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Sex steroid-induced neuroplasticity and behavioral activation in birds

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Abstract

The brain of adult homeothermic vertebrates exhibits a higher degree of morphological neuroplasticity than previously thought and this plasticity is especially prominent in birds. In particular, incorporation of new neurons is widespread throughout the adult avian forebrain and the volume of specific nuclei varies seasonally in a prominent manner. We review here work on steroid-dependent plasticity in birds based on two cases: the medial preoptic nucleus (POM) of Japanese quail in relation to male sexual behavior and nucleus HVC in canaries that regulates song behavior. In male quail, POM volume changes seasonally and in castrated subjects testosterone almost doubles POM volume within two weeks. Significant volume increases are, however, already observable after one day. The Steroid Receptor Coactivator-1 is part of the mechanism mediating these effects. Increases in POM volume reflect changes in cell size or spacing and dendritic branching but are not associated with an increase in neuron number. In contrast, seasonal changes in HVC volume reflect incorporation of newborn neurons in addition to changes in cell size and spacing. These are induced by treatments with exogenous testosterone or its metabolites. Expression of doublecortin, a microtubule-associated protein, is increased by testosterone in HVC but not in the adjacent nidopallium suggesting that neuron production in the subventricular zone, the birthplace of newborn neurons, is not affected. Together these data illustrate the high degree of plasticity that extends into adulthood and is characteristic of avian brain structures. Many questions do still remain concerning the regulation and specific function of this plasticity.

Keywords

Japanese quail; Songbirds - Sexual behavior; Preoptic area; Song control system; HVC; Doublecortin

Steroids, brain plasticity and behavior

The analysis of the interrelationships among hormones, brain and behavior has now been a vibrant field of study for more than half a century and has identified a variety of brain mechanisms that seem to generalize to most vertebrates (Beach 1981). Behavioral neuroendocrinology, as such an analysis is now commonly termed, has attracted a large number of scientists coming from the ethological tradition who always studied a diversity of

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animal species, including birds in particular (Marler, 2005). There are many advantages to studying birds (Konishi *et al.*, 1989; Wingfield, 2005) including the fact, highly relevant for the present topic, that they often display prominent annual cycles of reproductive activity. In many species living in the temperate zone, gonadal weight varies more than ten fold and sometimes close to one hundred fold between periods of sexual quiescence in the winter or the summer molt and during the spring time when reproduction takes place. Concentrations of sex steroids similarly display marked seasonal variations and, as a consequence, changes of very large amplitude are also observed in a variety of steroid-dependent responses (Wingfield & Farner, 1993; Dawson *et al.*, 2001; Ball & Balthazart, 2002; Wingfield & Silverin, 2002).

It is only recently that scientists have recognized the existence of significant morphological and physiological brain plasticity in adult homeothermic vertebrates. Physiological plasticity generally refers to enduring changes in synaptic physiology that can occur after different patterns of stimulation. Long-term potentiation (LTP) and long-term depression (LTD) are two well-known examples of this phenomenon (Bear & Malenka, 1994; Huang, 1998; Malenka & Bear, 2004). Morphological plasticity refers to measurable changes in morphology including cell size, cell number, cell shape and the connectivity among brain nuclei. Although variation in synaptic plasticity has been documented in birds (Wieraszko & Ball, 1991; 1993; Ding & Perkel, 2004; Meitzen *et al.*, 2009), this review will focus on morphological plasticity and focus on work completed in our labs in recent years.

The lack of substantial behavioral recovery after brain damage produced by accidental trauma or neurodegenerative diseases was, for a long time, taken as evidence for the absence of marked plasticity in the adult central nervous system of many vertebrate species including humans. It has, however, been acknowledged during the last few decades that mature adult neurons in the adult mammalian brain change their size and anatomical relationships with adjacent glial cells or reorganize their dendritic tree within hours to days (e.g., Theodosis & Poulain, 1987; Garcia-Segura *et al.*, 1988; Theodosis & Poulain, 1992). While neurogenesis was previously thought to be completely absent in adult homeothermic vertebrates, a limited amount of neurogenesis has been identified in specific brain regions of the adult mammalian brain (Altman & Das, 1965; Altman, 1969; Kemperman, 2006; Gould, 2007), possibly including humans (Eriksson *et al.*, 1998; Bhardwaj *et al.*, 2006). Neurogenesis and neural plasticity are, to an even greater extent, also observed in birds and several aspects of this plasticity seem to be related to the prominent annual cycles of gonadal activity.

In this review, we summarize work from our laboratories on two quite different neural systems displaying such prominent steroid-dependent seasonal plasticity: the medial preoptic nucleus (POM) located at the boundary of the telencephalon and diencephalon in Japanese quail (*Coturnix japonica*) and the telencephalic nucleus HVC (used as a proper name; see Reiner *et al.*, 2004) in canaries (*Serinus canaria*). These two nuclei vary in size as a function of the environmental or endocrine conditions to which the subjects are exposed. The functions of these morphological changes are obviously quite diverse. The preoptic region regulates a diverse set of behaviors and physiological processes but our studies in quail of the POM, a well-defined nucleus in the preoptic region, have focused on the role played by this area in the activation by testosterone of an instinctive “innate” behavior, male copulation (Panzica *et al.*, 1996b; Balthazart & Ball, 2007). In contrast, testosterone action in HVC modulates aspects of singing (Nottebohm *et al.*, 1976; Bottjer & Johnson, 1997; Fee *et al.*, 2004; Meitzen *et al.*, 2007), a learned behavior used in the defense of the breeding territory and/or the attraction of a sexual partner (Collins, 2004). Testosterone action in this nucleus therefore seems to be related to song behavior, especially the quality of song produced (Brenowitz, 2008). The cellular mechanisms mediating plasticity in these two

nuclei are additionally quite different and a major part of this review will be devoted to contrasting these two examples of hormone-regulated brain plasticity.

Plasticity in the medial preoptic nucleus of Japanese quail

Seasonality in Japanese quail

Japanese quail breed seasonally with a relatively long breeding season that in the north temperate zone can last from March until October (Robinson and Follett, 1982). They are highly photoperiodic and they respond to increased daylengths (i.e. photoperiods longer than 12.5L:11.5D) with a dramatic growth of the testes (Follett, 1976). They remain photosensitive as long as day length remains long and only regress their gonads when the photoperiod duration decreases in the fall (they show relative photorefractoriness; Robinson and Follett, 1982; Follett, 1984). Although temperature only plays a minor role in the control of seasonal cycles, cold temperatures have been shown to precipitate gonadal regression under photoperiods that are close to the stimulatory threshold (Tsuyoshi and Wada, 1992). A male may attain his maximal reproductive development in response to photoperiod alone and no additional effect of the presence of females on gonadal development can be detected (Delville *et al.*, 1984). However, we showed previously that sexual maturation occurs more rapidly in young males exposed to marginally stimulating photoperiods (12L:12D) if they are raised in the presence of females (Delville *et al.*, 1984).

The medial preoptic nucleus: a key center in the control of male reproductive behavior

The medial preoptic nucleus (POM) is a dense cluster of neurons situated on both sides of the third ventricle at the level of the preoptic area, the most rostral part of the diencephalon that is functionally associated with hypothalamic activity (Butler & Hodos, 2005). The POM extends in the rostro-caudal direction through most of the preoptic region, starting a few hundred microns caudally to the tractus septopallio-mesencephalicus and ending at the level of the anterior commissure (Fig. 1A)(Panzica *et al.*, 1996b). Working in collaboration with the laboratory of Gian Carlo Panzica and Carla Viglietti-Panzica at the University of Turin in Italy, we discovered in the mid 1980s that the volume of this nucleus based on standard Nissl-staining methods is significantly larger in male than in female quail (Viglietti-Panzica *et al.*, 1986).

Investigations were then initiated to identify the mechanisms controlling this brain sex difference and its functional significance. The presence of a sexually differentiated structure (volume larger in males than in females) that was also shown to be sensitive to testosterone indeed suggested that the POM might play a role in the control of male copulatory behavior in quail (which is present in males but not females, suppressed by castration and increased by exogenous testosterone).

Anatomically the POM is well positioned to regulate male-typical sexual behaviors in that it is located in the middle of the preoptic area that is known to be a key area for the activation of male sexual behavior in mammals as well as in birds (Larsson & Heimer, 1964; Lisk, 1967; Barfield, 1969; Hutchison, 1971; Balthazart *et al.*, 2004; Balthazart & Ball, 2007). A suite of experiments based on electrolytic lesions, stereotaxic implantation of testosterone, histological reconstruction of the lesion/implantation sites and correlation of these sites with behavioral activity, and finally identification of the inputs and outputs of the nucleus by retrograde and anterograde tract tracing clearly demonstrated during the next 10 years that the POM is part of a complex neural circuit that controls the expression of male sexual behavior in quail (see Panzica *et al.*, 1996b; Balthazart & Ball, 2007 for review).

This work has been reviewed generally on several occasions in the past (Panzica *et al.*, 1996b; Balthazart and Ball, 1998a) and, more recently, more specialized reviews have

appeared that have focused on the role of the preoptic aromatase activity in the control of sexual behavior (Balthazart *et al.*, 2004), on the neuroanatomical circuit in which POM is integrated (Ball and Balthazart, 2004) or on the functional specialization of sub-regions in the POM and their homology with the medial part of the preoptic area in other vertebrate species (Balthazart and Ball, 2007). Due to space limitations, all this information will not be reviewed again here but we will briefly recapitulate some of the key findings so that the reader can better appreciate the functional implications of the morphological plasticity of the POM that is the focus of the present review. References to the original papers establishing these conclusions in quail can be found in the above-mentioned review papers and will therefore not be listed here in detail.

One can enumerate at least 10 key finding relating to POM connectivity or function that highlight the importance of this nucleus in the control of sexual behavior and demonstrate its similarity and presumably homology with the medial part of the preoptic area in mammals and other tetrapods, namely:

1. Electrolytical lesions of the quail POM block or delay activation of male copulatory behavior,
2. Conversely, stereotaxic implantation of crystalline testosterone in the POM activates male copulatory behavior in castrated male quail. Therefore the POM is a critical, necessary and sufficient site for the activation of male copulation by testosterone like the medial preoptic area of mammals and reptiles (Baum, 2002; Hull, 2005; Hull and Rodriguez-Manzo, 2009),
3. Stereotaxic implants of aromatase inhibitors in POM inhibit male copulatory behavior. Testosterone must therefore be aromatized into an estrogen within POM itself to exert its behavioral effects,
4. Estrogens produced by testosterone aromatization act locally in the POM to activate copulatory behavior and accordingly, large numbers of neurons in the POM express high densities of estrogen receptors of the alpha and of the beta subtype. The same endocrine specificity has been demonstrated in rats and various species of mammals (Naftolin and MacLusky, 1984; Lephart, 1996; Celotti *et al.*, 1997)
5. Testosterone and its metabolite, estradiol increase aromatase activity by enhancing the transcription of the corresponding gene and thus the concentration of the enzymatic protein. This control of aromatase activity by sex steroids contributes to the long-term (seasonal) changes in quail sexual behavior (Balthazart and Ball, 1998b; Balthazart and Ball, 2006).
6. Additionally, the preoptic aromatase activity is also regulated by calcium-dependent phosphorylation processes that modify enzymatic activity within minutes without changing the concentration of the enzymatic protein. The resulting rapid local changes in estradiol bioavailability modulate sexual behavior expression on a short-term scale (within 15–30 min) and this locally produced estradiol is therefore acting more like a neurotransmitter/neuromodulator than as a classical hormone (Balthazart and Ball, 2006),
7. The POM receives inputs from multiple brain areas and presumably receives in this way indirect inputs from multiple sensory modes (olfactory, visual, acoustic, ...),
8. The POM also sends outputs to various brain structures including the mesencephalic periaqueductal gray that is well established in mammals as a pre-motor center involved in a variety of motor responses, including sexual responses (Rizvi *et al.*, 1992; Rizvi *et al.*, 1996; Bandler and Shipley, 1994; Murphy *et al.*,

1999). POM is thus, like the medial preoptic area of rats and other mammals, ideally positioned to integrate sensory inputs, endocrine signals and generate pre-motor outputs (Simerly and Swanson, 1986),

9. The POM is also a key site for the activation by sex steroids of appetitive aspects of male sexual behavior,
10. There is an anatomical specificity within POM between subregions that are more directly connected to the activation of appetitive sexual behavior and of copulation per se (consummatory sexual behavior). The rostral part of the POM appears to be more directly involved in the former while the caudal part of the nucleus would rather be functionally related to the latter aspects of this behavior. A similar distinction has been suggested in other vertebrate species including namely, song sparrows (Riters et al., 2004), rats (Van de Poll and Van Dis, 1979; Lisciotto and Morrell, 1994) and hamsters (Swann, 1997; reviewed in Balthazart and Ball, 2007).

Control by steroids of the sex difference in POM volume

Contrary to what had been described for the sexually dimorphic nucleus (SDN) of the preoptic area in rats where the volumetric sex difference is the result of a differential organization by steroids during the embryonic-early neonatal period (Gorski, 1984), the sex difference affecting the quail POM volume reflects a differential activation of this brain area by testosterone in males and females. Castration/ovariectomy reduces its volume to basal values in both sexes and treatment with a same dose of testosterone markedly increases this volume in both sexes (+40% in males; +120% in females) to levels typical of sexually mature males (Fig. 2B left; Panzica *et al.*, 1987; Panzica *et al.*, 1991). POM volume in males also varies with the duration of the photoperiod to which birds were exposed, the volume is small under short-days and significantly increases under long days that stimulate the secretion of testicular testosterone (Panzica *et al.*, 1987; Thompson & Adkins-Regan, 1994). Additionally, the volume of this nucleus is not affected by injections of estradiol benzoate in embryos (Panzica *et al.*, 1987), a treatment that markedly and irreversibly modifies the behavior of the birds hatched from these treated eggs (Adkins, 1975; Balthazart *et al.*, 1992). The quail POM is thus, from this point of view, more similar to the rat medial preoptic nucleus, MPN (Bloch & Gorski, 1988; Balthazart & Ball, 2007) than to the rat SDN: the volume of both nuclei (POM & MPN) is affected by steroids during adult life and their volumetric sex difference does not result from organizational effects of steroids taking place during early life. POM and MPN based on their anatomical position and other features are presumably homologous structures but POM is not homologous to the rat SDN (Balthazart & Ball, 2007).

Cellular mechanisms underlying POM plasticity

Another line of research investigated the cellular bases of changes in POM volume triggered by exposure to testosterone. Analyses of Nissl-stained sections showed that, in males, exogenous testosterone treatment of castrates increased the neuronal cross-section area increased by 36 % (Panzica *et al.*, 1991). Assuming a nearly spherical shape of the neurons, this 36% increase in surface would correspond to a 58% change in volume and could thus explain the global increase of the volume of the nucleus (Fig. 1B right). Surprisingly, however, little change in neuronal size was observed in females after testosterone treatment while the total volume of the POM more than doubled (Panzica *et al.*, 1991). Another mechanism should thus be involved in females to mediate these volumetric changes. Preliminary data had suggested that testosterone also produces an enlargement of the glial compartment that could potentially explain the increase in POM volume observed in females (Viglietti-Panzica *et al.*, 1991; Aste, N., Castagna, C., Balthazart, J. and Panzica, G.C.,

Unpublished data). This line of research was, however, never continued and the mechanisms underlying testosterone-induced changes in the female POM remain unknown at this time.

Changes in POM volume after treatment with testosterone were also accompanied by significant changes in the optical density of Nissl staining within the nucleus (Balthazart *et al.*, 1991). This suggested that the steroid was affecting the intracellular organization of neurons in the nucleus and presumably increasing protein synthesis in the nucleus. Changes in the ultrastructural organization of the POM after exposure to testosterone were therefore investigated by quantitative electron microscopy. Testosterone was shown to significantly increase the density of intracellular organelles involved in protein synthesis and secretion such as ribosomes, rough endoplasmic reticulum, Golgi complexes and vesicles (Panzica *et al.*, 1996a). Focusing on synaptic contacts, another study determined that in the POM of male quail gonadectomy decreases and testosterone treatment restores the number of axosomatic synapses per neuron without affecting the density of axodendritic and axospinal synapses. The cross sectional area of synaptic boutons was also decreased by castration and increased by testosterone (Castagna *et al.*, 1999). Changes in circulating testosterone concentrations are thus associated in the quail POM with a marked reorganization of synaptic contacts that presumably contributes, among other things, to the role played by the POM in the control of male copulatory behavior.

Although the POM displays a great deal of plasticity in response to steroids, no changes in neuronal numbers are expected in this nucleus during adulthood. Ongoing experiments on quail embryos indicate that neurons in POM are already post-mitotic by embryonic day 5 or 6 while glial cells become post-mitotic between days 12 and 14 (Bardet *et al.*, 2009; Mouriec *et al.*, 2010). No neurogenesis has been detected, under physiological conditions, in the preoptic area or hypothalamus of other bird species such as songbirds or ring doves (Alvarez-Buylla & Nottebohm, 1988; Cao *et al.*, 2002). Lesions to the ventromedial nucleus of ring doves, however, do induce a wave of neurogenesis that is critical for behavioral recovery (Cao *et al.*, 2002; Chen *et al.*, 2006). Similar experiments involving labeling new neurons with a marker of cell division such as tritiated thymidine or bromodeoxyuridine (BrdU) (these are thymidine analogs that are incorporated during the synthesis of DNA preceding mitosis) have not been specifically performed in quail but doublecortin, a specific marker of young neurons in birds and mammals (see following section on songbirds) is clearly not expressed in the adult quail preoptic area while this marker is quite abundant in the telencephalon. This confirms the presence of neurogenesis in the telencephalon but its complete absence in the diencephalon and in the POA (Balthazart, J. and Charlier, T.D., unpublished data, see figure 2).

Steroid action in the POM is also associated with pronounced neurochemical changes. The quail POM is for example outlined by a dense cluster of neurons immunoreactive for the enzyme aromatase that catalyzes the transformation of androgens such as testosterone into estrogens such as 17 β -estradiol (Balthazart *et al.*, 1996b). The number of these aromatase-immunoreactive (ARO-ir) neurons markedly changes in parallel with circulating concentrations of testosterone (Fig. 1C). Castration reduces this number 3 to 4 fold and this decrease is completely reversed by treatment with exogenous testosterone (Fig. 1D left; Aste *et al.*, 1994; Balthazart *et al.*, 1996b). The effects are more prominent in the lateral as compared to the medial part of the nucleus (Fig. 1D right; Aste *et al.*, 1994). The lateral POM seems to be preferentially involved in the activation of male copulatory behavior and this represents one of the multiple arguments supporting the notion that aromatase activity in the preoptic area is one key factor limiting the action of testosterone on male sexual behavior (see Balthazart & Ball, 2007 for discussion).

Similar testosterone-dependent changes also affect other neurotransmitter or peptidergic systems such as the innervation of POM by vasotocinergic fibers or by neurotensin. This work has, however been reviewed before (Panzica *et al.*, 1990; Panzica *et al.*, 1996b; Aste *et al.*, 1997) and will not be discussed here again.

Effects of testosterone require recruitment of the steroid receptor coactivator-1 (SRC-1)

Many effects of steroid hormones are mediated via the activation of specific receptors that are part of the nuclear receptors superfamily (Tsai & O'Malley, 1994; Mangelsdorf *et al.*, 1995). Upon binding to their ligand, these receptors experience a number of biochemical changes and recruit a series of specific proteins that enhance (activators) or repress (inhibitors) gene transcription (McKenna *et al.*, 1999; McKenna & O'Malley, 2002; Tetel *et al.*, 2009).

In quail specifically, recent experiments showed that the behavioral and neurochemical effects of testosterone and its metabolite estradiol in the POM depend on the presence of the steroid receptor coactivator-1 (SRC-1). Inhibition of the expression of SRC-1 by intracerebroventricular injection of antisense oligodeoxynucleotides partly blocked the activation of androgen-dependent (strutting, crowing) and estrogen-dependent (the copulatory sequence *sensu stricto*) aspects of male sexual behavior (Charlier *et al.*, 2005). Interestingly the infusion of SRC-1 antisense in the third ventricle also blocked to a large extent the testosterone-induced increase of POM volume, of the number and density of ARO-ir cells and of the density of VT-ir fibers (Charlier *et al.*, 2005). The reduction of sexual behavior induced by the down-regulation of SRC-1 expression was thus associated with significant neuroanatomical and neurochemical changes in the preoptic region.

Subsequent experiments have shown, however, that significant decreases in copulatory behavior can be obtained by infusion of SRC-1 antisense in the third ventricle without affecting the expression of aromatase in this brain area (Charlier *et al.*, 2006). The only possible interpretation of this dissociation between the effects of testosterone on male copulatory behavior and on the anatomical and neurochemical plasticity in the POM is that the activation of copulatory behavior requires a large variety of neurochemical effects in the POM, some identified (aromatase or VT induction) and others not, and that inhibition of one or several components of this network is sufficient to block behavior. Our first experiment suggested that the inhibition of male sexual behavior following injections of SRC-1 antisense was mediated, at least in part, by the decrease of preoptic aromatase expression. However, the two subsequent experiments indicated that sexual behavior is inhibited by injections of SRC-1 antisense in the absence of detectable changes in measures of brain aromatase activity or in the volume of the POM. It is thus likely that the antisense treatment additionally affected the control by steroids of other neurochemical systems of the preoptic area besides aromatase (e.g., various neuropeptides or neurotransmitters) and that these changes played a major role in the suppression of behavior following SRC-1 depletion (see Charlier *et al.*, 2006 for discussion).

Morphological plasticity in POM is unusually fast

In these SRC-1 experiments, separate groups of birds were injected with SRC-1 antisense oligonucleotides (AS group) or with scrambled oligonucleotides (SC group) as a control. After the final behavioral test, brains were collected for histological analysis as described above. A sub-group of birds from the AS group were however kept alive to test whether their behavior would recover if AS injections were interrupted and replaced with SC injections (AS-SC group). Quite interestingly, the behavior of these birds recovered very rapidly so that only two days after the interruption of the AS treatment, their sexual behavior

was expressed with frequencies equal to or exceeding those of control SC animals (Charlier *et al.*, 2005; Charlier *et al.*, 2006).

Western blot analysis showed that, in the first of these experiments, the AS-SC birds had, after two days, a stronger expression of SRC-1 compared to control AS animals, probably as a result of compensatory mechanisms activated during the antisense treatment. Additionally, the neuroanatomical and neurochemical effects of the antisense treatment were no longer apparent 2 days after the cessation of the antisense injections. The volume of the POM actually had increased by more than 60 percent within these two days, suggesting the existence of an unexpectedly rapid neuroplasticity (Charlier *et al.*, 2005).

We wondered whether this rapid neural reaction was linked to aspects of the SRC-1 modulation or reflected the normal rate of testosterone-induced effects on POM volume. In previous work, the effects of testosterone on POM volume were only quantified after exposure to the steroid for one or two weeks (Panzica *et al.*, 1987; Panzica *et al.*, 1991), which was the typical latency employed to investigate steroid hormone effects on brain plasticity. No information was thus available on the real time-course of testosterone action on POM volume and we decided to investigate this question experimentally.

Brains from castrated male quail were collected after 1, 2, 7 and 14 days of testosterone treatment (CX+T) and compared to brains of untreated castrates (CX) collected after 1 or 14 days (Charlier *et al.*, 2008). POM volumes defined either by Nissl staining or by aromatase immunohistochemistry increased in a time-dependent fashion in CX+T subjects and almost doubled after 14 days of treatment with testosterone while no change was observed in CX birds (Fig. 3C, D, E). A significant increase in the average POM volume was detected after only one day of testosterone treatment. The optical density of Nissl and aromatase staining were also increased after one or two days of testosterone treatment. Activation of male copulatory behavior followed these morphological changes with a latency of approximately one day (Fig. 3A, B). This rapid neurochemical and neuroanatomical plasticity observed in the quail POM thus seems to limit the activation of male sexual behavior and offers an excellent model to analyze features of steroid-regulated brain structure and function that determine behavior expression. The mechanisms mediating this rapid plasticity remain unclear at this time and it is in particular unknown whether these mechanisms mediating changes in POM volume (increase in cell size, neuropil volume, dendritic branching, extracellular space?) are the same the first few days of testosterone action and later on.

To analyze the potential contribution of variations in extra- vs. intra-cellular space to the rapid increase in POM volume induced by testosterone, the brain of castrated male quail was repeatedly analyzed by *in vivo* diffusion-weighted magnetic resonance imaging (DW-MRI) before and after 1, 2, 7 and 14 days of exposure to the steroid (Van der Linden *et al.*, 2006). Coronal slices with an image resolution of 100×100×500 µm through the whole telencephalon were obtained and accurately co-registered to allow voxel-wise paired comparisons. Apparent Diffusion Coefficient (ADC) of the POM region significantly increased after one day of T treatment and this effect apparently persisted during the whole experiment. By contrast, no changes in ADC were detected in regions that are not supposed to be steroid-sensitive such as the nucleus rotundus or the nucleus mesencephalicus lateralis, pars dorsalis (Van der Linden *et al.*, 2006). These data, although preliminary, suggest that testosterone rapidly increases the extracellular water volume in POM specifically, as a result of either cell shrinkage or increase in the space between cells, so that changes in the ratio of extra- to intra-cellular water mediate, at least in part, the fast plasticity of the POM volume observed after exposure to testosterone. This interpretation is, however, tentative and based only on the most plausible interpretation of the changes in ADC. It should be confirmed by histological work analyzing more directly the cellular changes that are triggered in the short-

term by testosterone. Increases in cellular volumes or in cell spacing could also explain rapid increases in POM volume.

More work is obviously needed to specify the cellular mechanisms that mediate the fast and slower plasticity induced by steroids in the POM. It is clear however that this plasticity is not based on adult neurogenesis involving neuronal death following decreases in testosterone concentration and neurogenesis in subjects exposed to higher concentrations of the steroid. Importantly, these rapid plastic changes in POM might not be an isolated phenomenon at least in birds. Evidence has been accumulating indicating that testosterone can also influence other aspects of brain morphology much more rapidly than previously thought, namely in songbirds (Bernard *et al.*, 1997; Tramontin *et al.*, 2000; Thompson *et al.*, 2007).

Seasonal changes in the song control circuit of songbirds

The analysis of mechanisms that control singing behavior in songbirds led to the discovery in the mid 1970s of a complex network of interconnected nuclei that control both the acquisition (learning) and expression of song (Nottebohm *et al.*, 1976). This so-called song control system consists of at least two major pathways. One pathway HVC→RA→ICo→nXIIts (HVC= proper name, previously acronym of High Vocal Center, RA= nucleus robustus of the arcopallium, ICo= midbrain nucleus intercollicularis; nXIIts= motor nucleus of the 12th nerve innervating the syrinx) is clearly involved in song production based on lesion, electrophysiological and immediate early gene induction studies (Nottebohm *et al.*, 1976; Jarvis & Nottebohm, 1997; Kimpo & Doupe, 1997; Margoliash, 1997; Wild, 1997). The other pathway HVC→area X→DLM→LMAN→RA (area X is a specialized subregion of the medial striatum of the basal ganglia; DLM is the medial part of the dorsolateral anterior thalamic nucleus; LMAN in the telencephalon is the lateral part of the magnocellular nucleus of the anterior nidopallium) is required for song learning, maintenance, and feedback effects on song behavior (Bottjer & Arnold, 1997; Doupe & Solis, 1997; Jarvis *et al.*, 1998; see Brenowitz *et al.*, 1997; Zeigler & Marler, 2008 for general reviews on this brain circuit and its function).

Fernando Nottebohm discovered that several nuclei in this network display an unusual level of neural plasticity across seasons in male canaries, *Serinus canaria* (Nottebohm, 1981). In the past two or three decades, seasonal variation in the brain of songbirds such as canaries has thus emerged as one of the best model systems for the study of naturally occurring brain plasticity (Tramontin & Brenowitz, 2000). Seasonal changes in the song system were first described based on dramatic changes in nucleus volume (Nottebohm, 1981). In canaries, for example, the volume of song nuclei based on the reconstruction of Nissl-defined boundaries is twice as large in males collected in the spring than in those collected in the fall (Nottebohm, 1981).

These volumetric changes occur essentially and were studied in most detail in three nuclei: HVC, RA and area X. They were originally studied by standard general histology techniques such as the Nissl stain but were later confirmed and enhanced by chemical neuroanatomical techniques such as immunohistochemistry or quantitative receptor autoradiography for various neurochemical markers. These two techniques showed that the volume changes that had been identified either based on seasonal changes or on the manipulations of testosterone concerned populations of cells expressing specific markers such as alpha 2 adrenergic receptors (Bernard & Ball, 1995; Ritters *et al.*, 2002) or receiving a specific innervation by neurotransmitters (e.g., nuclei labeled by dense networks of fibers immunoreactive for tyrosine hydroxylase, the rate limiting enzyme for catecholamines, (Appeltants *et al.*, 2001).

Several of these nuclei and their seasonal variations in volume can also be identified by the presence of dense neuronal populations expressing androgen receptors (Soma *et al.*, 1999).

Seasonal changes identified by nuclear magnetic resonance imaging

More recently, the development of *in vivo* imaging techniques raised the possibility that song control nuclei could be visualized and measured without having to collect the brains of subjects, thus opening a whole range of new experimental possibilities including the measurement of brain changes within the same subjects. In collaboration with the Bio-Imaging lab headed by Professor Annemie Van der Linden at the University of Antwerp, Belgium, we explored the applicability of such techniques to the study of the song system. An *in-vivo* Magnetic Resonance imaging (MRI) procedure was developed on live anesthetized canaries to obtain three-dimensional high quality images of the entire brain with an image resolution of 78 microns. This technique provided images with a high anatomical resolution that allowed the delineation of a large number of brain structures but song control nuclei could not be identified in these images (Van der Linden *et al.*, 1998).

Several of these nuclei could later be visualized in starlings (*Sturnus vulgaris*), canaries and zebra finches (*Taeniopygia guttata*) by stereotaxic injections into HVC of manganese, a calcium analogue. Manganese is a paramagnetic tract-tracing agent that is taken up by cells at the site of injection and then transported anterogradely to the axon terminals. These terminals become densely labeled with manganese and after a few hours both RA and area X can be visualized by *in vivo* MRI. Structures highlighted by manganese accumulation assumed the expected three-dimensional shape of RA and area X previously identified by histological methods (Van der Linden *et al.*, 2002; Tindemans *et al.*, 2003; Van der Linden *et al.*, 2004; Van Meir *et al.*, 2004). The volume of these nuclei could be accurately measured and this could be done repeatedly in the same subjects thus allowing us for the first time to measure seasonal variations in volumes within particular individuals. Several studies using these techniques have now been published and were recently reviewed (Van der Linden *et al.*, 2004; Van der Linden *et al.*, 2009). It could, for example, be confirmed that testosterone increases RA and area X volumes in female starlings by imaging the same birds before and after exposure to the exogenous steroid (Van Meir *et al.*, 2004). This technique furthermore allowed the establishment of correlations between the changes in song control nuclei and the vocal behavior produced by the birds between two imaging sessions (Van Meir *et al.*, 2006).

More recent studies using another MRI technique called Diffusion Tensor Imaging (DTI) also identified in starlings an extensive seasonal plasticity in song control nuclei and in fibers tracts connecting them as well as in other fibers tracts that were not previously thought to be influenced by steroids (De Groof *et al.*, 2006; De Groof *et al.*, 2008; De Groof *et al.*, 2009).

Sex steroid hormones and song behavior

The relation between sex steroid hormone action and singing behavior was first discovered during field studies correlating seasonal changes in physiology with changes in song behavior. These data have been reviewed previously (see for example Ball, 1999) but some of the key features will be briefly summarized here. First, in many species of temperate-zone songbirds males sing at higher rates in the spring as compared to other seasons (e.g., Cox, 1944; Slagsvold, 1977; see Catchpole & Slater, 2008 for a review). These seasonal differences in male song are positively correlated with dramatic seasonal variations in aspects of reproductive physiology such as gonadal size and sex steroid secretion in the plasma (Wingfield & Farner, 1993). Note, however, that some songbird species sing at high rates outside the breeding season but still exhibit seasonal cycles in gonad activity that are

similar to other temperate-zone species. In such species there is little or no correlation between endocrine activity of the gonad and song. Studies in song sparrows (*Melospiza melodia*) indicate, however, that the brain in this species expresses a high activity of the enzyme 3 β -hydroxysteroid dehydrogenase (3 β HSD) that is able to catalyze the transformation of a steroid precursor of adrenal origin dehydroepiandrosterone (DHEA) into active androgens and estrogens (Soma *et al.*, 2008). The 3 β HSD activity increases in the fall at a time when plasma testosterone concentration decreases markedly but concentration of DHEA remains high (Pradhan *et al.*, 2010). Fall singing and the associated aggressive behavior can still be activated by sex steroid but these steroids are produced in the brain by local metabolism of an adrenal precursor rather than in the testes.

Song output is thus related to measures of reproductive physiology but there is not necessarily a strong causal relationship between changes in sex steroids concentrations and behavioral outputs such as described above for male-typical copulatory behaviors in Japanese quail (Balthazart & Ball, 1998a) or for lordosis behavior in female rats (Pfaff *et al.*, 1994). The hormonal control of song behavior appears to be a clear case of a hormone-enhanced behavior but other factors and stimuli promote song production in males. Gonadal steroids increase the probability and intensity of behavioral responses to the appropriate stimulus but their presence may not be essential for behavioral activation. One should thus not be surprised to occasionally observe substantial song production in association with low steroid hormone concentrations (see (Ball *et al.*, 2002; Ball *et al.*, 2004) for additional discussion).

The discovery of a dense expression of sex steroid receptors (androgen receptors [AR] and estrogen receptors of the alpha subtype [ER α]) provided a second type of argument suggesting a direct role of sex steroids in the control of song behavior (Arnold *et al.*, 1976; Nordeen *et al.*, 1987; Gahr *et al.*, 1993). The distribution of these receptors has now been studied in a variety of species by *in vivo* autoradiography, by immunohistochemistry of the receptor protein and by *in situ* hybridization histochemistry for the corresponding mRNA. These data have been reviewed on multiple occasions (Schlinger, 1997; Ball & Balthazart, 2002; Ball *et al.*, 2002; Ball *et al.*, 2004; Schlinger & Brenowitz, 2009) and all confirm the presence of AR in multiple song control nuclei including HVC, RA, LMAN, ICO and nXIIIts along with the presence of ER α in ICo and the HVC of some species (Gahr *et al.*, 1993).

Finally, castration of songbirds, associated or not with sex steroid replacement has been performed on a limited number of species but these studies all confirm the functional relationship between steroids and song behavior. For example, in zebra finches castration greatly reduces but does not eliminate male-typical song (e.g., Arnold, 1975; Harding *et al.*, 1983) while in red-winged blackbirds castration eliminates adult song production. Replacement studies also indicate that both androgenic and estrogenic metabolites of testosterone are needed to completely restore high rates of singing (e.g., Harding *et al.*, 1983; Harding *et al.*, 1988).

It is also important to note that endocrine manipulations affecting the rate of song production often, but not always, modify in parallel the volume of key song control nuclei such as HVC, RA and area X. The seasonal volumetric changes can often be mimicked in male song birds by castration, producing a regressed state of the nuclei, and testosterone replacement, resulting in the development of these nuclei into a condition similar to that observed during the spring reproductive period (see Ball *et al.*, 2004 for review).

Recent research employing local implants indicates that changes in multiple nuclei might relate to the direct action of testosterone on HVC which would then indirectly (transsynaptically) affect the growth of the two primary HVC targets, RA and area X (For

review, see: Brenowitz, 2008). These changes are usually assumed to take days to weeks to occur but morphological changes on a much faster time scale have also been reported (Tramontin *et al.*, 2000; Thompson *et al.*, 2007).

What are the cellular targets of steroid action?

Studies of North-American sparrow species (song and white-crowned sparrows) identified cellular mechanisms critically implicated in these volumetric changes and demonstrated that different processes mediate in different nuclei the seasonal and testosterone-induced changes in morphology. They include for all nuclei variations in neuronal soma size, in neuronal density (cell spacing per unit volume) and in the complexity of neuronal branching (see Brenowitz, 2004; 2008 for review). In HVC however, the cellular basis of these volumetric differences is also related to variation in neuronal number (Smith *et al.*, 1997; Tramontin & Brenowitz, 2000; Brenowitz, 2004). Thus an important process underlying volume changes in HVC is the incorporation of new neurons. No neurogenesis apparently takes place in RA and while there is evidence for adult neurogenesis in area X this process does not seem to change seasonally so it cannot be invoked to explain volumetric changes in this nucleus (Thompson & Brenowitz, 2005).

Neuronal incorporation seems to concern exclusively HVC neurons projecting to RA (Alvarez-Buylla *et al.*, 1988; Scharff *et al.*, 2000; Scotto-Lomassese *et al.*, 2007). Neurons projecting to area X are produced before hatching and not replaced in adulthood (Alvarez-Buylla *et al.*, 1988; Kirn *et al.*, 1999; Wilbrecht & Kirn, 2004). In the canary HVC approximately 50 to 60% of neurons project to RA while 20% project to area X and the rest of the population represents interneurons. It has been estimated that nearly 50 % of the neurons connecting HVC to RA are replaced during the six-month period between spring and fall (Wilbrecht & Kirn, 2004). This represents a truly unusual level of brain plasticity and neuronal turnover.

The discovery of a very high rate of neurogenesis in the canary HVC (and more generally in its telencephalon) (Goldman & Nottebohm, 1983; Alvarez-Buylla & Nottebohm, 1988) led to the reconsideration of what had been viewed as a dogma stating that no neurogenesis takes place in the brain of adult homeothermic vertebrates. This broadly accepted notion was then experimentally scrutinized and a significant amount of neurogenesis was also (re-)discovered in the brain of mammals (see Kemperman, 2006 for review) though this phenomenon is more restricted than in the avian brain. The regulation and significance of this adult neurogenesis are still being investigated today. In canaries specifically, understanding how environmental and endocrine factors facilitate the recruitment of new neurons into HVC represents a major goal of our research program and data collected to this date will be reviewed below.

Multiple cellular processes must take place before new neurons are incorporated into a functional network in HVC. Each of these events can potentially vary in a seasonal manner and be specifically controlled by the action of sex steroids (testosterone or its estrogenic metabolites) as well as by social and environmental factors that have been shown to affect HVC volume. The production of new neurons at the level of the ventricle wall involves the division of progenitor cells from the ventricle wall (vimentin-positive radial glial cells) that can then follow either a neuronal or a glial cell fate. These newborn neurons must migrate to their final destination (HVC in this case), and stop their migration at this target. They must finally differentiate from their migratory phenotype into a mature neuronal phenotype and establish axonal connection with their targets (in this case RA). They will then live with this phenotype for a certain time at this location before dying, often by apoptosis (See figure 4).

At any time, a variable number (N) of neurons (N1 to N5 in figure 4) will be present at each different stage. Not all neurons produced at the ventricle wall will migrate to HVC and only a fraction of them will be recruited and differentiate at this location. Furthermore, these neurons may exist at a given stage for a variable amount of time (t1 to t3 in figure 3). The intersection of these two sets of parameters (numbers and time) obviously creates a complex dynamical process that contributes to the seasonal fluctuations of HVC volume. This process is difficult to study in physiologically relevant conditions but by studying the fate of neurons labeled with tritiated thymidine during their last DNA synthesis phase a substantial amount of useful information has already been collected.

Initial time-course studies indicated that one hour and one day after injections of tritiated thymidine all ^3H -labeled cells were found at the wall of the lateral ventricles. Elongated migrating cells began to appear in the areas adjacent to the ventricle three days later and then they progressively invaded most of the telencephalon as time progressed, suggestive of a migration wave. Between 20 and 40 days post injection, the undifferentiated elongated cells decreased in number while there was a concomitant increase in the number of ^3H -labeled differentiated neurons (Alvarez-Buylla & Nottebohm, 1988; Nottebohm, 2004). The number of labeled cells then decreased with time: for example the number of labeled neuron 40 days after the injections was only one third of the number of migrating cells that had been observed at day 20 (Alvarez-Buylla & Nottebohm, 1988).

Additional work using the same methods helped determine the survival time of these newborn neurons. The migration from the ventricle wall to HVC appears to take approximately one week but many cells reaching HVC die between the age of 2 and 3 weeks during the process of differentiation and while establishing connections to RA. If new RA projecting HVC neurons survive past their first month, they then have a life span ranging from 4 to at least 8 months (see Wilbrecht & Kirn, 2004 for review).

Several endocrine or environmental signals have now been shown to affect HVC size but there is still little information concerning the specific stage at which they act in the neurogenesis process. Original studies indicated that the number of labeled cells at the ventricle wall counted soon after an injection of ^3H -thymidine was not affected by testosterone treatment of female canaries suggesting that the steroid was not affecting the rate of cell division (Rasika *et al.*, 1994). The same study also indicated that testosterone increases the survival of these new neurons. In contrast, in one study of first year male starlings, we found that during the fall testosterone treatment increases the number of bromodeoxyuridine (BrdU)-labeled cells (another analog of thymidine used to label cells in their S phase just before mitosis) in the area immediately adjacent to the ventricular wall. The lack of effect of this steroid on cell proliferation in the ventricle wall might thus not apply to both sexes or to every species and physiological condition (Absil *et al.*, 2003a). In this study the counting area was positioned on the ventricular epithelium so that the vast majority of counted cells were within the proliferative area and included few or no migrating/differentiating neuron. However, since birds were killed two weeks after the last BrdU injection, it cannot be excluded that testosterone caused a differential migration away from the ventricle rather than a differential rate of proliferation. At the other end of a neuron's life cycle, it has been also established that the number of pyknotic cells in the HVC of canaries varies seasonally (Kirn *et al.*, 1994) and very elegant studies by Constance Scharff and collaborators actually demonstrated that neuronal death in HVC promotes the recruitment of new neurons in this nucleus (Scharff *et al.*, 2000). Many aspects of the factors controlling this process remain, however, unknown. Testosterone and other factors could obviously influence the rate of transition between different life stages of the new neurons (% moving from N1 to N2 to N3 to N4 to N5 in figure 4) as well as the length of time during

which these cells remain at a given stage (t1 to t3). Additional work would thus be in order to study these mechanisms.

Biochemical and cellular mechanisms

The intracellular signals that are activated by testosterone or environmental factors to promote cell division, migration, recruitment, differentiation and survival have been only partly identified. One mechanism through which testosterone promotes changes in the volume of the song control system is via enhancement of the secretion of the Brain Derived Neurotrophic Factor, BDNF (Rasika *et al.*, 1999). Indeed: testosterone increases BDNF concentration in the female canary HVC, HVC neurons express the BDNF receptor TrkB, infusion of BDNF in the absence of testosterone increases neuronal survival in HVC and finally infusion of an antibody to BDNF into HVC blocks the effects of testosterone on neuron survival (reviewed in Nottebohm, 2004). Testosterone action in HVC also seems sufficient to release BDNF in RA and cause via transsynaptic mechanisms the seasonal development of this nucleus (Wissman & Brenowitz, 2009).

Interestingly, the act of singing *per se* also increases BDNF secretion in HVC. This was originally demonstrated in the Nottebohm laboratory (Li *et al.*, 2000; Alvarez-Borda & Nottebohm, 2002) and we subsequently showed that various manipulations affecting the rate of singing independently of changes in plasma testosterone concentrations affect in parallel HVC growth and BDNF concentration in this nucleus (Sartor *et al.*, 2002; Sartor, 2004; Sartor & Ball, 2005). Therefore testosterone can act on neuronal survival in HVC in two complementary ways: a direct route in which testosterone increases BDNF expression (Alvarez-Borda & Nottebohm, 2002) and an indirect route in which testosterone increases singing motivation possibly through its action in the medial preoptic area (Riters & Ball, 1999; Riters *et al.*, 2000; Riters & Alger, 2004) which in turn increases song rate and BDNF secretion (reviewed in Ball *et al.*, 2004; Ball *et al.*, 2008).

More recently, it was also demonstrated that much of this BDNF production originates in endothelial cells surrounding blood vessels located within HVC and that testosterone markedly promotes the development of these blood vessels through an increase in Vascular Endothelial Growth Factor (VEGF) secretion that then acts on the VEGF tyrosine kinase receptor (VEGF-R) expressed by these endothelial cells (Louissaint *et al.*, 2002). This leads to a model of testosterone action on HVC growth (based mainly on experiments performed in females), which states that testosterone stimulates HVC growth by promoting an increased transcription of VEGF, VEGF receptor and subsequently BDNF which enhances the recruitment and survival of new neurons in HVC. A recent study confirmed that inhibition of VEGF signaling through the VEGF receptor-2 blocks the effects of testosterone on song production and HVC morphology. Concurrent treatment with BDNF (through *in vivo* plasmid transfection) restored the effects of the steroid in agreement with the postulated sequence of events (Hartog *et al.*, 2009).

Migration signals: a role for reelin?

Studies described above tie the survival of new neurons to concomitant effects on HVC volume but do not discriminate between mechanisms that could lead to the presence of a higher number of newborn neurons in HVC. Specific mechanisms must indeed exist to control the migration of new neurons, instruct them to stop this migration in HVC and then promote their integration and survival into functional circuits. We are currently investigating in this context the presence and regulation in HVC and adjacent areas of proteins that are known to control this type of mechanism in the developing mammalian brain.

Reelin is a large glycoprotein, defective in reeler mice, that is assumed to play a key role in determining the final location of migrating neurons in the developing brain (Schiffmann *et al.*, 1997; Tissir & Goffinet, 2003; Forster *et al.*, 2006). The protein is secreted into the extracellular matrix and binds to the membrane receptors ApoER2 and VLDLR, which causes the association of an intracellular protein, Disabled-1 (Dab-1) to the intra-cytoplasmic tails of the receptors. The phosphorylation of Dab-1 is then the first event in a chain of reactions in the cytoskeleton of the neuron, ultimately resulting in the arrest of its movements and the determination of its final position (Forster *et al.*, 2006).

Since adult songbirds display an active neurogenesis so that new neurons must obviously find a final target, we hypothesized that reelin expression persists in the adult songbird brain. Our first study in male starlings demonstrated that reelin-immunoreactive (Re-ir) structures (cells and associated fibers) are indeed broadly distributed throughout the brain, especially in the lateral nidopallium with an especially dense cluster of reelin-ir cells and fibers present in HVC (Absil *et al.*, 2003b). Interestingly reelin expression in HVC was sharply decreased by a two week treatment with testosterone (Absil *et al.*, 2003b)(decrease in the fractional area covered by immunoreactive cells and fibers), a finding consistent with the idea that seasonal changes in reelin expression might modulate the arrest of migration and recruitment of new neurons within HVC (more neurons are incorporated during the fall when plasma testosterone levels are low (Kirn *et al.*, 1994) and reelin expression is presumably high).

Based on these findings, we initiated studies of reelin in canaries and surprisingly discovered a prominent species difference: in starlings, reelin expression is denser in HVC than in the surrounding nidopallium, but the opposite is observed in canaries where HVC is almost devoid of reelin-ir structures (Balthazart *et al.*, 2008b). Dab-1, the intracellular messenger of reelin was similarly expressed in lower density in HVC than in the adjacent nidopallium of the canary brain.

We then wondered whether three-dimensional changes in the structure of these two proteins possibly associated with their action could modify their immunoreactivity making, them undetectable so that they could in fact be present in HVC but undetectable by immunohistochemistry due to their activation. Analysis of the corresponding mRNA by radioactive *in situ* hybridization histochemistry, however, confirmed the lower expression of reelin and Dab-1 mRNA in HVC than in the nidopallium. So striking was the difference that it allowed this song control nucleus to be easily distinguished from the surrounding tissue in the corresponding autoradiograms (Balthazart *et al.*, 2008b).

In summary, these studies identified a major species difference affecting reelin expression in canaries as compared to starlings. In both species, reelin is expressed differently in HVC and in the surrounding nidopallium suggesting that it might play a role in neuronal recruitment. However, one species is a negative image of the other and while testosterone inhibited reelin expression in starlings, one experiment suggested the absence of any effect in canaries (Balthazart *et al.*, 2008b). It is therefore difficult to make specific predictions about what will be the role of this protein in songbirds until more studies have been carried out.

Doublecortin and neuronal recruitment in HVC

Neurogenesis, neuronal recruitment and survival have traditionally been studied as described above by techniques that label new neurons during their DNA synthesis phase before mitosis by injections of ³H-thymidine or more recently of bromodeoxyuridine (BrdU) that can later be visualized by immunohistochemistry. The numbers of labeled cells are then quantified after various survival times. In order to obtain with this approach detailed information on multiple aspects of the dynamics of new neurons' incorporation requires the use of multiple

groups of birds injected and killed at various times during an experiment that is designed to affect the number of HVC neurons. It is often difficult to determine whether a given factor has affected neurogenesis *sensu stricto* (the rate of division of progenitors) or neuronal migration and recruitment or survival, acknowledging that these different actions are not mutually exclusive. To be detected, a change in one of these processes must have occurred at the right time in relation to the initiation of the experimental treatment, the injection of BrdU and the time of brain collection from the subjects.

Several endogenous markers can be used to label new cells in the adult brain at various stages of their early life. This is the case for the nuclear protein Ki67 or the Proliferating Cell Nuclear Antigen (PCNA) that are expressed at specific stages of proliferation in progenitors and thus provide an index of the rate of cell proliferation in a given brain area (Kee *et al.*, 2002; Mandyam *et al.*, 2007). Doublecortin (DCX) is another of these endogenous markers, used to identify young neurons. DCX is a microtubule-associated protein and as such represents an integral part of the cellular machinery mediating neuronal migration (Bai *et al.*, 2003; Jin *et al.*, 2004; Moores *et al.*, 2004). The analysis of its expression can thus provide an additional window on the mechanisms mediating changes in HVC volume.

In the mammalian brain, DCX is expressed in post-mitotic young neurons during their migration and the early stages of differentiation. This expression then disappears in mature neurons (Francis *et al.*, 1999; Brown *et al.*, 2003; Rao & Shetty, 2004). Employing one or several of these endogenous markers allows progress in the understanding of the mechanisms mediating the incorporation of new neurons because experiments analyzing the effects of a given experimental factor only require taking into account the length of time between exposure to this factor and the time of brain collection from the birds. In contrast, looking at a similar problem with ³H-thymidine or BrdU labeling requires consideration of both the time since injection of thymidine/BrdU and the time since beginning of treatment. Furthermore, a marker like DCX provides an integrated view of all new neurons present at a given location at a given time while numbers of BrdU-positive cells only provide a snapshot of a selection of these cells that were born at a specific time (within a few hours after the injection, since BrdU is rapidly metabolized and thus labels cells during only a short period) (Packard *et al.*, 1973; Miller & Nowakowski, 1988; Boswald *et al.*, 1990).

Because the adult canary brain produces large numbers of new neurons we investigated whether these newborn neurons expressed DCX as is the case in the developing mammalian brain. DCX or its mRNA had been identified in the developing or early postnatal brain in chickens (Hannan *et al.*, 1999; Capes-Davis *et al.*, 2005) and zebra finches (Kim *et al.*, 2006) and our initial immunohistochemical studies indeed identified large populations of DCX-immunoreactive (ir) neurons in many areas of the canary brain (Boseret *et al.*, 2007). As is the case in mammals, we found two types of DCX-ir neurons. Some displayed a characteristic fusiform shape (Figure 4, bottom panel middle) and obviously represent migrating cells while others were observed during the early stages of differentiation when neurons acquire a multipolar shape (Figure 4, bottom panel right). Densely stained DCX-ir cells were found exclusively in parts of the telencephalon that are known to incorporate new neurons in adulthood, in particular the nidopallium. Within this brain region, the boundaries of the song control nucleus HVC could be clearly distinguished from surrounding structures by a higher density of DCX-ir structures.

DCX is a widely recognized marker of newborn neurons in the mammalian brain and it was expressed specifically in regions of the canary brain that display a high level of neurogenesis in adulthood (Boseret *et al.*, 2007). It was important though to confirm its specificity as a marker of new neurons in canaries. Therefore, in a subsequent experiment, two batches of

male canaries were injected with BrdU, their brains were collected either 10 or 30 days later and then sections through HVC were double-labeled by immunohistochemistry (Balthazart *et al.*, 2008a). A very large number of BrdU-labeled cell nuclei and DCX-ir cells were detected at the level of the ventricular wall and in the nidopallium, including HVC. At the 10-day time point more than 70% of the DCX fusiform cells in HVC clearly contained BrdU, demonstrating that the vast majority of these DCX fusiform cells represent new neurons that were born (or more precisely entered the DNA synthesis phase) during BrdU injections or very soon thereafter. From day 10 to day 30, the percentage of DCX-ir fusiform cells containing BrdU decreased, while the number of DCX-ir round cells containing BrdU increased (from 5 to 24%). These results are thus fully consistent with the notion that many, if not all, DCX-ir cells in the canary HVC represent neurons that went through their natal division (and DNA synthesis phase) shortly before brain collection.

We then investigated in several experiments changes in DCX expression in male canaries that were exposed to physiological situations that are known to affect HVC volume (Balthazart *et al.*, 2008a). As illustrated in figure 5, the number of round DCX-ir cells was increased specifically in the HVC of males treated with testosterone as compared to control castrates. In castrated males in which plasma testosterone concentrations were clamped to a stable level by a subcutaneous implant of testosterone, the number of round DCX-ir cells in HVC was higher in birds living in a cage with a female (M-F) as compared to males paired with another male (M-M). Elongated fusiform DCX-ir cells that are presumably migrating neurons were also more numerous under these conditions (Testosterone-treated > Control and M-F>M-M).

In another experiment, we also found that round multipolar DCX-ir cells that represent differentiating neurons were more numerous in photostimulated and in photosensitive non-photostimulated birds than in matched photorefractory subjects (Balthazart *et al.*, 2008a; Data not shown). In all these cases, the changes in DCX-ir cell numbers paralleled the changes in HVC volume (see left two columns in figure 5 for the first two of these experiments). These studies thus demonstrate that, in canaries, the endocrine state (Testosterone-treated vs. Control) as well as the social (M-F vs. M-M) or photoperiodic condition (photosensitive/photostimulated vs. photorefractory) affect the expression of a protein involved in neuronal migration specifically in brain areas that incorporate new neurons in parallel with changes in HVC size. The DCX gene may thus be a target by which testosterone and social/environmental stimuli induce seasonal changes in the volume of song nuclei.

Quite interestingly, all these treatments generally did not affect the numbers of fusiform or round DCX-ir cells located in the nidopallium adjacent to HVC (sampled area of the same surface just lateral or ventral to the song control nucleus; see figure 5). The anatomical specificity of these experimental effects (see in but not around HVC) provides important information concerning the mechanisms that mediate the increases in HVC volume.

New neurons incorporated in HVC and in these adjacent areas presumably originate from broad sections of the lateral ventricle but their specific origin has not been univocally identified at this time. Cell divisions are not equally distributed along the wall of the third ventricle and areas of dense mitotic activity, termed "hot spots" (Alvarez-Buylla *et al.*, 1990) have been identified. It is not currently known whether HVC neurons preferentially originate in these "hot spots". Time-course studies after BrdU injections suggest that in the coronal plane newborn cells progressively invade the entire nidopallium from its most medial to most lateral aspects (Alvarez-Buylla & Nottebohm, 1988). In addition, a recent BrdU study indicated that HVC recruits similar numbers of migrating neurons as surrounding brain tissue (Vellema *et al.*, 2010) and mathematical modeling suggests that

recruitment of cells in and around HVC is best described by a random displacement from broad sections of the ventricle rather than by a directional migration perpendicular to the overlying ventricle wall (Vellema *et al.*, 2010).

Analysis of DCX-ir cells in our experiments (Balthazart *et al.*, 2008a) lead to similar conclusions. In the control condition of the testosterone experiment, a similar number of DCX-ir cells (round or fusiform) was found in, lateral, or ventral to HVC (Figure 5 top). After treatment with testosterone, an increase in both types of DCX-ir cells (fusiform and round) was found specifically in HVC but the treatment did not affect adjacent areas. Given that new neurons apparently reach HVC and adjacent tissue by random movement, these data indicate that the increase in HVC volume and in the number of DCX-ir cells in the nucleus cannot be the result of an effect of the steroid on the production of new neurons (otherwise a similar effect would be seen in adjacent areas) but should result from a direct effect of testosterone on the recruitment of new neurons in the nucleus.

A similar situation was observed for round DCX cells in the experiment comparing birds housed in M-M vs. M-F pairs. The numbers of DCX-ir cells were similar in and around HVC in the M-M condition and the increase in HVC volume observed in the M-F condition was associated with a specific increase of DCX-ir round cells in HVC but not in areas ventral or lateral to this nucleus. This again indicates a specific effect of the social condition on the recruitment and/or differentiation of new neurons in the song control nucleus. In contrast, the data for fusiform cells is slightly more complex in that changes in numbers of DCX cells seem to take place both in and around the nucleus. It must be noted, however, that these effects are statistically significant only for cell numbers in HVC. Additionally, this experiment lacked a control group raised in isolation so that it is impossible to know whether housing in M-F pairs increased HVC volume and numbers of DCX-ir cells or conversely whether housing in M-M condition decreased these measures. This absence of a control group complicates the interpretation of the cell dynamics that were observed. Additional work is in progress to address these questions.

In a final experiment, we tested whether testosterone or its androgenic (e.g. 5 α -dihydrotestosterone, DHT) and estrogenic (estradiol, E₂) metabolites are responsible for the changes in HVC volume and the associated changes in numbers of DCX-ir cells (Yamamura *et al.*, 2009). This clearly indicated that the synergistic action of DHT and E₂ is required to mediate these effects, each metabolite in isolation being partly effective or not effective at all (Sartor *et al.*, 2005; Yamamura *et al.*, 2009). More central to the present discussion, this experiment confirmed that effects of testosterone on the numbers of fusiform or round DCX-ir cells are observed specifically in HVC and do not affect adjacent areas. This provides strong support for the notion that testosterone acts specifically on the recruitment and differentiation of new neurons in HVC without affecting their rate of production at the ventricular level. Interestingly, testosterone also increases the total number of pyknotic cells in the entire HVC suggesting that the steroid might also affect the survival rate of neurons in this nucleus (Barker *et al.*, 2010). Studies based on more specific markers of apoptosis (e.g., activated caspase-3 immunocytochemistry, TUNEL labeling) should, however, be performed to confirm this conclusion.

General conclusions

This review of two types of morphological changes affecting nuclei implicated in the control of species-specific behaviors clearly highlights the high degree of morphological neuroplasticity exhibited by the avian brain in response to changes in the steroidal or social environment. We know at this point that many of the molecular markers of this neuroplasticity are homologous in birds and mammals (e.g. markers of proliferation such as

PCNA, proteins present in young neurons such as DCX, markers of apoptosis such as activated caspase-3) so that the brain of avian species represents an outstanding experimental model to analyze the underlying cellular and molecular mechanisms that mediate this plasticity. These similarities also suggest that the mechanisms that will be identified are likely to be transposable to mammals.

The neuroplasticity described here can be extremely rapid: significant changes in POM volume are observed after 24 hours of exposure to testosterone and similarly rapid changes in HVC size have been reported: a significant decrease in HVC volume is already observed 12 hours after castration and removal of a testosterone subcutaneous implant (Thompson *et al.*, 2007). The cellular mechanisms mediating such rapid changes in brain structure remain unknown. We have collected preliminary data indicating a potential role of the redistribution of intra- vs. extra-cellular water in POM but these changes need to be confirmed by more extensive work. In the longer term, variation in water distribution must obviously be consolidated by more stable changes in cell size, structure and eventually numbers. In the quail POM, neurogenesis does not mediate the variations in global volume of the nucleus but, in contrast, seasonal variations in the incorporation of new neurons does seem to contribute very significantly to the control of HVC volume in songbirds. These changes in neurogenesis can be directly induced by sex steroids but steroid-independent effects resulting from modifications in the social environment or from the act of singing itself have also been described indicating that a complex network of chemical and environmental factors can indeed modulate neurogenesis in the adult brain. The prominence of this phenomenon in birds makes them outstanding model systems for such studies.

The cellular mechanisms that mediate changes in neuron numbers within a given structure are equally complex. Current data suggest that, in the song control nucleus HVC, steroids mainly affect the numbers of neurons that are being recruited and differentiate within the nucleus with possible concurrent changes in cell death. Current studies do not, however, discriminate between effects that would increase the attraction of new neurons in the nucleus and effects that would lengthen the duration during which these cells are observed at a given stage (changes in N_x mediated by changes in t_x in figure 4). Detailed time-course studies combining labeling of dividing cells with BrdU and specific markers of cells at different life stages should be performed to answer these questions.

Finally, many questions remain concerning the specific function of this neuronal plasticity. In quail the variation in POM volume is positively correlated in most circumstances with the activation of male-typical sexual behavior but establishing a direct link between the cellular changes associated with the increase in POM volume (not all of which have been described yet) and behavioral activation has been elusive (Balthazart *et al.*, 1996a). Similarly, seasonal variation in HVC volume seems to be most closely correlated in a positive fashion with seasonal variation in song quality as measured by variation in stereotypy (Brenowitz, 2008). However, again precise links between the cellular changes underlying this change in brain nucleus volume and behavior are difficult to establish. A number of studies have now demonstrated the existence of specific correlations between rates of neurogenesis in particular brain areas and behavioral or physiological changes (see for example (Scharff *et al.*, 2000) or (Hartog *et al.*, 2009) in song birds, and (Kemperman, 2006) for a general review) but the demonstration of a direct causal link between these events is often missing (Scharff, 2000). Correlations between neurogenesis and phenotypic changes have, however, become more and more sophisticated. For example, a recent clever study in mice manipulated prolactin signaling and abolished increased paternal olfactory neurogenesis and the subsequent adult offspring recognition (Mak & Weiss, 2010). This study thus claims that adult neurogenesis in paternal mice is linked to offspring recognition. Similar studies that can manipulate adult neurogenesis and other cellular events associated with adult

morphological plasticity and link them to specific behavioral events certainly represents one of the current frontiers in the field.

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References

- Absil P, Pinxten R, Balthazart J, Eens M. Effect of age and testosterone on autumnal neurogenesis in male European starlings (*Sturnus vulgaris*). *Behav Brain Res*. 2003a; 143:15–30. [PubMed: 12842292]
- Absil P, Pinxten R, Balthazart J, Eens M. Effects of testosterone on Reelin expression in the brain of male European starlings. *Cell Tissue Res*. 2003b; 312:81–93. [PubMed: 12712319]
- Adkins EK. Hormonal basis of sexual differentiation in the Japanese quail. *J Comp Physiol Psychol*. 1975; 89:61–71. [PubMed: 1159113]
- Altman J. Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. *J Comp Neurol*. 1969; 137:433–457. [PubMed: 5361244]
- Altman J, Das GD. Post-natal origin of microneurons in the rat brain. *Nature*. 1965; 207:953–956. [PubMed: 5886931]
- Alvarez-Borda B, Nottebohm F. Gonads and singing play separate, additive roles in new neuron recruitment in adult canary brain. *J Neurosci*. 2002; 22:8684–8690. [PubMed: 12351743]
- Alvarez-Buylla A, Nottebohm F. Migration of young neurons in adult avian brain. *Nature*. 1988; 335:353–354. [PubMed: 3419503]
- Alvarez-Buylla A, Theelen M, Nottebohm F. Birth of projection neurons in the higher vocal center of the canary forebrain before, during, and after song learning. *Proc Natl Acad Sci USA*. 1988; 85:8722–8726. [PubMed: 3186755]
- Alvarez-Buylla A, Theelen M, Nottebohm F. Proliferation “hot spots” in adult avian ventricular zone reveal radial cell division. *Neuron*. 1990; 5:101–109. [PubMed: 2369518]
- Appeltants D, Ball GF, Balthazart J. The distribution of tyrosine hydroxylase in the canary brain: demonstration of a specific and sexually dimorphic catecholaminergic innervation of the telencephalic song control nuclei. *Cell Tissue Res*. 2001; 304:237–259. [PubMed: 11396718]
- Arnold AP. The effects of castration and androgen replacement on song courtship, and aggression in zebra finches (*Poephila guttata*). *J Exp Zool*. 1975; 191:309–326. [PubMed: 1092802]
- Arnold AP, Nottebohm F, Pfaff DW. Hormone concentrating cells in vocal control areas of the brain of the zebra finch (*Poephila guttata*). *J Comp Neurol*. 1976; 165:487–512. [PubMed: 1262541]
- Aste N, Panzica GC, Aimar P, Viglietti-Panzica C, Harada N, Foidart A, Balthazart J. Morphometric studies demonstrate that aromatase-immunoreactive cells are the main target of androgens and estrogens in the quail medial preoptic nucleus. *Exp Brain Res*. 1994; 101:241–252. [PubMed: 7843311]
- Aste N, Viglietti-Panzica C, Balthazart J, Panzica GC. Testosterone modulation of peptidergic pathways in the septo-preoptic region of male Japanese quail. *Poultry and Avian Biology Reviews*. 1997; 8:77–93.
- Bai J, Ramos RL, Ackman JB, Thomas AM, Lee RV, LoTurco JJ. RNAi reveals doublecortin is required for radial migration in rat neocortex. *Nat Neurosci*. 2003; 6:1277–1283. [PubMed: 14625554]
- Ball, GF. Neuroendocrine basis of seasonal changes in vocal behavior among songbirds. In: Hauser, M.; Konishi, M., editors. *The design of animal communication*. MIT Press; Cambridge, MA: 1999. p. 213–253.

- Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Ritters LV, Balthazart J. Seasonal Plasticity in the song control system. Multiple brain sites of steroid hormone action and the importance of variation in song behavior. *Ann N Y Acad Sci.* 2004; 1016:586–610. [PubMed: 15313796]
- Ball, GF.; Balthazart, J. Neuroendocrine mechanisms regulating reproductive cycles and reproductive behavior in birds. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior.* Academic Press; San Diego, CA: 2002. p. 649-798.
- Ball GF, Balthazart J. Hormonal regulation of brain circuits mediating male sexual behavior in birds. *Physiol Behav.* 2004; 83:329–346. [PubMed: 15488549]
- Ball GF, Ritters LV, Balthazart J. Neuroendocrinology of song behavior and avian brain plasticity: multiple sites of action of sex steroid hormones. *Front Neuroendocrinol.* 2002; 23:137–178. [PubMed: 11950243]
- Ball, GF.; Ritters, LV.; MacDougall-Shackleton, SA.; Balthazart, J. Sex differences in brain and behavior and the neuroendocrine control of the motivation to sing. In: Zeigler, HP.; Marler, P., editors. *Neuroscience of birdsong.* Cambridge; Cambridge: 2008. p. 320-331.
- Balthazart J, Baillien M, Cornil CA, Ball GF. Preoptic aromatase modulates male sexual behavior: slow and fast mechanisms of action. *Physiol Behav.* 2004; 83:247–270. [PubMed: 15488543]
- Balthazart J, Ball GF. The Japanese quail as a model system for the investigation of steroid-catecholamine interactions mediating appetitive and consummatory aspects of male sexual behavior. *Ann Rev Sex Res.* 1998a; 9:96–176. [PubMed: 10349027]
- Balthazart J, Ball GF. New insights into the regulation and function of brain estrogen synthase (aromatase). *Trends Neurosci.* 1998b; 21:243–249. [PubMed: 9641536]
- Balthazart J, Ball GF. Is brain estradiol a hormone or a neurotransmitter? *Trends Neurosci.* 2006; 29:241–249. [PubMed: 16580076]
- Balthazart J, Ball GF. Topography in the preoptic region: differential regulation of appetitive and consummatory male sexual behaviors. *Front Neuroendocrinol.* 2007; 28:161–178. [PubMed: 17624413]
- Balthazart J, Boseret G, Konkle AT, Hurley LL, Ball GF. Doublecortin as a marker of adult neuroplasticity in the canary song control nucleus HVC. *Eur J Neurosci.* 2008a; 27:801–817. [PubMed: 18333960]
- Balthazart J, De Clerck A, Foidart A. Behavioral demasculinization of female quail is induced by estrogens: Studies with the new aromatase inhibitor, R76713. *Horm Behav.* 1992; 26:179–203. [PubMed: 1612564]
- Balthazart J, Sante P, Ball GF. Testosterone effects on the staining density and autoradiographic investigations of the α 2-adrenergic receptor in the medial preoptic nucleus of the Japanese quail: relationship to the activation of reproductive behavior. *Arch Int Physiol Bioch.* 1991; 99:385–392.
- Balthazart J, Tlemcani O, Ball GF. Do sex differences in the brain explain sex differences in the hormonal induction of reproductive behavior? What 25 years of research on the Japanese quail tells us. *Horm Behav.* 1996a; 30:627–661. [PubMed: 9047287]
- Balthazart J, Tlemcani O, Harada N. Localization of testosterone-sensitive and sexually dimorphic aromatase-immunoreactive cells in the quail preoptic area. *J Chem Neuroanat.* 1996b; 11:147–171. [PubMed: 8906458]
- Balthazart J, Voigt C, Boseret G, Ball GF. Expression of reelin, its receptors and its intracellular signaling protein, Disabled1 in the canary brain: Relationships with the song control system. *Neuroscience.* 2008b; 153:944–962. [PubMed: 18448255]
- Bandler R, Shipley MT. Columnar organization in the midbrain periaqueductal gray: modules for emotional expression? *Trends Neurosci.* 1994; 17:379–389. [PubMed: 7817403]
- Bardet SM, Woods J, Balthazart J. Extended neural proliferation in the embryonic medial preoptic nucleus in female quail. *Abst Soc Behav Neuroendo (P2.33).* 2009
- Barfield RJ. Activation of copulatory behavior by androgen implanted into the preoptic area of the male fowl. *Horm Behav.* 1969; 1:37–52.
- Barker, JM.; Yamamura, T.; Balthazart, J.; Ball, GF. Testosterone increases cell turnover in song nucleus HVC and increases cell recruitment into Area X of adult female canaries. *Abstract. Conf. on Adult Neurogenesis: structure and function; Frauenchiemsee, Germany.* 2010.

- Baum, MJ. Neuroendocrinology of sexual behavior in the male. In: Becker, JB.; Breedlove, SM.; Crews, D.; McCarthy, MM., editors. Behavioral Endocrinology. MIT Press; Cambridge, Mass: 2002. p. 153-203.
- Beach FA. Historical origins of modern research on hormones and behavior. *Horm Behav.* 1981; 15:325–376. [PubMed: 7035327]
- Bear MF, Malenka RC. Synaptic plasticity: LTP and LTD. *Curr Opin Neurobiol.* 1994; 4:389–399. [PubMed: 7919934]
- Bernard DJ, Ball GF. Two histological markers reveal a similar photoperiodic difference in the volume of the high vocal center in male European starlings. *J Comp Neurol.* 1995; 360:726–734. [PubMed: 8801262]
- Bernard DJ, Wilson FE, Ball GF. Testis-dependent and -independent effects of photoperiod on volumes of song control nuclei in American tree sparrows (*Spizella arborea*). *Brain Res.* 1997; 760:163–169. [PubMed: 9237531]
- Bhardwaj RD, Curtis MA, Spalding KL, Buchholz BA, Fink D, Bjork-Eriksson T, Nordborg C, Gage FH, Druid H, Eriksson PS, Frisen J. Neocortical neurogenesis in humans is restricted to development. *Proc Natl Acad Sci U S A.* 2006; 103:12564–12568. [PubMed: 16901981]
- Bloch GJ, Gorski RA. Cytoarchitectonic analysis of the SDN-POA of the intact and gonadectomized rat. *J Comp Neurol.* 1988; 275:604–612. [PubMed: 3192759]
- Boseret G, Ball GF, Balthazart J. The microtubule-associated protein doublecortin is broadly expressed in the telencephalon of adult canaries. *J Chem Neuroanat.* 2007; 33:140–154. [PubMed: 17367992]
- Boswald M, Harasim S, Maurer-Schultze B. Tracer dose and availability time of thymidine and bromodeoxyuridine: application of bromodeoxyuridine in cell kinetic studies. *Cell Tissue Kinet.* 1990; 23:169–181. [PubMed: 2357716]
- Bottjer SW, Arnold AP. Developmental plasticity in neural circuits for a learned behavior. *Annu Rev Neurosci.* 1997; 20:459–481. [PubMed: 9056722]
- Bottjer SW, Johnson F. Circuits, hormones, and learning: Vocal behavior in songbirds. *J Neurobiol.* 1997; 33:602–618. [PubMed: 9369462]
- Brenowitz E, Margoliash D, Nordeen KW. The neurobiology of birdsong. *J Neurobiol.* 1997; 33:495–709. [PubMed: 9369455]
- Brenowitz EA. Plasticity of the adult avian song control system. *Ann N Y Acad Sci.* 2004; 1016:560–585. [PubMed: 15313795]
- Brenowitz, EA. Plasticity of the song control system in adult birds. In: Zeigler, HP.; Marler, P., editors. Neuroscience of birdsong. Cambridge University Press; Cambridge: 2008. p. 332-349.
- Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol.* 2003; 467:1–10. [PubMed: 14574675]
- Butler, AB.; Hodos, W. Comparative vertebrate neuroanatomy Evolution and adaptation. Wiley-Interscience; New York: 2005.
- Cao J, Wenberg K, Cheng MF. Lesion induced new neuron incorporation in the adult hypothalamus of the avian brain. *Brain Res.* 2002; 943:80–92. [PubMed: 12088841]
- Capes-Davis A, Tolhurst O, Dunn JM, Jeffrey PL. Expression of doublecortin (DCX) and doublecortin-like kinase (DCLK) within the developing chick brain. *Dev Dyn.* 2005; 232:457–467. [PubMed: 15614772]
- Castagna C, Obole A, Viglietti-Panzica C, Balthazart J, Panzica GC. Effects of testosterone on the synaptology of the medial preoptic nucleus of male Japanese quail. *Brain Res Bull.* 1999; 50:241–249. [PubMed: 10582522]
- Catchpole, CK.; Slater, PJB. Bird song Biological themes and Variations. Cambridge University Press; Cambridge UK: 2008.
- Celotti F, Negri-Cesi P, Poletti A. Steroid metabolism in the mammalian brain: 5 alpha-reduction and aromatization. *Brain Res Bull.* 1997; 44:365–375. [PubMed: 9370201]
- Charlier TD, Ball GF, Balthazart J. Inhibition of steroid receptor coactivator-1 blocks estrogen and androgen action on male sex behavior and associated brain plasticity. *J Neurosci.* 2005; 25:906–913. [PubMed: 15673671]

- Charlier TD, Ball GF, Balthazart J. Rapid action on neuroplasticity precedes behavioral activation by testosterone. *Horm Behav.* 2008; 54:488–495. [PubMed: 18452920]
- Charlier TD, Harada N, Ball GF, Balthazart J. Targeting steroid receptor coactivator-1 expression with locked nucleic acids antisense reveals different thresholds for the hormonal regulation of male sexual behavior in relation to aromatase activity and protein expression. *Behav Brain Res.* 2006; 172:333–343. [PubMed: 16797739]
- Chen G, Bonder EM, Cheng MF. Lesion-induced neurogenesis in the hypothalamus is involved in behavioral recovery in adult ring doves. *J Neurobiol.* 2006; 66:537–551. [PubMed: 16555235]
- Collins, S. Vocal fighting and flirting: the functions of birdsong. In: Marler, P.; Slabbekoorn, H., editors. *Nature's Lusic, The Science of Birdsong.* Elsevier; Amsterdam: 2004. p. 39-79.
- Cox PR. A statistical investigation into bird-song. *British Birds.* 1944:38.
- Dawson A, King VM, Bentley GE, Ball GF. Photoperiodic control of seasonality in birds. *J Biol Rhythms.* 2001; 16:365–380. [PubMed: 11506381]
- De Groof G, Verhoye M, Poirier C, Leemans A, Eens M, Darras VM, Van der Linden A. Structural changes between seasons in the songbird auditory forebrain. *J Neurosci.* 2009; 29:13557–13565. [PubMed: 19864568]
- De Groof G, Verhoye M, Van Meir V, Balthazart J, Van der Linden A. Seasonal rewiring of the songbird brain: an in vivo MRI study. *Eur J Neurosci.* 2008; 28:2475–2485. discussion 2474. [PubMed: 19032586]
- De Groof G, Verhoye M, Van Meir V, Tindemans I, Leemans A, Van der Linden A. In vivo diffusion tensor imaging (DTI) of brain subdivisions and vocal pathways in songbirds. *Neuroimage.* 2006; 29:754–763. [PubMed: 16242349]
- Delville Y, Sulon J, Hendrick JC, Balthazart J. Effect of the presence of females on the pituitary-testicular activity in male Japanese quail (*Coturnix coturnix japonica*). *Gen Comp Endocrinol.* 1984; 55:295–305. [PubMed: 6479577]
- Ding L, Perkel DJ. Long-term potentiation in an avian basal ganglia nucleus essential for vocal learning. *J Neurosci.* 2004; 24:488–494. [PubMed: 14724247]
- Doupe AJ, Solis MM. Song-and order-selective neurons develop in the songbird anterior forebrain during vocal learning. *J Neurobiol.* 1997; 33:694–709. [PubMed: 9369467]
- Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, Gage FH. Neurogenesis in the adult human hippocampus. *Nat Med.* 1998; 4:1313–1317. [PubMed: 9809557]
- Fee MS, Kozhevnikov AA, Hahnloser RH. Neural mechanisms of vocal sequence generation in the songbird. *Ann N Y Acad Sci.* 2004; 1016:153–170.
- Follett BK. Plasma follicle-stimulating hormone during photoperiodically induced sexual maturation in male Japanese quail. *J Endocrinol.* 1976; 69:117–126. [PubMed: 1270949]
- Follett, BK. Birds. In: Lamming, GE., editor. *Marshall's physiology of reproduction.* Longman Greene; Edinburgh: 1984. p. 283-350.
- Forster E, Jossin Y, Zhao S, Chai X, Frotscher M, Goffinet AM. Recent progress in understanding the role of Reelin in radial neuronal migration, with specific emphasis on the dentate gyrus. *Eur J Neurosci.* 2006; 23:901–909. [PubMed: 16519655]
- Francis F, Koulakoff A, Boucher D, Chafey P, Schaar B, Vinet MC, Friocourt G, McDonnell N, Reiner O, Kahn A, McConnell SK, Berwald-Netter Y, Denoulet P, Chelly J. Doublecortin is a developmentally regulated, microtubule-associated protein expressed in migrating and differentiating neurons. *Neuron.* 1999; 23:247–256. [PubMed: 10399932]
- Gahr M, Guttinger HR, Kroodsma DE. Estrogen receptors in the avian brain: Survey reveals general distribution and forebrain areas unique to songbirds. *J Comp Neurol.* 1993; 327:112–122. [PubMed: 8432903]
- Garcia-Segura LM, Hernandez P, Olmos G, Tranque PA, Naftolin F. Neuronal membrane remodelling during the oestrus cycle: a freeze-fracture study in the arcuate nucleus of the rat hypothalamus. *J Neurocytol.* 1988; 17:377–383. [PubMed: 3171611]
- Goldman SA, Nottebohm F. Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc Natl Acad Sci U S A.* 1983; 80:2390–2394. [PubMed: 6572982]

- Gorski, RA. Critical role of the medial preoptic area in the sexual differentiation of the brain. In: De Vries, GJ.; De Bruin, JPC.; Uylings, HBM.; Corner, MA., editors. Sex differences in the brain. Elsevier; Amsterdam: 1984. p. 129-146.
- Gould E. How widespread is adult neurogenesis in mammals? *Nat Rev Neurosci.* 2007; 8:481–488. [PubMed: 17514200]
- Hannan AJ, Henke RC, Seeto GS, Capes-Davis A, Dunn J, Jeffrey PL. Expression of doublecortin correlates with neuronal migration and pattern formation in diverse regions of the developing chick brain. *J Neurosci Res.* 1999; 55:650–657. [PubMed: 10082087]
- Harada N, Abe-Dohmae S, Loeffen R, Foidart A, Balthazart J. Synergism between androgens and estrogens in the induction of aromatase and its messenger RNA in the brain. *Brain Res.* 1993; 622:243–256. [PubMed: 8242362]
- Harding CF, Sheridan K, Walters MJ. Hormonal specificity and activation of sexual behavior in male zebra finches. *Horm Behav.* 1983; 17:111–133. [PubMed: 6862388]
- Harding CF, Walters MJ, Collado D, Sheridan K. Hormonal specificity and activation of social behavior in male red-winged blackbirds. *Horm Behav.* 1988; 22:402–418. [PubMed: 3169703]
- Hartog TE, Dittrich F, Pieneman AW, Jansen RF, Frankl-Vilches C, Lessmann V, Lilliehook C, Goldman SA, Gahr M. Brain-derived neurotrophic factor signaling in the HVC is required for testosterone-induced song of female canaries. *J Neurosci.* 2009; 29:15511–15519. [PubMed: 20007475]
- Huang EP. Synaptic plasticity: going through phases with LTP. *Cur Biol.* 1998; 8:R350–R352.
- Hull, EM.; Rodriguez-Manzo, G. Male sexual behavior. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior.* Academic Press; San Diego, CA: 2009. p. 5-65.
- Hull, EM.; Wood, RI.; McKenna, KE. The neurobiology of male sexual behavior. In: Neill, JD., editor. *Knobil and Neill's Physiology of Reproduction.* 3. Academic Press; San Diego, New York: 2005. p. 1729-1824.
- Hutchison JB. Effects of hypothalamic implants of gonadal steroids on courtship behaviour in Barbary doves (*Streptopelia risoria*). *J Endocrinol.* 1971; 50:97–113. [PubMed: 4931797]
- Jarvis ED, Nottebohm F. Motor-driven gene expression. *Proc Natl Acad Sci USA.* 1997; 94:4097–4102. [PubMed: 9108111]
- Jarvis ED, Scharff C, Grossman MR, Ramos JA, Nottebohm F. For whom the bird sings: context-dependent gene expression. *Neuron.* 1998; 21:775–788. [PubMed: 9808464]
- Jin K, Mao XO, Cottrell B, Schilling B, Xie L, Row RH, Sun Y, Peel A, Childs J, Gendeh G, Gibson BW, Greenberg DA. Proteomic and immunochemical characterization of a role for stathmin in adult neurogenesis. *Faseb J.* 2004; 18:287–299. [PubMed: 14769823]
- Kee N, Sivalingam S, Boonstra R, Wojtowicz JM. The utility of Ki-67 and BrdU as proliferative markers of adult neurogenesis. *J Neurosci Methods.* 2002; 115:97–105. [PubMed: 11897369]
- Kemperman, G. Adult neurogenesis. Stem cells and neuronal development in the adult brain. Oxford University Press; New York: 2006.
- Kim YH, Peregrine J, Arnold AP. The distribution of expression of doublecortin (DCX) mRNA and protein in the zebra finch brain. *Brain Res.* 2006; 1106:189–196. [PubMed: 16814268]
- Kimpo RR, Doupe AJ. FOS is induced by singing in distinct neuronal populations in a motor network. *Neuron.* 1997; 18:315–325. [PubMed: 9052801]
- Kirn J, O'Loughlin B, Kasparian S, Nottebohm F. Cell death and neuronal recruitment in the high vocal center of adult male canaries are temporally related to changes in song. *Proc Natl Acad Sci USA.* 1994; 91:7844–7848. [PubMed: 8058721]
- Kim JR, Fishman Y, Sasportas K, Alvarez-Buylla A, Nottebohm F. Fate of new neurons in adult canary high vocal center during the first 30 days after their formation. *J Comp Neurol.* 1999; 411:487–494. [PubMed: 10413781]
- Konishi M, Emlen ST, Ricklefs RE, Wingfield JC. Contributions of bird studies to biology. *Science.* 1989; 246:465–472. [PubMed: 2683069]
- Larsson K, Heimer L. Mating behaviour of male rats after lesions in the preoptic area. *Nature.* 1964; 202:413–414. [PubMed: 14152848]

- Lephart ED. A review of brain aromatase cytochrome P450. *Brain Res Rev.* 1996; 22:1–26. [PubMed: 8871783]
- Li XC, Jarvis ED, Alvarez-Borda B, Lim DA, Nottebohm F. A relationship between behavior, neurotrophin expression, and new neuron survival. *PNAS.* 2000; 97:8584–8589. [PubMed: 10890902]
- Lisciotto CA, Morrell JI. Sex differences in the distribution and projections of testosterone target neurons in the medial preoptic area and the bed nucleus of the stria terminalis of rats. *Horm Behav.* 1994; 28:492–502. [PubMed: 7729818]
- Lisk RD. Neural localization for androgen activation of copulatory behavior in the male rat. *Endocrinology.* 1967; 80:754–761. [PubMed: 6022056]
- Louissaint AJ, Rao S, Leventhal C, Goldman SA. Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. *Neuron.* 2002; 34:945–960. [PubMed: 12086642]
- Mak GK, Weiss S. Paternal recognition of adult offspring mediated by newly generated CNS neurons. *Nat Neurosci.* 2010; 13:753–758. [PubMed: 20453850]
- Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. *Neuron.* 2004; 44:5–21. [PubMed: 15450156]
- Mandyam CD, Harburg GC, Eisch AJ. Determination of key aspects of precursor cell proliferation, cell cycle length and kinetics in the adult mouse subgranular zone. *Neuroscience.* 2007; 146:108–122. [PubMed: 17307295]
- Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM. The nuclear receptor superfamily: the second decade. *Cell.* 1995; 83:835–839. [PubMed: 8521507]
- Margoliash D. Functional organization of forebrain pathways for song production and perception. *J Neurobiol.* 1997; 33:671–693. [PubMed: 9369466]
- Marler P. Ethology and the origins of behavioral endocrinology. *Horm Behav.* 2005; 47:493–502. [PubMed: 15777816]
- McKenna NJ, Lanz RB, O'Malley BW. Nuclear receptor coregulators: cellular and molecular biology. *Endocr Rev.* 1999; 20:321–344. [PubMed: 10368774]
- McKenna NJ, O'Malley BW. Minireview: Nuclear receptor coactivators - An update. *Endocrinology.* 2002; 143:2461–2465. [PubMed: 12072374]
- Meitzen J, Moore IT, Lent K, Brenowitz EA, Perkel DJ. Steroid hormones act transsynaptically within the forebrain to regulate neuronal phenotype and song stereotypy. *J Neurosci.* 2007; 27:12045–12057. [PubMed: 17978046]
- Meitzen J, Weaver AL, Brenowitz EA, Perkel DJ. Plastic and stable electrophysiological properties of adult avian forebrain song-control neurons across changing breeding conditions. *J Neurosci.* 2009; 29:6558–6567. [PubMed: 19458226]
- Miller MW, Nowakowski RS. Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. *Brain Res.* 1988; 457:44–52. [PubMed: 3167568]
- Moore CA, Perderiset M, Francis F, Chelly J, Houdusse A, Milligan RA. Mechanism of microtubule stabilization by doublecortin. *Mol Cell.* 2004; 14:833–839. [PubMed: 15200960]
- Mouriec, K.; Bardet, SM.; Balthazart, J. Ontogeny of sex differences in steroid-sensitive regions in the quail brain (*Coturnix Japonica*). *Abst. Conf. Adult Neurogenesis: structure and function; Frauenchiemsee, Germany.* 2010.
- Murphy AZ, Rizvi TA, Ennis M, Shipley MT. The organization of preoptic-medullary circuits in the male rat: Evidence for interconnectivity of neural structures involved in reproductive behavior, antinociception and cardiovascular regulation. *Neurosci.* 1999; 91:1103–1116.
- Naftolin, F.; MacLusky, NJ. Aromatization hypothesis revisited. In: Serio, M.; Motta, M.; Zanisi, M.; Martini, L., editors. *Sexual differentiation: basic and clinical aspects.* Raven Press; New York: 1984. p. 79-91.
- Nordeen KW, Nordeen EJ, Arnold AP. Estrogen accumulation in zebra finch song control nuclei: implications for sexual differentiation and adult activation of song behavior. *J Neurobiol.* 1987; 18:569–582. [PubMed: 3694195]

- Nottebohm F. A brain for all seasons: Cyclical anatomical changes in song-control nuclei of the canary brain. *Science*. 1981; 214:1368–1370. [PubMed: 7313697]
- Nottebohm F. The road we travelled: discovery, choreography, and significance of brain replaceable neurons. *Ann N Y Acad Sci*. 2004; 1016:628–658. [PubMed: 15313798]
- Nottebohm F, Stokes TM, Leonard CM. Central control of song in the canary, *Serinus canarius*. *J Comp Neurol*. 1976; 165:457–486. [PubMed: 1262540]
- Packard DS Jr, Menzies RA, Skalko RG. Incorporation of thymidine and its analogue, bromodeoxyuridine, into embryos and maternal tissues of the mouse. *Differentiation*. 1973; 1:397–404. [PubMed: 4802502]
- Panzica GC, Castagna C, Aste N, Viglietti-Panzica C, Balthazart J. Testosterone effects on the neuronal ultrastructure in the medial preoptic nucleus of male Japanese quail. *Brain Res Bull*. 1996a; 39:281–292. [PubMed: 8705315]
- Panzica GC, Viglietti-Panzica C, Aste N, Andreone C, Fasolo A. Neuropeptides in the male quail medial preoptic nucleus. An immunocytochemical study. *Neuroendocrinol*. 1990; 52:68–60.
- Panzica GC, Viglietti-Panzica C, Balthazart J. The sexually dimorphic medial preoptic nucleus of quail: a key brain area mediating steroid action on male sexual behavior. *Front Neuroendocrinol*. 1996b; 17:51–125. [PubMed: 8788569]
- Panzica GC, Viglietti-Panzica C, Calcagni M, Anselmetti GC, Schumacher M, Balthazart J. Sexual differentiation and hormonal control of the sexually dimorphic preoptic medial nucleus in quail. *Brain Res*. 1987; 416:59–68. [PubMed: 3620956]
- Panzica GC, Viglietti-Panzica C, Sanchez F, Sante P, Balthazart J. Effects of testosterone on a selected neuronal population within the preoptic sexually dimorphic nucleus of the Japanese quail. *J Comp Neurol*. 1991; 303:443–456. [PubMed: 2007660]
- Pfaff, DW.; Schwartz-Giblin, S.; McCarthy, MM.; Kow, LM. Cellular and molecular mechanisms of female reproductive behaviors. In: Knobil, E.; Neill, JD., editors. *The physiology of reproduction*. Raven Press; New York: 1994. p. 107-220.
- Pradhan DS, Newman AE, Wacker DW, Wingfield JC, Schlinger BA, Soma KK. Aggressive interactions rapidly increase androgen synthesis in the brain during the non-breeding season. *Horm Behav*. 2010; 57:381–389. [PubMed: 20116379]
- Rao MS, Shetty AK. Efficacy of doublecortin as a marker to analyse the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. *Eur J Neurosci*. 2004; 19:234–246. [PubMed: 14725617]
- Rasika S, Alvarez-Buylla A, Nottebohm F. BDNF mediates the effects of testosterone on the survival of new neurons in an adult brain. *Neuron*. 1999; 22:53–62. [PubMed: 10027289]
- Rasika S, Nottebohm F, Alvarez-Buylla A. Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. *Proc Natl Acad Sci USA*. 1994; 91:7854–7858. [PubMed: 8058723]
- Reiner AD, Perkel J, Bruce L, Butler A, Csillag A, Kuenzel W, Medina L, Paxinos G, Shimizu T, Striedter G, Wild M, Ball GF, Durand S, Gunturkun O, Lee DW, Mello CV, White SA, Hough G, Kubikova L, Smulders TV, Wada K, Dugas-Ford J, Husband S, Yamamoto K, Yu J, Siang C, Jarvis ED. Revised nomenclature for avian telencephalon and some related brainstem nuclei. *J Comp Neurol*. 2004; 473:377–414. [PubMed: 15116397]
- Riters LV, Alger SJ. Neuroanatomical evidence for indirect connections between the medial preoptic nucleus and the song control system: possible neural substrates for sexually motivated song. *Cell Tissue Res*. 2004; 316:35–44. [PubMed: 14968358]
- Riters LV, Ball GF. Lesions to the medial preoptic area affect singing in the male European starling (*Sturnus vulgaris*). *Horm Behav*. 1999; 36:276–286. [PubMed: 10603291]
- Riters LV, Eens M, Pinxten R, Ball GF. Seasonal changes in the densities of $\alpha 2$ -noradrenergic receptors are inversely related to changes in testosterone and the volumes of song control nuclei in male European starlings. *J Comp Neurol*. 2002; 444:63–74. [PubMed: 11835182]
- Riters LV, Eens M, Pinxten R, Duffy DL, Balthazart J, Ball GF. Seasonal changes in courtship song and the medial preoptic area in male European starlings (*Sturnus vulgaris*). *Horm Behav*. 2000; 38:250–261. [PubMed: 11104643]

- Robinson JE, Follett BK. Photoperiodism in Japanese quail: the termination of seasonal breeding by photorefractoriness. *Proc R Soc Lond B Biol Sci.* 1982; 215:95–116. [PubMed: 6127699]
- Sartor, JJ. Department of Psychological and Brain Sciences. Johns Hopkins University; Baltimore: 2004. The role of variation in singing behavior in the regulation of seasonal neuroplasticity in European starlings.
- Sartor JJ, Ball GF. Social suppression of song is associated with a reduction in volume of a song-control nucleus in European starlings (*Sturnus vulgaris*). *Behav Neurosci.* 2005; 119:233–244. [PubMed: 15727528]
- Sartor JJ, Balthazart J, Ball GF. Coordinated and dissociated effects of testosterone on singing behavior and song control nuclei in canaries (*Serinus canaria*). *Horm Behav.* 2005; 47:467–476. [PubMed: 15777813]
- Sartor JJ, Charlier T, Pytte CL, Balthazart J, Ball GF. Converging evidence that song performance modulates seasonal changes in the avian song control system. *Soc Neurosci Abstr.* 2002 2002 CD-ROM, ProgramNo.781.710.
- Scharff C, Kirn JR, Grossman M, Macklis JD, Nottebohm F. Targeted neuronal death affects replacement and vocal behavior in adult songbirds. *Neuron.* 2000; 25:481–492. [PubMed: 10719901]
- Schiffmann SN, Bernier B, Goffinet AM. Reelin mRNA expression during mouse brain development. *Eur J Neurosci.* 1997; 9:1055–1071. [PubMed: 9182958]
- Schlinger BA. Sex steroids and their actions on the birdsong system. *J Neurobiol.* 1997; 33:619–631. [PubMed: 9369463]
- Schlinger, BA.; Brenowitz, EA. Neural and hormonal control of birdsong. In: Pfaff, DW.; Arnold, AP.; Etgen, AM.; Fahrbach, SE.; Rubin, RT., editors. *Hormones, Brain and Behavior*. Academic Press; San Diego: 2009. p. 898-941.
- Scotto-Lomassese S, Rochefort C, Nshdejan A, Scharff C. HVC interneurons are not renewed in adult male zebra finches. *Eur J Neurosci.* 2007; 25:1663–1668. [PubMed: 17408434]
- Slagsvold T. Bird song activity in relation to breeding cycle, spring weather, and environmental physiology. *Ornis Scand.* 1977; 8:197–222.
- Smith GT, Brenowitz EA, Wingfield JC. Seasonal changes in the size of the avian song control nucleus HVC defined by multiple histological markers. *J Comp Neurol.* 1997; 381:253–261. [PubMed: 9133567]
- Soma KK, Hartman VN, Wingfield JC, Brenowitz EA. Seasonal changes in androgen receptor immunoreactivity in the song nucleus HVC of a wild bird. *J Comp Neurol.* 1999; 409:224–236. [PubMed: 10379916]
- Soma KK, Scotti MA, Newman AE, Charlier TD, Demas GE. Novel mechanisms for neuroendocrine regulation of aggression. *Front Neuroendocrinol.* 2008; 29:476–489. [PubMed: 18280561]
- Tetel MJ, Auger AP, Charlier TD. Who's in charge? Nuclear receptor coactivator and corepressor function in brain and behavior. *Front Neuroendocrinol.* 2009; 30:328–342. [PubMed: 19401208]
- Theodosia DT, Poulain DA. Oxytocin-secreting neurones: a physiological model for structural plasticity in the mammalian brain. *TINS.* 1987; 10:426–430.
- Theodosia DT, Poulain DA. Neuronal-glial and synaptic plasticity of the adult oxytocinergic system: Factors and consequences. *Ann NY Acad Sci.* 1992; 652:303–325. [PubMed: 1320829]
- Thompson CK, Bentley GE, Brenowitz EA. Rapid seasonal-like regression of the adult avian song control system. *Proc Natl Acad Sci U S A.* 2007; 104:15520–15525. [PubMed: 17875989]
- Thompson CK, Brenowitz EA. Seasonal change in neuron size and spacing but not neuronal recruitment in a basal ganglia nucleus in the avian song control system. *J Comp Neurol.* 2005; 481:276–283. [PubMed: 15593375]
- Thompson RR, Adkins-Regan E. Photoperiod affects the morphology of a sexually dimorphic nucleus within the preoptic area of male Japanese quail. *Brain Res.* 1994; 667:201–208. [PubMed: 7697357]
- Tindemans I, Verhoye M, Balthazart J, Van Der Linden A. In vivo dynamic ME-MRI reveals differential functional responses of RA- and area X-projecting neurons in the HVC of canaries exposed to conspecific song. *Eur J Neurosci.* 2003; 18:3352–3360. [PubMed: 14686908]

- Tissir F, Goffinet AM. Reelin and brain development. *Nat Rev Neurosci.* 2003; 4:496–505. [PubMed: 12778121]
- Tramontin AD, Brenowitz EA. Seasonal plasticity in the adult brain. *TINS.* 2000; 23:251–258. [PubMed: 10838594]
- Tramontin AD, Hartman VN, Brenowitz EA. Breeding conditions induce rapid and sequential growth in adult avian song control circuits: A model of seasonal plasticity in the brain. *J Neurosci.* 2000; 20:854–861. [PubMed: 10632615]
- Tsai MJ, O'Malley BW. Molecular mechanisms of action of steroid/thyroid receptor superfamily members. *Annu Rev Biochem.* 1994; 63:451–486. [PubMed: 7979245]
- Tsuyoshi H, Wada M. Termination of LH secretion in Japanese quail due to high-and low-temperature cycles and short daily photoperiods. *Gen Comp Endocrinol.* 1992; 85:424–429. [PubMed: 1577245]
- Van der Linden, A.; De Groof, G.; Charlier, TD.; Balthazart, J. Rapid testosterone-induced Apparent Diffusion Coefficient (ADC) changes in the sexually dimorphic medial preoptic nucleus of male Japanese quail. *Abst. of the Soc. for Neurosci Meeting*, 660.668/DD612; 2006.
- Van der Linden A, Van Meir V, Boumans T, Poirier C, Balthazart J. MRI in small brains displaying extensive plasticity. *Trends Neurosci.* 2009; 32:257–266. [PubMed: 19307029]
- Van der Linden A, Van Meir V, Tindemans I, Verhoye M, Balthazart J. Applications of manganese-enhanced magnetic resonance imaging (MEMRI) to image brain plasticity in song birds. *NMR Biomed.* 2004; 17:602–612. [PubMed: 15761949]
- Van der Linden A, Verhoye M, Van Audekerke J, Peeters R, Eens M, Newman SW, Smulders T, Balthazart J, DeVoogd TJ. Non invasive in vivo anatomical studies of the oscine brain by high resolution MRI microscopy. *J Neurosci Methods.* 1998; 81:45–52. [PubMed: 9696309]
- Van der Linden A, Verhoye M, Van Meir V, Tindemans I, Eens M, Absil P, Balthazart J. In vivo manganese-enhanced magnetic resonance imaging reveals connections and functional properties of the songbird vocal control system. *Neuroscience.* 2002; 112:467–474. [PubMed: 12044464]
- Van Meir V, Pavlova D, Verhoye M, Pinxten R, Balthazart J, Eens M, Van der Linden A. In vivo MR imaging of the seasonal volumetric and functional plasticity of song control nuclei in relation to song output in a female songbird. *Neuroimage.* 2006; 31:981–992. [PubMed: 16529952]
- Van Meir V, Verhoye M, Absil P, Eens M, Balthazart J, Van der Linden A. Differential effects of testosterone on neuronal populations and their connections in a sensorimotor brain nucleus controlling song production in songbirds: a manganese enhanced-magnetic resonance imaging study. *Neuroimage.* 2004; 21:914–923. [PubMed: 15006658]
- Vellema M, van der Linden A, Gahr M. Area-specific migration and recruitment of new neurons in the adult songbird brain. *J Comp Neurol.* 2010; 518:1442–1459. [PubMed: 20187140]
- Viglietti-Panzica C, Panzica GC, Fiori MG, Calcagni M, Anselmetti GC, Balthazart J. A sexually dimorphic nucleus in the quail preoptic area. *Neurosci Ltrs.* 1986; 64:129–134.
- Viglietti-Panzica C, Spigolon S, Aste N, Balthazart J, Panzica GC. Ultrastructural modifications of the male quail medial preoptic nucleus after adult testosterone treatment. *European Journal of Neuroscience, Suppl.* 1991; 4:225.
- Wieraszko A, Ball GF. Long-term enhancement of synaptic responses in the songbird hippocampus. *Brain Res.* 1991; 538:102–106. [PubMed: 2018921]
- Wieraszko A, Ball GF. Long-term potentiation in the avian hippocampus does not require activation of the N-methyl-D-aspartate (NMDA) receptor. *Synapse.* 1993; 13:173–178. [PubMed: 8095355]
- Wilbrecht L, Kim JR. Neuron addition and loss in the song system: regulation and function. *Ann N Y Acad Sci.* 2004; 1016:659–683. [PubMed: 15313799]
- Wild JM. Neural pathways for the control of birdsong production. *J Neurobiol.* 1997; 33:653–670. [PubMed: 9369465]
- Wingfield JC. Historical contributions of research on birds to behavioral neuroendocrinology. *HormBehav.* 2005; 48:395–402.
- Wingfield, JC.; Farner, DS. Endocrinology of reproduction in wild species. In: Farner, DS.; King, J.; Parkes, KC., editors. *Avian Biology*. Vol. 9. Academic Press; New York: 1993. p. 163-327.

- Wingfield, J.C.; Silverin, B. Ecophysiological studies of hormone-behavior relations in birds. In: Pfaff, D.W.; Arnold, A.P.; Etgen, A.M.; Fahrbach, S.E.; Rubin, R.T., editors. *Hormones, Brain and Behavior*. Academic Press; San Diego, CA: 2002. p. 587-647.
- Wissman AM, Brenowitz EA. The role of neurotrophins in the seasonal-like growth of the avian song control system. *J Neurosci*. 2009; 29:6461–6471. [PubMed: 19458217]
- Yamamura, T.; Balthazart, J.; Ball, G.F. The roles of estrogenic and androgenic metabolites of testosterone in the regulation of doublecortin in the song system of adult female canaries. *Abst Soc for Neuroscience nbr 584.20*; Chicago Ill: 2009.
- Zeigler, H.P.; Marler, P. *Neuroscience of birdsong*. Cambridge University Press; Cambridge UK: 2008.

Figure 1.

Testosterone-induced plasticity in the medial preoptic nucleus (POM) of the Japanese quail. **A.** Localization of the POM in the quail brain. The nucleus runs through most of the preoptic area from the level of the tractus septopallio-mesencephalicus (TSM) to the level of the anterior commissure (CA). Gray area denote the presence of dense populations of aromatase cells in the POM and bed nucleus of the stria terminalis, medial part (BSTm). E: entopallium; FPL: fasciculus prosencephali lateralis; Hp: hippocampus; N: nidopallium; SL: lateral septal nucleus; SM: medial septal nucleus; VLT: nucleus ventrolateralis thalami. **B.** Effect of gonadectomy associated or not with a treatment by testosterone on the total volume of the POM (left) and on the average neuronal size in the lateral POM, reflected by the cross sectional area. **C.** Histological sections through the left POM stained by immunohistochemistry for aromatase illustrating the decrease in number of aromatase-immunoreactive (ARO-ir) cells observed when intact birds are castrated (CX) and the recovery following treatment with exogenous testosterone (CX+T). Magnification bar= 400 μm. **D.** Effect of gonadectomy associated or not with a treatment by testosterone on the average numbers of ARO-ir cells in the POM (left) and on the specific numbers of these

cells located in the medial or lateral parts of the nucleus (right). Data in the two parts of this panel come from different studies in which quantification was performed by different methods so that absolute numbers of ARO-ir cells cannot be directly compared. All quantitative data are means $\pm$ SEM. Redrawn from data in (Panzica *et al.*, 1987; Harada *et al.*, 1993; Aste *et al.*, 1994) and (Balthazart & Ball, 2007).

Figure 2.

Doublecortin (DCX) expression in the quail brain. DCX-immunoreactive cells are present in large numbers at the level of the lateral ventricle in the telencephalon (A) as well as at the lateral border of the nidopallium where they seem to be migrating in a tangential direction (B). In contrast, no clearly immunoreactive cells are seen in the medial preoptic area where only a few spots of non specific staining can be detected (C). CA: anterior commissure; VL: lateral ventricle; VIII: third ventricle. Magnification bar = 200 μ m (Balthazart, J. and Charlier, T.D., unpublished data).

Figure 3.

Rapid effects of testosterone on two aspects of male sexual behavior, female neck grab (A) and cloacal contact movements (B) and on the volume of the quail POM defined in Nissl stained sections (C) and based on the dense group of aromatase-immunoreactive cells that identifies the POM (D). Panel E shows photomicrographs illustrating the group of aromatase-immunoreactive cells located in the POM in males that were castrated (CX) or CX and treated with testosterone (CX+T) for one or fourteen days (d). Groups of CX subjects were tested as killed at day 1 or 14 (CX) while CX+T birds were studied at days 1, 2, 7 and 14. All quantitative data are means $\pm$ SEM. Redrawn from data in (Charlier *et al.*, 2008).

Figure 4.

Schematic representation of the different steps leading from the division of progenitor cells to the incorporation of new neurons and their death in a given brain nucleus (top) and photomicrographs of representative cells at different stages of this process. At any given point in time, various numbers of cells are engaging into a neuronal (as opposed to glial) fate of differentiation (N1), migrate toward their final target (N2), are recruited and differentiate in this target nucleus (N3), are incorporated in this target as mature functional neurons (N4) and finally die by apoptosis or necrosis (N5). Only a fraction of neurons at a given stage will progress to the next stage and they will stay in each condition for variable durations (t_1 , t_2 , t_3). Testosterone could potentially modulate each of these processes and their duration. So far evidence suggests that effects of the steroid take place mainly at the level of the differentiation and survival of neurons (arrows) but actions at other levels cannot be excluded (dotted arrows). The photomicrographs in the bottom row represent cells labeled by BrdU in the ventricle wall (left), an elongated (fusiform) migrating young neuron labeled by immunocytochemistry for DCX (middle left), a slightly older neuron that has started its differentiation but is still immunoreactive for DCX (middle right) and an apoptotic cell identified by its pyknotic nucleus (right).

Figure 5. Effects of testosterone (Testosterone vs. Control; top) or of the social environment (Male-Male [M-M] vs. Male-Female [M-F] housing; bottom) on the volume of HVC (left) and on the number of fusiform (middle) or round (right) doublecortin (DCX)-immunoreactive cells in HVC and in two equivalent areas located laterally (LHVC) or ventrally (v.HVC) to HVC. The brain drawing in the top-right side schematically illustrates the distribution of DCX-immunoreactive cells in the brain and the location of areas in which systematic quantification was performed. See also text for additional explanations. *= $p < 0.05$ compared to the other experimental group. Redrawn from data in (Balthazart *et al.*, 2008a).