lesioned). The length of the arrow corresponds to the length of the mean vector with an arrow equal to the radius of the circle having a mean vector value of 1.0. It can be noticed that unshifted birds are homeward oriented while the clock-shifted birds show an appropriate counter-clockwise shift in orientation thereby indicating a correct use of sun compass orientation.

use the sun compass as a directional reference to learn the directional relationships among stimuli in space (6).

4. *Hippocampal formation and the use of the sun compass.* - Recent findings show that hippocampal lesions have a devastating effect on the ability of pigeons to use the sun compass to associate a stimulus (food) with a compass direction, indicating that hippocampal lesions impair sun compass use in the context of learning (6). This result is consistent with the hypothesis that navigational learning deficits following hippocampal lesions are a consequence of an inability to use the sun compass as a directional reference to directionally locate stimuli in space. However, it is necessary to distinguish between having a sun compass that cannot be used as a directional reference for learning, and not having a sun compass for general orientation. Indeed, there is evidence from homing pigeons with ablations of the hippocampal formation that the sun compass is present, but it cannot be used in learning a navigational map. The finding that experienced pigeons subjected to hippocampal lesions continue to orient homeward from distant, unfamiliar release sites (9) certainly suggests that the lesioned birds possess a functional sun compass for orientation. In addition, experiments performed with hippocampal ablated, clock-shifted pigeons clearly demonstrate the presence in these birds of a func-

tional sun compass that is used for orientation. The clock-shift procedure is the standard procedure by which one can demonstrate sun compass orientation (53). Briefly, by altering an animal's internal clock, one also alters how the animal interprets the direction of the sun. For example, if a pigeon's internal clock is shifted by six hours, such that its subjective day begins at midnight, when released from an unfamiliar location the pigeon would display orientation that is shifted by about 90 degrees counter-clockwise compared to a non-shifted bird. In a recent experiment (Bingman, Gagliardo and Ioalè, unpublished results), experienced pigeons received hippocampal ablation and half of them were then subjected to such a six hour clock-shift procedure. When released from distant, unfamiliar location, unshifted birds were homeward oriented, while the clock-shifted birds (hippocampal lesioned birds and untreated controls) showed an appropriate counter-clockwise shift in orientation (Fig. 7). The shifted orientation of the clock-shifted, hippocampal ablated pigeons unequivocally demonstrates that hippocampal lesion does not impair sun compass orientation. These findings suggest that the hippocampal formation is not involved in the operation of the sun compass, but it plays a fundamental role in a cognitive process in which the sun compass is specifically used to *learn* about the location of stimuli in space (map learning).

PERSPECTIVES

The data reported in the present review show that a considerable amount of information is now available on the anatomical and neurochemical characteristics of the avian hippocampal formation as well as on the roles played by this structure in spatial cognition. It appears that, in general, both the anatomical/neurochemical characteristics and the role in spatial memory of the hippocampal formation of homing pigeons are similar to those of mammals (4, 12). In addition, with respect to mammals, homing pigeons offer the possibility of studying hippocampal functions in a "real world" setting. It is now tempting to design experimental approaches using the homing behavior of pigeons as an experimental model for the identification of specific transmitter systems that may be involved in hippocampal functions related to spatial cognition. For example, a recent study on the distribution of preproenkephalin (the precursor of enkephalin peptides) mRNA in the chicken and pigeon telencephalon shows that there are many more cells expressing this mRNA in the pigeon hippocampal formation than in the chicken (43). This finding suggests a species-specific neurochemical organization in the pigeon hippocampal formation that may be related to a role of enkephalin peptides in behavioral functions characteristic of the pigeon, such as the homing behavior. In addition, this observation is consistent with the experimental evidence of an involvement of opioid peptides in the homing behavior of pigeons, because the administration of the potent opioid antagonist naloxone affects the initial orientation performance of homing pigeons (52). Furthermore, there is evidence in rats that cholinergic afferents to the hippocampus may play a role in spatial navigation

(2, 20, 61) as evaluated in a water maze task (44). These studies, however, have pointed out that disruption of the hippocampal cholinergic system *per se* is not sufficient to significantly impair spatial performance (61), and suggest that cholinergic projections to the cortex are also necessary to perform spatial memory tasks (2). These findings from studies in birds and in rats may constitute the basis for an investigation of the role of peptidergic and cholinergic systems in the operation of a navigational map in homing pigeons.

From a developmental point of view, a major objective of future researches is the identification of transmitter systems that may be involved in the acquisition of the navigational map by homing pigeons. Studies on the development of transmitter-identified neuronal populations in the pigeon hippocampal formation demonstrate that these populations frequently undergo a major postnatal reorganization, and the adult pattern of distribution of transmitter-containing cells is usually attained by the third week after hatching (23). Thus, the chemo-anatomical organization of the pigeon hippocampal formation is not completely in place for the first few weeks of life. Since, as reported above, the hippocampal formation of homing pigeons plays a key role in the acquisition of the navigational map, the possibility exists that this and perhaps other related cognitive abilities may not be fully available to the pigeon until the various transmitter systems of the hippocampus have completed their maturation. While no data are at present available on neurogenetic processes and circuit maturation in the pigeon hippocampal formation, its protracted chemoanatomical development suggests the possibility of a delayed formation of cognitive maps. It is interesting to note that, generally, the time required for the maturation of specific brain regions varies with species but it seems to be correlated with behavioral changes characteristic of that species. In this sense, a delayed maturation of the hippocampal neurochemical organization may be related to the fact that pigeons would not be able to actually use a navigational map until they have developed the motor circuitries and the body structures that allow them to fly. Therefore, a mature organization of the hippocampal formation and the ability to learn a navigational map would be required only some time before pigeons develop their ability to fly. Behavioral studies are consistent with this hypothesis. Indeed, homing pigeons begin to fly around the loft area from the age of 6 weeks, and at about 15 weeks they have changed from juvenile to adult navigation strategy (67).

A further issue that needs to be clarified to improve our knowledge of the role of the hippocampal formation in spatial memory processes related to the homing behavior of pigeons is the possibility of a differential contribution to spatial cognition by the two major subdivisions of the avian hippocampus. Indeed, the data derived from pathway connection and neurochemical studies clearly indicate that hippocampus proper and area parahippocampalis represent two strictly interrelated, yet distinct, structures. The lesion studies conducted up to now have been performed without a clear attempt at discerning the involvement of each of these structures in spatial cognition, and the reported findings are usually the result of combined lesions of both hippocampus proper and area parahippocampalis. Further studies are therefore needed to resolve the specific roles of different hippocampal subdivisions. Such studies may take advantage from the use of specific neurotoxic agents directed to specific neuronal populations that are characteristically expressed in one or the other of these subdivisions.

In conclusion, studies of the homing pigeon hippocampal formation have provided important insights into the roles played by this brain structure in spatial learning and cognition. The anatomical and functional findings constitute a reliable background for further studies aimed at elucidating the contribution of different transmitter systems of the hippocampal formation in the learning and in the operation of navigational maps.

SUMMARY

The rich ethological tradition that characterizes the homing behavior of pigeons offers an excellent opportunity to examine the importance of the hippocampal formation for the regulation of spatial cognitive mechanisms. The present review summarizes both anatomical and behavioral data obtained in researches on the pigeon hippocampal formation that have been performed over the last 12 years. Pathway connection studies and investigations on the neurochemical organization of the avian hippocampal formation show that this structure shares many similarities with the mammalian hippocampus and provide the basis for structural as well as functional homology. The initial research on the role of the hippocampal formation in the homing behavior showed that this brain structure is likely to be involved in phenomena of spatial cognition. Therefore, the homing behavior of pigeons has been extensively used as an experimental model to investigate the role of the hippocampal formation in spatial cognition related to a naturally occurring behavior. These studies have revealed that the hippocampal formation plays a fundamental role in the learning of a navigational map based on atmospheric odors, but it doesn't seem to be involved in the *operation* of such a map. In contrast, both the *learning* and the *operation* of a navigational map based on the recognition of familiar landmarks require a functional hippocampal formation. Further investigations indicated that these functions of the hippocampal formation are mediated by its involvement in the use of the sun compass, and suggested that the hippocampal formation plays a fundamental role in a cognitive process in which the sun compass is specifically used to *learn* about the location of stimuli in space. The studies reviewed in the present paper have provided a considerable amount of experimental data both on the anatomical/neurochemical organization of the avian hippocampal formation and on the role played by this brain structure in spatial cognition. The future development of these researches will need to consider the contribution to hippocampal function of specific transmitter systems that are involved in hippocampal circuitry. In particular, the afferent cholinergic system and some of the peptidergic systems intrinsic to the hippocampal formation deserve particular attention in view of their possible involvement in the acquisition and/or operation of spatial cognitive abilities by homing pigeons.

REFERENCES

1. BENOWITZ, L. and KARTEN, H. The tractus infundibuli and other afferents to the parahippocampal region of the pigeon. *Brain Res.*, **102**: 174-180, 1976.
2. BERGER-SWEENEY, J., HECKERS, S., MESULAM, M.-M., WILEY, R.G., LAPPI, D. A. and SHARMA, M. Differential effects on spatial navigation of immunotoxin-induced cholinergic lesions of the medial septal area and nucleus basalis magnocellularis. *J. Neurosci.*, **14**: 4507-4519, 1994.
3. BINGMAN, V.P. Spatial organization in birds. Pp. 423-447. In: KESNER, R. and OLTON, D. (Eds.), *Neurobiology of Comparative Cognition*. Hillsdale, Erlbaum Press, 1990.
4. BINGMAN, V.P. The importance of comparative studies and ecological validity for understanding hippocampal structure and cognitive function. *Hippocampus*, **2**: 213-219, 1992.
5. BINGMAN, V.P. AND IOALÈ, P. Initial orientation in anosmic homing pigeons based on information gathered at familiar release sites remains homeward directed following clock shift. *Behaviour*, **110**: 205-218, 1989.
6. BINGMAN, V.P. and JONES, T.J. Hippocampal lesions disrupt sun compass directional learning in homing pigeons. J. *Neurosci.*, **14**: 6687-6694, 1994.
7. BINGMAN, V. P. and MENCH, J. Homing behavior of hippocampus and parahippocampus lesioned pigeons following short-distance releases. *Behav. Brain Res.*, **40**: 227-238, 1990.
8. BINGMAN, V.P. and YATES, G. Hippocampal lesions impair navigational learning in experienced homing pigeons. *Behav. Neurosci.*, **106**: 229-232, 1992.
9. BINGMAN, V.P., BAGNOLI, P., IOALÈ, P. and CASINI, G. Homing behavior in pigeons after telencephalic ablations. *Brain Behav. Evol.*, **24**: 94-106, 1984.
10. BINGMAN, V.P., IOALÈ, P., CASINI, G. and BAGNOLI, P. Dorsomedial forebrain ablations and home loft association behavior in homing pigeons. *Brain Behav. Evol.*, **26**: 1-9, 1985.
11. BINGMAN, V.P., IOALÈ, P., CASINI, G. and BAGNOLI, P. Hippocampal ablated homing pigeons show a persistent impairment in the time taken to return home. *J. Comp. Physiol. A*, **163**: 559-563,1988.
12. BINGMAN, V.P., BAGNOLI, P., IOALÈ, P. and CASINI, G. Behavioral and anatomical studies of the avian hippocampus. Pp. 379-394. In: CHAN-PALAY, V. and KOEHLER, C. (Eds.), *The Hippocampus, New Vistas*. Vol. 52. *Neurology and Neurobiology*. New York, Liss, 1989.
13. BINGMAN, V.P., IOALÈ, P., CASINI, G. and BAGNOLI, P. The avian hippocampus: Evidence for a role in the development of the homing pigeon navigational map. *Behav. Neurosci.*, **104**: 906-911, 1990.
14. BRAITHWAITE, V.A. AND GUILFORD, T. Viewing familiar landscapes affects pigeon homing. *Proc. Acad. Roy. Lond., B*, **245**: 183-186, 1991.
15. BRUCE, L. AND BUTLER, A. Telencephalic connections in lizards. I. Projections to cortex. *J. Comp. Neurol.*, **229**: 585-601, 1984.
16. CASINI, G., BINGMAN, V. and BAGNOLI, P. Connections of the pigeon dorsomedial forebrain studied with WGA-HRP and 3H-proline. *J. Comp. Neurol.*, **245**: 454-470, 1986.
17. CASINI, G., MOLNAR, M., DAVIS, M.B. AND BAGNOLI, P. Posthatching development of preproenkephalin mRNA-expressing cell populations in the pigeon telencephalon. *Dev. Brain Res.*, **84**: 233-244, 1995.
18. CRAIGIE, E. The hippocampal and parahippocampal cortex of the emu (Dromiceius). *J. Comp. Neurol.*, **61**: 563-591, 1935.

19. DELIUS, J. and BENNETTO, K. Cutaneous sensory projections to the avian forebrain. *Brain Res.*, **37**: 205-221, 1972.
20. DUNBAR, G.L., RYLETT, R.J., SCHMIDT, B.M., SINCLAIR, R. C. and WILLIAM, L. R. Hippocampal choline acetyltransferase activity correlates with spatial learning in aged rats. *Brain Res.*, **604**: 266-272, 1993.
21. DURWARD, A. Observations on the cell masses in the cerebral hemisphere of the New Zealand Kiwi (Apteryx australis). *J. Anat., Lond.*, **46**: 437-477, 1932.
22. ERICHSEN, J.T., BINGMAN, V.P. AND KREBS, J.R. The distribution of neuropeptides in the dorsomedial telencephalon of the pigeon (Columba livia): a basis for regional subdivision. *J. Comp. Neurol.*, **314**: 478-492, 1991.
23. ERICHSEN, J.T., CIOCCHETTI, A., FONTANESI, G. and BAGNOLI, P. Neuroactive substances in the developing dorsomedial telencephalon of the pigeon (Columba livia): Differential distribution and time course of maturation. *J. Comp. Neurol.*, **345**: 537-561, 1994.
24. GALLISTEL, C.R. *The Organization of Learning*. Cambridge, Bradford Brooks/MIT Press, 1989.
25. IOALE', P., NOZZOLINI, M. and PAPI, F. Homing pigeon do extract diretional information from olfactory stimuli. Beh*av. Ecol. and Sociobiol.*, **26**: 301-305, 1990.
26. JACOBS, L.F.,GAULIN, S.J.C., SHERRY, D.F. and HOFFMAN, G.E. Evolution of spatial cognition: sex-specific patterns of spatial behavior predict hippocampal size. *Proc. Natl. Acad. Sci. USA*, **87**: 6349-6352, 1990.
27. JARRAD, L.E. On the role of the hippocampus in learning and memory in the rat. *Behav. Neural. Biol.*, **60**: 9-26, 1993.
28. JONES, D. and SMITH, B. The hippocampus and its response to differential enveronments. *Progr. Neurobiol.*, **15**: 19-69, 1980.
29. KARTEN, H. and HODOS. W. *A Stereotaxic Atlas of the Brain of the Pigeon (Columba livia).* Baltimore, John Hopkins University Press, 1967.
30. KARTEN, H., HODOS, W., NAUTA, W. AND REVZIN, A. Neuronal connections of the visual Wulst of the avian telencephalon: experimental studies in the pigeon (Columba livia) and owl (Speotito cunicularia). *J. Comp. Neurol.*, **150**: 253-276, 1973.
31. KRAMER, G. Die sonnenorientierund der V*ogel. Verh. Dtsch Zool. Ges.*, **52**: 77-84, 1953.
32. KRAMER, G. Recent experiments in bird orientation. *Ibis*, **101**: 399-416, 1959.
33. KRAYNIAK, P.F. AND SIEGEL, A. Efferent connections of the hippocampus and adjacent regions in the pigeon. *Brain Behav. Evol.*, **15**: 372-338, 1978.
34. KREBS, J.R., ERICHSEN, J.T. and BINGMAN, V.P. The distribution of neurotransmitters and neurotransmitter-related enzymes in the dorsomedial telencephalon of the pigeon (Columba livia). *J. Comp. Neurol.*, **314**: 467-477, 1991.
35. KREBS, J., SHERRY, D., HEALY, S., PERRY,V.H. and VACCARINO, A. Hippocampal specializationof food-storing birds. *Proc. Natl. Acad. Sci. USA* , **86**: 1388-1392, 1989.
36. KRUSHINSKAYA, N. Some complex forms of feeding behavior of nutcracker Nucifraga caryocatectes, after removal of old cortex. *Zh. Evol. Biokim. Fisiol.*, **11**: 563-568, 1966.
37. LIPP, H.P., SCHWEGLER, H., HEIMRICH, B., CERBONE, A. and SADILE A.G. Strain-specific correlation between hippocampal structural straits and habituation in spatial novelty situation. *Behav. Brain Res.*, **24**: 111-123, 1987.
38. LOHMAN, A.H.M. and MENTINK, G. M. Some cortical connections of the Tegu lizard (Tupinambis Teguixin). *Brain Res.*, **45**: 325-344, 1972.
39. LOHMAN, A.H.M. and VAN WOERDEN-VERKLEY, I. Further studies on the cortical connections of the Tegu lizard. *Brain Res.*, **103**: 9-28, 1976.
40. LUSCHI, P. and DALL'ANTONIA, P. Anosmic pigeons orient from familiar sites by relying on the map-and-compass mechanism. *Anim. Behav.*, **46**: 1195-1203, 1993.

41. MICELI, D., GIOANNI, H., REPERANT, J. and PEYRICHOUX, J. The avian visual Wulst: I. An anatomical study of afferent and efferent pathways. II. An electrophysiological study of the functional properties of single neurons. Pp. 223-254 In: GRANDA, A. and MAXWELL, J. (Eds.), *Neuronal Mechanisms of Behaviour in the Pigeon*. New York, Plenum, 1979.
42. MOLLA', R., RODRIGUEZ, J., CALVET, S. and GARCIA-VERDUGO, J.M. Neuronal types of the cerebral cortex of the adult chicken (Gallus gallus). A Golgi study. *J. Hirnforsch.*, **27**: 381-390, 1986.
43. MOLNAR, M., CASINI, G., DAVIS, B.M., BAGNOLI, P. and BRECHA, N.C. Distribution of preproenkephalin mRNA in the chicken and pigeon telencephalon. *J. Comp. Neurol.*, **348**: 419-432, 1994.
44. MORRIS, R.G.M. Spatial localization does not depend on the presence of local cues. *Learn. Mot.*, **12**: 239-260, 1981.
45. MORRIS, M., GARRUD, R., RAWLINS, P. and O'KEEFE, J. Place navigation impaired in rats with hippocampal lesions. *Nature*, **297**: 681-683, 1982.
46. NADEL, L. The hippocampus and space rivisited. *Hippocampus*, **1**: 221-229, 1991.
47. NICOLL, R.A., KAUER, J.A. and MALENKA, R.C. The current exitement in long term potentiation. *Neuron*, **1**: 97-103, 1988.
48. O'KEEFE, J. and CONWAY, D. Hippocampal place units in the freely moving rat: Why they fire when they fire. *Exp. Brain Res.*, **31**: 573-590, 1978.
49. O'KEEFE, J. and NADEL, L. *The Hippocampus as a Cognitive Map*. Oxford, Clarendon Press, 1978.
50. OLTON, D.S., BECKER, J. and HANDELMANN, G. Hippocampus, space and memory. *Behav. Brain Sci.*, **2**: 313-365, 1979.
51. PAPI, F. Olfactory navigation in birds. *Experientia*, **46**: 352-363, 1990.
52. PAPI, F. and LUSCHI, P. Pigeon navigation: naloxone injection and magnetic disturbance have a similar effect on initial orientation. *Rend. Fis. Acc. Lincei* ,**9**: 473-477, 1990.
53. SCHMIDT-KOENIG, K. *Avian Orientation and Navigation*. New York, Academic Press, 1979.
54. SCHMIDT-KOENIG, K. and WALCOTT, C. Tracks of pigeons homing with frosted lenses. *Animal Behav.*, **26**: 480-486, 1978.
55. SHERRY, D. and VACCARINO, A. L. The hippocampus and memory for food caches in Black-capped chickadees. *Behav. Neurosci.*, **103**: 308-318, 1989.
56. SHERRY, D., VACCARINO, A.L., BUCKENHAM, K. and HERZ, R.S. The hippocampal complex of food-storing birds. *Brain Behav. Evol.*, **34**: 308-317, 1989.
57. STREIT, P., STELLA, M. AND CUENOD, M. Transneuronal labelling in the pigeon visual system. *Neuroscience*, **5**: 763-775, 1980.
58. SWANSON, L.W., TEYLER, T.J. and THOMPSON, R.F. Hippocampal LTP: mechanisms and functional implications for memory. *Neurosci. Res. Prog. Bull.*, **20**: 617-769, 1982.
59. TAUBE, J.S., MULLER, R.U. and RANCK JR., J.B. Head-direction cells recorded from the postsubiculum in freely moving rats. I. Description and quantitative analysis. *J. Neurosci.*, **10**: 420-435, 1990.
60. TEYLER, T.J. and DISCENA, P. Long-term potentiation as a candidate for mnemonic device. *Brain Res. Rev.*, **7**: 15-28, 1984.
61. TORRES, E.M., PERRY, T.A., BLOKLAND, A., WILKINSON, L.S., LAPPI, D.A. and DUNNET, S.B. Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. *Neuroscience*, **63**: 95-122, 1994.
62. WALLRAFF, H.G. Navigation by homing pigeons. *Ethol. Ecol. Evol.*, **2**: 81-115, 1990.
63. WALLRAFF, H.G., KIEPENHEUER, J. and STRENG, A. Further experiments on olfactory navigation and non-olfactory pilotage by homing pigeons. *Behav. Ecol. Sociobiol.*, **32**: 387-390, 1993.

64. WIERASZKO, A. and BALL, G.F. Long-term enhancement of synaptic responses in the songbird hippocampus. *Brain Res.*, **528**: 102-106, 1991.
65. WIERASZKO, A. And BALL, G.F. Long term potentiation in the avian hippocampus does not require activation of the N-methyl-D-aspartate (NMDA) receptor. *Synapse*, **13**: 173-178,1993.
66. WILTSCHKO, R. The role of experience in avian navigation and homing. Pp. 250-269. In: BERTHOLD, P. (Ed.), *Orientation in Birds*. Basel, Birkausser Vergal, 1991.
67. WILTSCHKO, R. and WILTSCHKO, W. Pigeon homing: change in navigational strategy during ontogeny. *Anim. Behav.*, **33**: 583-590, 1985.
68. WILTSCHKO, W. and BALDA, R.P. Sun compass orientation in seed-caching Scrub Jays (Aphelocoma coerulescens). *J. Comp. Physiol. A*, **164**: 717-721, 1989.
69. ZEIER, H. and KARTEN, H. The archistriatum of the pigeon. Organization of afferent and efferent connections. *Brain Res.*, **31**: 313-326, 1971.