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BRAIN DIMORPHISMS AND SEX: A REVIEW

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ABSTRACT: In this article we review evidence from studies of fish, amphibians, reptiles, birds and mammals which bears on the question of whether differences in sexual behaviour are reflected by differences in central nervous system (CNS) structure. Neural structures in fish demonstrate the existence of both inter- and intra-sexual dimorphisms related to dimorphic behaviours, as well as environmentally triggered changes in the size of neural structures. Seasonal changes in neural structure in amphibians have demonstrated a strong correlation between sexually dimorphic brain structures and sex-specific behaviours. While in reptiles there are some examples of sexually dimorphic CNS structures, *C.uniparens* demonstrates that differences in brain morphology are not necessary to display sexually distinct behaviour. Birds demonstrate the clearest sex related brain-behaviour differences; the song control nuclei exhibit substantial differences in size between the sexes varying in magnitude in relation to the amount of sexual dimorphism in song production. There are sexually dimorphic areas in the mammalian brain, in areas associated with sexual and maternal behaviour, which are correlated with differences in hormonal environments during ontogeny. No single phyletic trend is obvious, though this could be the consequence of a small number of taxa examined or the different aims of the studies. It appears that sexuality has not necessarily evolved linearly from a particular primitive vertebrate ancestor but is manifested variously in different vertebrate classes, most likely as the result of distinct environmental pressures.

INTRODUCTION

Debate as to whether gender differences are socially constructed or biologically ordained is far from resolved. Following the publication of Margaret Mead's Sex and Temperament (1935), the prevailing view

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was that cultural factors and early experiences served to define gender. More recently, especially with the development of the Gay Rights movement in the United States, biological factors have been seen as prominent (LeVay, 1996).

The issue is further complicated by the suspicion that sex is not a bipolar phenomenon, a matter of being either totally male or female. The usual attributes of one's sex, viz. genitals, gonads, karyotype, predominate hormones, etc., may be discordant, suggesting that sex may need to be viewed as having a number of different dimensions. The practical problems this can pose are illustrated by the dilemma of Olympic officials at the 1976 games who were faced with cases of putative female athletes with XY karyotype in their hair cells (reviewed in Klopfer, 1982).

We review evidence that bears on the question of whether differences in sexual behavior are reflected by differences in the structure of particular areas in the central nervous system, or whether brain dimorphisms map onto some other attribute of sex. We also consider whether the dimorphic structures are the cause or the effect of differences in sexual behavior. We examine studies of fish, amphibians, reptiles, birds, and mammals.

FISH

Structural dimorphisms

Neural structures in fish demonstrate the existence of both inter- and intra-sexual dimorphisms. For example the plainfin midshipman, *Porichthys notatus*, has two different male morphs - Type I and Type II, which not only present with distinct sexual behaviors, but also underlying dimorphic neural structures. Type I males are larger than Type II males and represent about 90% of the reproductively active male population (Bass, 1992). While the Type I males build nests and guard their eggs, the Type II males "sneak-spawn" leaving the protective responsibilities to their larger male counterparts (Bass & Marchaterre, 1989; Brantley & Bass, 1991). Despite their smaller body size, Type II males' ratio of gonad size to body size is nine times greater than that of Type I males (Bass & Anderson, 1991). This ratio in females is fifteen times greater than in Type I males.

The vocal behavior of the plainfin midshipman is also dimorphic. While all three morphs generate grunting sounds, Type I males' grunts

are louder and more rhythmic in aggressive situations (Brantley & Bass, 1991). Type I males also generate a hum, a sound of higher amplitude and longer duration than a grunt. This hum is used to attract gravid females to their nest (Brantley & Bass, 1991). Electrical stimulation can cause females to produce sound. The only difference in the electrically stimulated sounds generated by the three morphs is a 20% higher frequency in the Type I male (Bass & Baker, 1990).

The acoustic systems responsible for these dimorphic vocalizations provide an ideal basis for examining the relationship between behavior and neural differences (Bass, 1989). Sonic muscles (SM) attached to each lateral face of the swimbladder contract to produce both hums and grunts (Bass & Baker, 1990). SM's receive input from pacemaker neurons in the brain, via sonic nerves, and together they regulate firing frequency. Each sonic nerve which innervates a SM is a fusion of two occipital nerve roots which exit the brain laterally; these roots originate in the sonic motor nucleus (Bass & Baker, 1990). The vocal control system is anatomically similar in females and Type II males, both of which are less developed than in Type I males. The Type I males' SM mass is six times larger than that of Type II males and females (correcting for body size) (Bass & Marchaterre, 1989; Brantley & Bass, 1991). Additionally Type I males have larger muscle fiber number, diameter, and proportion of myofibers to myofibrils (Bass & Marchaterre, 1989).

More pertinent to the discussion are inter- and intra- sexual dimorphisms in the sonic and pacemaker neurons (Bass & Baker, 1990). Both average diameter of somata and primary dendrites of the sonic motoneurons are significantly larger in Type I males than in females and Type II males. Pacemaker cells also have a larger average soma and dendrite size in Type I males than in females. The ratio of soma diameter, however, to average diameter of the dendrites is not significantly different among the three morphs. This suggests that both the sonic and pacemaker neurons in Type I males are larger but equally scaled versions of those found in Type II males and females (Bass & Baker, 1990).

Functional dimorphisms

Sexual brain dimorphisms in fish also consist of differential neurosecretory activity, including brain peptides such as Substance P and galanin. (Weld & Maler, 1992; Cornbrooks & Parsons, 1991). The dimorphic distribution of these peptides is thought to play a key role in

different reproductive behaviors. However it should be noted that, for most studies cited throughout this review, staining methodology does not differentiate between neurons which actually secrete a particular neuropeptide and neurons which may have a high accumulation of that neuropeptide.

Weakly electric fish generate sexually dimorphic electric organ discharges (EODs) as a means of courtship, territorial, electrolocation, and communication signals. The EODs they produce are of either pulse-type, which are made of brief, irregular rhythmic pulses, or wave-type, which consist of longer duration and very regularly rhythmic pulses (Bullock & Heiligenberg, 1986). Courtship EODs are sexually dimorphic. Female EODs, in pulse fish, are of shorter duration than males (Bullock & Heiligenberg, 1986; Hagedorn & Heiligenberg, 1986). Some female fish, that communicate with wave-type EODs, have discharges of greater frequency and shorter duration than males. Other fish show the reverse pattern (Bullock & Heiligenberg, 1985).

The EOD is controlled by a midline medullary pacemaker nucleus, which receives primary input from the prepacemaker nucleus (PPN) (Kawasaki, Male, Rose & Heiligenberg, 1988). The PPN determines transient changes in EOD frequency called "chirps" in Gymnotiforms (Kawasaki et al., 1988). Weld & Maler (1992) found that Substance P-like immunoreactivity (Spli) was differentially distributed in the PPN between males and females. Dimorphic Spli innervation of this diencephalic cell group arises in either the hypothalamus lateralis (Hl) or the hypothalamus ventralis (Hv). Male Gymnotiforms have more abundant Spli cell bodies and fibers in the Hl, and the Hv was intensely stained in males while devoid of Spli in females (Weld & Maler, 1992). Additionally, Spli distribution is sexually dimorphic in some hypothalamic and septal nuclei. These results suggest that a tachykinin may play an important role in the regulation of sexual differences in fish behavior, EODs (Weld & Maler, 1992).

The male sailfin molly, *Poecilia latpinna*, possesses a sexually dimorphic galanin-like immunoreactivity (GAL-LI) fiber system in the brain extending from mesencephalic levels to caudal structures of the spinal cord (Cornbrooks & Parsons, 1991). The optic tectum, torus semicircularis, brainstem tegmentum, and spinal cord contain higher GAL-Li levels in the male molly than the female (Cornbrooks & Parsons, 1991). Comparative studies looked at GAL-LI distribution in the spinal cord of the goldfish and neonatal mollies, which revealed no sexual dimorphisms. In fact neonatal mollies lacked spinal GAL-LI completely. Thus this unique sexually dimorphic distribution of GAL-

LI, as well as the extensive fiber network present only in the adult male, suggest a role for galanin in sexually dimorphic behavioral displays (Cornbrooks & Parsons, 1991).

Brain-behaviour relationships

Fish display a sexually dimorphic distribution of gonadotropin-releasing hormone (GnRH) cells throughout the brain (Grober & Bass, 1991). This sex difference is related to dimorphic reproductive behaviour in various fish, including sex and role changing fish. In certain species, individuals differentiate as one sex and then change sex later in life. Fish which undergo sex change are called sequential hermaphrodites, of which there are two patterns - protogyny and protandry (Shapiro, 1994). Protogynous species include individuals which change from female to male, whereas individuals in protandric species change form male to female. In these species, changes in reproductive behaviour correlates with the number of GnRH releasing cells within the brain (Grober & Bass, 1991; Shapiro, 1994).

Sex change in fish is manifested in changes in gonad type, external appearance (coloration), and behaviour. The latter two characteristics are secondary sex characteristics, dependent on androgens (Shapiro, 1994). Sex and role reversal is correlated with a change in brain structure, the distribution of GnRH releasing cells, resulting in the altered release of gonadotropin releasing hormone from the brain. Increased gonadotropin levels caused by increases in GnRH are thought to induce sex change. Experiments with human chorionic gonadotropin (HCG), show that this hormone causes females to change in colour and gonad type (Koulish & Kramer, 1989). Gonadotropin levels are regulated by interactions involving the hypothalamus, pituitary, and gonads. Thus sex change is most likely an example of a sexually dimorphic neuroendocrine feedback loop controlling the secretion of gonadotropins.

Exogenous factors have been shown to influence the development of structural dimorphisms. In Davis and Fernald's studies (1990) on the African chichlid fish, *Haplochromis burtoni,* maturation of males is strongly influenced by the growth of gonadotropin releasing hormone releasing (GnRH-ir) preoptic neurons. Further the growth of the neurons is partially determined by the social environment; no neuronal growth took place when fish were housed in an environment that encourages aggression. This inhibition of neuronal growth appeared to stunt sexual maturation. Growth of neurons and sexual maturation

occurred only when these fish were housed with other fish their age, as opposed to older fish, a presumably less aggressive environment. Thus the fish's social environment affects neuron size which in turn affects the fish's sexual maturation.

In further studies with this species, territorial males were found to have larger gonadotropin releasing hormone (GnRH) releasing neurons in the preoptic area than non-territorial males. A change from territorial to non-territorial status resulted in a reduction of GnRH-ir neurons, whereas a change from non-territorial to territorial status resulted in an expansion (Francis, Soma & Fernald, 1993).

Steroid hormones have been shown to change both the EOD and physiology of the electric organ in several species which demonstrate a sexually dimorphic EOD in the field (Meyer & Zakon, 1982). Landsman, Harding, Moller, and Thomas (1990) performed a study examining the effects of implanted testosterone, dihydrotestoserone and estradiol on EODs and morphology of the weakly electric fish, *Gnathonemus petersii*. They found androgens significantly increased the duration of phases 2 and 3 of the juvenile and adult EOD, and decreased the peak power spectrum frequency (PPSF) of the Fourier transformation (Landsman, et al. 1990). Additionally, androgen induced the male-like indentation of the dorsal margin of the anal fin in juveniles and females (Landsman, et al. 1990). The findings of lengthened EOD duration and decreased PPSF are congruent with seasonal sex differences in the *G. petersii*. These effects were androgen specific and did not occur in individuals implanted with estradiol; estradiol induced a slight increase in PPSFs of adults. Estrogen has been demonstrated in previous studies to influence the PPSFs of other weakly electric fish (Meyer, 1983).

Like the plainfin midshipman, there are two male morphs in the bluehead wrasse - primary and terminal (or secondary males). Sex reversal also occurs in the bluehead wrasse. Grober and Bass (1991) found that luteinizing hormone releasing hormone (LHRH) secreting cells were distributed diffusely in the brains of all three morphs (female, primary male, and terminal male). Although no qualitative differences were found, there were quantitative differences in distribution in the preoptic area and the hypothalamus between the three sexual phases. Terminal males have a significantly greater number of LHRH preoptic cells (2-3 times greater) than primary phase males and females (Grober & Bass, 1991). Grober, Jackson and Bass (1991) found 11-ketotestosterone induced increases in both female and primary male LHRH preoptic cell numbers to levels equivalent to terminal

males. However, 11-ketotestosterone had no effect on terminal phase males, suggesting that these individuals may have reached an end stage in developmental maturity.

This brain dimorphism in LHRH preoptic cell number corresponds with different reproductive strategies. Both primary and terminal males release sperm as females release eggs at the top of the water column (Shapiro, 1994). Terminal males, however, spawn with single females, while primary males spawn in groups of 5 to 40 males for every female (Shapiro, 1994). Shapiro (1994) hypothesizes that the LHRH cell number is more strongly correlated with the development of alternative male reproductive behaviors than with the process of sex change or reversal.

Gonadotropin releasing hormone immunoreactive (GnRH-ir) cells in the preoptic area (POA) of the plainfin midshipman demonstrate differences in cell size rather then cell number as a key component in sexual differentiation, though changes in both occur during development (Grober, Fox, Laughlin, & Bass, 1994). In the Type II morph, the ratio of GnRH-ir cell number increase relative to body size increase is disproportionately greater than in the other two morphs. Grober *et al.* (1994) suggest that the increases, which vary among the three morphs, are reflective of differences in the onset of sexual maturation. Gonadotropin releasing hormone cells in the terminal nerve(TN) and POA appear to play a role in the development and maintenance of teleost sexual behavior (Grober *et al.*, 1994). Significant differences in GnRH-ir cell size in the POA were found among all 3 adult morphs. Sex differences in GnRH-ir cell size nor cell number, however, were found in the TN. The differential timing of POA developmental changes may be a strategy for providing alternative male reproductive morphs.

Dimorphic sexual behaviors have been linked with both functional and structural dimorphisms in the brains of certain fish. In these fish there are often two male morphs, in addition to a female morph, and structural dimorphisms are related to behavior more than the condition of being male or female. Additionally, exogenous factors, such as living environment, can affect the size of neural structures and must also be considered in determining brain-behavior relationships. The causal nexus remains unclear between brain dimorphisms and behavioral displays.

AMPHIBIANS

Structural dimorphisms

Amphibians display sexually dimorphic behaviors which have been correlated with sex differences in both neural structures and circulating levels of hormones or neuropeptides. The clearest and most studied example is the sexually dimorphic anuran mating call. The African clawed frog, *Xenopus laevis*, produces three distinct vocalizations: the mate call, a repetitive trill composed of brief clicks, used by males to attract females; ticking, a slower trill of clicks, used by sexually unreceptive females when clasped; and sawing, an aggressive signal, used by paired sexually active males or androgen-treated females (Kelly, 1986). Male mate calls are acoustically distinguishable from female ticking.

Mate calls and ticking are produced by contractions of the laryngeal dilator muscles which receive input from motor neurons in cranial nerve nucleus IX-X (n. IX-X) of the caudal medulla (Kelly, 1980). n. IX-X receives afferents from inferior and medius reticular nuclei and from the pretrigeminal nucleus of the dorsal tegmental area of the medulla (DTAM); which in turn, are innervated by the ventrolateral striatum, the preoptic area and postero-central and ventral thalamus (Kelly, 1986). It is not yet clear how these CNS areas function to produce the different anuran vocalizations.

Both the larynx and CNS vocalization pathways are sexually distinct in the African clawed frog. The larynx of males is 2-3 times larger in males than females. This difference results from larger cartilage and muscle mass in males; males have an average of 32,000 muscle fibers while females have only 4,000 fibers (Kelly, 1986). There are also physiological sex differences in laryngeal muscle. Male muscle is made up of mostly fast-twitch, fatigue-resistant fibers; while, female muscle is predominantly slow-twitch fiber with some fast-twitch intermingled (Gray, Sassoon & Kelly, 1985). Projections from the preoptic area to the DTAM and recurrent collaterals from laryngeal motor neurons to DTAM are also dimorphic, with females showing a great reduction in size or complete absence (Weltz et al., 1985 cited in Kelly, 1986).

Kelly, Fenstemaker, Hannigan, & Shih (1988) morphologically categorized n.IX-X neurons in, *Xenopus laevis*, and examined them for sex differences. Multipolar n.IX-X neurons were classified based on somal shape (Type I-triangular, Type II-ovoid, Type III-elongated).

The frequency of different types of n.IX-X neurons and primary dendrites were the same between males and females. However, male n.IX-X neurons had a greater number of higher order dendrites, therefore more total dendritic segments, and the male mean length of the dendritic tree was 2.3 times that found in females (Kelly *et al*, 1988). Only males displayed fifth order branches of Type II and III cells.

As mentioned earlier, the anterior preoptic nucleus (APON) provides input to the DTAM. This area is thought to be a triggering center for male mate calling behavior in anuran species. The APON has been found to be sexually dimorphic in many vertebrate species including the Japanese toad, *Bufo japonicus*. Takami & Urano (1984) tested the hypothesis that the APON was responsible for evoking male mating behavior by comparing nuclear volumes between male and female, and also between post-breeding and hibernating *B. japonicus*. The male APON was 125% larger in hibernating than post-breeding toads, whereas in females it did not fluctuate in size. Additionally, the male APON was significantly larger in male than female animals. The ratio of male to female nuclear volume was 1.39 and 1.25 in hibernating and post-breeding toads, respectively (Takami and Urano, 1984). Similar sex differences were found in the nuclear volume of two morphologically and probably functionally related nuclei, the amygdala subnuclei, Am and Al.

Sexual dimorphisms have also been found in amphibian sensory systems, for example, the vomeronasal system in the red-backed salamander, *Plethodon cinerus*. In salamanders, odorants are received through nasolabial grooves and are delivered to vomeronasal receptors in a process known as nose tapping (Dawley & Crowder, 1995). During the breeding season males may use nose tapping to locate and identify potential mates. Vomeronasal size in *P. cinerus* is correlated with overall body size and sex. Male vomeronasal organs are significantly larger than females throughout the year, and this difference is the result of a greater number of cells per section of vomeronasal epithelium (Dawley & Crowder, 1995). In both sexes the vomeronasal organ is largest in the summer, which most likely corresponds to an increase in organ cells during the prebreeding season.

Functional dimorphisms

The neuropeptide arginine vasotocin (AVT) has been shown to influence sexual behavior in a variety of vertebrates, including

amphibians (Moore & Deviche, 1987). Boyd, Tyler & DeVries (1992) used immunocytochemistry to compare AVT distribution between males and females in the bullfrog, *Rana catesbeiana*. While AVT-immunoreactive cells (AVT-ir) were found diffusely throughout the brain, in both hypothalamic and extrahypothalamic areas, there were clear sexual differences in specific brain areas. Males and females demonstrated sex differences in the amygdala pars lateralis and habenular nucleus, with males having a denser distribution of AVT-ir. Additionally, males had larger AVT-ir cell bodies than females in the suprachiasmatic nucleus. No differences were found in the dorsomedial or ventromedial septum nor in the magnocellular preoptic nucleus.

Brain-behavior relationships

Observations of seasonal changes in neural structure and neuropeptide levels, as well as experimental hormonal manipulations, in amphibians have demonstrated a strong correlation between sexually dimorphic brain structures and sex-specific behaviors. Female African clawed frogs are unable to produce male-typical mating calls, even when administered androgen (Kelly, 1986). It appears that females lack the morphology to produce the repetitive trill involved in the male mating call. When the female laryngeal nerve is stimulated at the rate of a male mate call, only one click is produced as successive stimuli act to maintain tonic tension in the arytenoid discs of the larynx (Kelly, 1986). It may be that sexual dimorphism of the CNS vocal control pathway preserves reproduction in the African clawed frog by preventing females from being able to mimic the male mating call. However, it appears that the female vocal limitation is manifested at least partially in the periphery.

Sexually dimorphic seasonal changes in cell number, perhaps cells containing steroid receptors, suggest a causative role for these structural changes in the production of sex-specific behavior (Takami & Urano, 1984). By comparing post-breeding Japanese toads with hibernating toads, seasonal variations in the volume of the anterior preoptic nucleus (APON) were discovered. Additionally, Takami and Urano (1984) found these seasonal changes in neural structure to precede physiological and behavioral changes in the breeding season. Given that the seasonal changes in the APON were only found in male toads, it is more likely that this area of the brain is involved in male-specific activity, either mate calling exclusively or even encompassing other areas of copulatory behavior.

Similarly, the vomeronasal organ was found to be more highly developed in males and seasonally variable in size (Dawley & Crowder, 1995). While the vomeronasal system is used throughout the year for territorial maintenance, it may be that courtship and mating in the breeding season require a different set of hormone receptors. These authors suggest that seasonal fluctuation in vomeronasal cell number may demonstrate neurogenesis of additional receptors used in mating. Males may require a more sensitive olfactory system for identifying potential mates, such that the sexual dimorphism of the vomeronasal system reflects a structural difference underlying sex differences in sensory ability.

Seasonally breeding amphibians show demonstrable fluctuations in gonadal steroid levels associated with sexual behavior and possibly sex differences in morphology. The red-backed salamander, with its sexually dimorphic vomeronasal organ, is a perfect example. In the summer season when the vomeronasal organs are largest in both sexes males have low androgen levels and high GnRH levels (Dawley & Crowder, 1995). Similarly, females have low estradiol and testosterone levels but high GnRH in summer months (Dawley & Crowder, 1995). These findings might suggest the existence of GnRH regulation of sex differentiation; however, further measurements of circulating hormones are needed to elucidate this relationship.

Functional dimorphisms, such as differences in neurosecretory activity, have also been shown to underlie sex-specific differences in mating behaviors. Arginine vasotocin (AVT) has been shown to influence male sexual behavior. Administration of AVT to male rough-skinned newts, *Taricha granulosa*, stimulates clasping behavior in intact newts and androgen primed castrates; whereas, injections of anti-AVT serum inhibits sexual behaviors (Moore & Miller, 1983). Further, it appears that AVT is acting centrally given that intracranial injections of AVT, at levels that are behaviorally ineffective in the periphery, are sufficient to stimulate sexual behaviors (Deviche, Propper & Moore, 1990). Seasonal changes in AVT also supports its role in the expression of sexual behaviors; irAVT concentrations were found to increase fivefold in the optic tectum in the spring when sexual behaviors are most prevalent in male newts (Zoeller & Moore, 1986). Similarly, infundibulum concentrations of irGnRH in male rough-skinned newts increased from the end of May to mid-June with a concomitant increase in plasma androgen, testis weight, and mating behavior (Deviche et al., 1990). These authors suggest that these neuropeptides might be acting directly or through intermediary hormones at multiple sites, such as sensory and motor pathways, to evoke and organize sexual behavior.

REPTILES

Structural dimorphisms

A substantial amount of the research in reptiles has been done on sex differences in the whiptail lizards (*Cnemidophorus* species) because they consist of both sexually distinct and uni-sexual species. For example, the all-female parthenogenetic species *C. uniparens* is the descendant of two sexually reproducing species, *C. inornatus* and another *C. nemidophorus* species (Densmore, Mortiz, Wright & Brown, 1989 cited to Kingston & Crews, 1994). These parthenogenetic females are capable of displaying both male and female-typical copulatory behavior depending on their hormonal status (Lindzey and Crews, 1988).

There are two primary areas involved in the control of *Cnemidophorus* species' mating behavior: the anterior hypothalamus-preoptic area (AH-POA) responsible for producing male-typical sexual behavior and the ventromedial hypothalamus (VMH) involved in female-typical sexual behavior (Wade, Huang & Crews, 1993). Activation of behavior is hormone dependent. Androgen administration in the AH-POA leads to male behavior in gonadectomized male individuals of either species (Mayo & Crews, 1987 cited in Wade et al., 1993). Likewise, estrogen implants in the VMH of either species leads to female-typical receptivity in females (Wade, Huang & Crews, 1993).

Distinct sexual brain dimorphism has been found only in the adults of the sexually reproducing *C. inornatus.* The anterior hypothalamus-preoptic area (AH-POA) is significantly larger in males while the ventromedial hypothalamus (VMH) is significantly larger in females of this species (Crews, Wade & Wilczynski, 1990). In the parthenogenetic species, *C. uniparens*, all individuals display the female brain plan, and there is not a substantial size change in the two critical areas when these individuals alternate their display of male and female mating behavior (Wade, Huang & Crews, 1993).

Thus, it appears that in the ancestral condition there were distinct sexually dimorphic brain areas involved in copulatory behavior. In the evolution of all-female parthenogens, there was a change to a more economical condition where the default (female) brain plan was able to produce both sex-typical behavior patterns given the correct hormonal environment. Therefore, differences in brain morphology do not necessarily correspond with nor are needed for differences in sexual behavior.

Functional dimorphisms

Arginine vasotocin (AVT) is a hormone which has been studied in a number of different reptiles species to determine its distribution and whether there is a sex difference in the cells which produce this peptide. AVT in nonmammalian vertebrates is similar in location and equivalent in function to the mammalian hormone vasopressin (Acher, 1974). Studies have demonstrated AVT's role in antidiuresis in reptiles, and many other important roles have been proposed for this hormone based on the functions vasopressin controls in mammals (Bons, 1983 according to Smeets, Sevensma and Jonker, 1990). These include: memory processes, passive avoidance behavior, thermoregulation, blood pressure, and uterine contractions (Smeets, Sevensma & Jonker, 1990; Propper, Jones & Lopez, 1992). Additionally, AVT may function extrahypothalamically as a neuro-transmitter (Propper et al., 1992). Given that AVT is involved in sexual behavior it is an important peptide to examine for sex differences in CNS distribution.

Stoll and Voorn (1985) have shown a sexual dimorphism in arginine vasotocin (AVT) cell distribution in the lizard, *Gekko gecko*. Male lizards have significantly higher levels of vasotocin (VT) innervation in the areas of the lateral septum, nucleus sphericus, and periaqueductal gray (Stoll & Voorn, 1985). Sexual dimorphism in VT distribution is similar to that in *Gekko gecko*, in the turtle *Pseudmys cripta elegans* and the snake *Python regius*. The most evident sex differences were found in the lateral septal nucleus and the periaqueductal gray areas, with males having greater VT innervation (Smeets, Sevensma & Jonker, 1990). Additionally, less prominent sexual dimorphism was seen in the ventral region, the lateral habenular nucleus, the ventral tegmental area, and the substantia nigra (Smeets, Sevensma & Jonker, 1990). The lizard, *Anolis carolinensis*, has more AVT-ir staining in males than females; specifically in the sexually dimorphic areas of the lateral, medial, and dorsal cortex (Propper et al., 1992).

Reptiles also exhibit sex differences in the central distribution of other neurochemicals including the neuropeptide Y (NPY) and gonadotropin releasing hormone (GnRH). For example given that the lizard, *Pordarcis hispanica*, is a seasonal breeder with hormonal fluctuations corresponding with sexual activity, many hormones such as NPY have been examined for the existence of sexual dimorphism. Results have shown sex differences in NPY distribution in the lateral septal nucleus (LSN) and the periventricular preoptic nucleus (PPN),

which are accentuated during the reproductive season; females have a greater amount of NPY reactive cells in the LSN whereas males show a greater amount of reactive cells in the PPN (Salom, Font & Martinez-Garcia, 1994). Tsai and Licht looked at GnRH distribution in the turtle, *Trachemys scripta*, they found: chicken-I GnRH and chicken-II GnRH had different distributions, CI-GnRH is most likely responsible for gonadotropin release, and there was a sex difference for the distribution of CI-GnRH with females having a greater concentration in the median eminence (Tsai & Licht, 1993).

Brain-behavior relationships

Many studies assume sexual differences in behavior represent an underlying difference in brain structure. Yet, Wade et al. (1993) found all-female parthenogens were capable of producing both male and female-typical behavior without a change in the size of critical brain areas. While much sexual behavior may indeed be related to differences in the size of brain areas between the sexes, this study alerts us to the fact that morphological changes are not necessary for functional changes (Wade et al., 1993).

Research on such species as the lizard, *Podarcis hispanica*, indicates the importance of controls for different seasonal effects on the size of hormonal-regulated structures. Some of the studies did not explicitly test for the persistence of sexual dimorphism in and out of the breeding season.

Differences in behavior between the sexes may be a result of different levels of circulating hormones (i.e. androgens), which mediate pituitary action rather than due to actual structural differences. Although within the reptilian taxa there are clear examples of sexually dimorphic neural structures, the all-female parthenogen whiptail lizard demonstrates that morphological changes are not necessary for behavioral changes (Wade et al., 1993). Additionally, the sex differences in neuropeptides may result from sex differences in body size, blood volume, and blood flow to specific brain structures.

BIRDS

Structural dimorphisms

The best examples of structural brain dimorphisms linked directly

to sexually dimorphic behavior are found in birds. More specifically, gross morphological sex differences have been found in the song control nuclei of birds of the order Passeriformes (songbirds). The brain areas and neural pathways involved in the learning and production of bird song were first described by Nottebohm and Arnold (1976) in canaries and zebra finches; they have since been clearly delineated in several other species. The main descending pathways for vocalization of song consist of: the ventral hyperstriatum (HVc), which sends an efferent projection to the robust nucleus of the archistriatum (RA), and the hypoglossal nucleus of the medulla (nXII) which is innervated directly by an RA projection. From there motor signals travel to and control neural output of the syrinx, the avian organ for vocal production. There also is a recursive loop involved in the process of song learning in which area X (a forebrain nucleus in the parolfactory lobe) receives information from the HVc. These are the brain regions which exhibit substantial differences in size between the sexes, and the sexual dimorphisms vary across species in relation to the degree of sexual dimorphism in song production (Brenowitz, Arnold & Lewin, 1985).

In most songbird species song production is sexually dimorphic, with males singing more than females, however birds of different species fall along a broad continuum. For example, male zebra finches sing in order to attract females and ward off competitive females whereas females do not sing at all (Adkins-Regan & Ascenzi, 1990). Females of this species do not sing even when testosterone propionate is implanted in adults (Adkins-Regan & Ascenzi, 1987). Similarly, male canaries have a complex song repertoire and female canaries do not normally sing. Although female canaries will sometimes sing when isolated from males or treated with testosterone in adulthood (Nottebohm, 1980). In these instances female song is simpler and less frequent.

In both the canary and zebra finch, the Hvc, RA, area X and nXII are significantly larger, have more neurons, larger neuronal somata and longer dendritic processes in males than in females (Nottebohm & Arnold, 1976). There were no such differences in other areas not involved in song production, and the sexual differences in volume were more pronounced in the zebra finch. These two findings suggest that the differences are specific to song area and related to dimorphisms in song production.

On the other hand, canaries are more sexually dimorphic than the bay wren. The bay wren is a tropical species in which both sexes

normally sing in duets. In this species there are no sex differences in song complexity, size of song nuclei or accumulation of steroid hormones by neurons in the song control nuclei (Brenowitz & Arnold, 1985a).

In the closely related Rufus and white wrens, males and females also exhibit song behavior in duets, with females having a less complex song. There was no sex difference observed in the proportion of tritium-testosterone labeled target cells in the higher vocal control center of the magnocellular nucleus of the anterior neostriatum(MAN). No information was given about the size of the song control circuitry (Brenowitz & Arnold, 1985b).

Although most work looking at sexually dimorphic neural structures in birds tends to focus on the song control pathways, additional sex differences in brain structure have been reported. One example involves asymmetry in visual pathways connecting the thalamus to the hyperstriatum in the chicken. The ipsilateral connections from the dorsolateral thalamus to the right hyperstriatum are present, whereas there are few contralateral connections to the left hyperstriatum, prior to day 21 (Rogers & Sink, 1988). This asymmetry, which is generated by asymmetrical light exposure of the embryo's eyes, is sexually dimorphic being more pronounced in males. Males treated with 17 beta-estradiol, 5 days prior to hatching, fail to develop a pronounced asymmetry in the thalamofugal projections (Rogers and Rajendra, 1992). This suggests that the asymmetry is dependent on circulating hormone levels, and the lesser degree of asymmetry in females is the result of higher estradiol levels in females prior to hatching.

Functional dimorphisms

Nitric oxide (NO) is hypothesized to play a role in synaptic plasticity. Given this fact, NO is an interesting compound to study for sex differences in the brain and its relationship to behavioral plasticity in song production. Staining for NADPH-diaphorase, an enzyme used in the synthesis of NO, showed that the proportion of stained cells decreases with development, mostly occurring prior to the auditory song learning phase (Wallhausser-Franke, Collins & Devoogd, 1995). When quantifying results for Area X, in zebra finches, it was found that males have a larger decrease in staining (56%) than females (23%) (Wallhausser-Franke et al., 1995). Additional sexual dimorphisms were found in the song control nuclei of adults, with male finches

having a lesser degree of staining in area X, the HVC and RA compared to females. This suggests that NO is more likely involved in early formation of song control circuitry than plasticity needed for song acquisition.

Development, organization, and activation of the song control system are influenced by steroid sex hormones. For this reason the accumulation of sex steroids in song control nuclei has been studied carefully. In canaries, using autoradiographic analysis of injected tritium-labeled testosterone (T), it was found that males and females have equal proportions of cells labeled by T or its metabolites in the four song control nuclei: HVC, MAN, RA, and nXII (Brenowitz & Arnold, 1992). Because males have larger HVC and RA than females, they have a greater absolute number of hormone-sensitive cells in these areas.

The pattern of hormone accumulation in canaries is similar to that of duetting bay wren, rufus, and white wren species which show no sex difference in the proportion of T labeled cells in the HVC or MAN. In contrast, zebra finches show a pronounced sex difference in T labeled cells in the HVC and MAN. This sex difference may reflect more pronounced development of efferent projections from HVC to both RA and Area X in males of the zebra finch species (Brenowitz & Arnold, 1992). Comparison amongst the preceding species suggests that song production can only occur if a sufficient percentage of neurons in song nuclei are hormone sensitive.

Brain-behavior relationships

Experimental hormone manipulation during early post hatching and/or in adulthood has been used extensively to study the effects of sex steroid hormones on the song control nuclei and singing behavior. For example female zebra finches treated with androgens in adulthood show neither changes in singing nor size of song control nuclei. Similarly castrated adult zebra finches, while showing a reduction in song, do not show a reduction in size of the song control circuitry (Adkins-Regan & Ascenzi, 1987). Thus hormonal activation in adulthood is not sufficient to produce singing or the sex differences in song nuclei. It appears that the sexually dimorphic neural circuitry for song is organized early in development and activated in adulthood by testosterone acting on the male-typical structure (Gurney & Konishi, 1980). Female zebra finches injected with estradiol post-hatching and given testosterone as adults, not only sang but also had RA and Hvc

sizes close to those of untreated males (Gurney & Konishi, 1980).

Adkins-Regan *et al* (1994), in studying the influential time for estrogen in the alteration of neuroanatomy and sexually dimorphic behaviors in the zebra finch, found that the first week post-hatching was the critical period for sexual differentiation. Females given estradiol benzoate (EB) during post-hatching week 1 were masculinized in terms of RA neuron soma size and density and dancing behavior, and were partially masculinized with respect to song nuclei size and singing. Males injected with EB during this time failed to mount. Thus both masculinization of females and demasculinization of males is possible during post-hatching week 1.

Nottebohm (1980) demonstrated that testosterone administration to canaries in adulthood can change both neural structures and behavior. Testosterone administered to adult gonadectomized females induced a 90% and 53% increase in size of the HVc and RA, respectively, as these birds acquired male-typical song. These results were similar in magnitude and opposite in direction to the reduction of HVc and RA volumes of males castrated during days 5-10 post-hatching.

Kirn and DeVoogd (1989) suggest that cell death plays a prominent role in the development of structural brain dimorphisms in birds. They examined rates of posthatch neurogenesis and cell death in the vocal control regions - HVC, RA and Area X of the zebra finch. Although the time course for these three areas differed, there were significantly higher numbers of pyknotic, degenerating cells, observed in the Hvc, RA and Area X for females compared to males. Peak levels of cell death occurred 4-6 weeks after hatching, after the onset of sex differences in steroid levels (Kirn & DeVoogd, 1989). Comparisons of cell death and cell incorporation suggest that adult sex differences may result from differential survival of neurons after hatching rather than differential proliferation.

Finally some bird species, including canaries, have been shown to have seasonal changes in brain area sizes that are correlated with behavioral changes. The HVc and RA of adult male canaries are 99% and 76% larger, respectively, in the spring than the fall (Nottebohm, 1981). When the photoperiod increases in spring, the testes grow and secrete androgens, the HVc and the RA double in size, singing increases, and the song repertoire enlarges (Nottebohm, 1981). This author suggests that the changes in song control areas reflect fluctuations in the number of synapses and this underlies the ability to acquire new motor coordinations, thus a structural change preceding a behavioral change.

MAMMALS

Structural dimorphisms

Sex related differences in brain structures are known in several mammalian taxa but have been most studied in rodents and carnivores. The areas of the brain with the greatest number of sexual dimorphisms are the hypothalamus and olfactory system. The vomeronasal system (VNS) is a highly sensitive, sexually dimorphic, olfactory pathway implicated in the control of reproductive behavior (Collado, Valencia, Del Abril, Rodriguez-Zafra, Perez-Laso, Segovia & Guillamon, 1993). The accessory olfactory bulb (AOB) is the first target of vomeronasal input form the vomeronasal organ. The rat AOB is sexually dimorphic: males have greater AOB volume, more mitral cells, and more light and dark granule cells than females (Perez-Laso, Valencia, Rodriguez-Zafra, Cales, Guillamon & Segovia, 1994). These sex differences appear to arise postnatally as a result of the hormonal environment.

The bed nucleus of the accessory olfactory tract (BAOT) is a forebrain group of cells which receives vomeronasal input from the AOB and sends efferents back to the AOB and medial preoptic area (MPA). The BAOT is involved in the control of reproductive and parental behavior (Collado et al., 1993). Male rats have greater BAOT volumes, number of neurons, and neuron to glia ratios than females.

The bed nucleus of the stria terminalis (BST) is a forebrain structure which connects olfactory nuclei with components of the amygdaloid complex, and is considered to be a secondary olfactory center (Segovia & Guillamon, 1993). Some of the divisions of the BST are sexually dimorphic. The medial division, posterior part (BSTMP) has a larger volume and more neurons in male rats than in females (Segovia & Guillamon, 1993). Analogous sex differences have been found in the guinea pig. Conversely the medial division, anterior part (BSTMA) showed a larger volume and greater number of neurons in female rats compared to males.

The medial preoptic area (MPA) receives olfactory input from the BSTMA and BSTMP by way of the stria terminalis. The MPA is involved in many behaviors including: maternal behavior, male and female copulatory behavior, and the cyclic release of gonadotropin in females (Segovia & Guillamon, 1993). Dorner and Staudt (1969, cited in Segovia & Guillamon, 1993) found that female rats presented a larger nuclear volume of the medial preoptic area-anterior hypothalamus continuum than males. Gorski et al. (1980) found a

group of cells in the male MPA that had higher staining than in female rats and named it the sexually dimorphic nucleus of the preoptic area (SDN-POA). This nucleus is 2.6 times larger, contains larger neurons, and displays greater neuronal density in males (Segovia & Guillamon, 1993). Female ferrets entirely lack a sexually dimorphic structure in the MPA, the male nucleus of the preoptic/anterior hypothalamic area (MN-POA/AH) (Cherry & Baum, 1990).

Other dimorphisms include the numerical densities in spine and shaft synapses in the Ventromedial nucleus (VMN) of the rat, which are higher in adult males than females (Miller & Aoki, 1991). This sexual dimorphism was evident by day 5 and persisted into adulthood.

Sex differences have also been found in two hypothalamic nuclei that receive vomeronasal input: the ventromedial hypothalamic nucleus (VMH) involved in feminine reproductive behavior and the premammillary nucleus, ventral part (PMV) which is a tonic center for gonadotropin secretion (Segovia & Guillamon, 1993). Female rats have larger VMH nuclei than males, while the volume of the VMH is significantly larger in males (Matsumoto & Arai, 1983). The size of the nuclei of PMV neurons is larger in male rats than females.

A final CNS structural dimorphism to consider involves a set of spinal motoneurons that innervate the muscles attached to the base of the rat penis. This group of neurons, is collectively named the spinal nucleus of the bulbocavernosus (SNB). These neurons control the muscle groups responsible for the external anal sphincter and penile erection. Adult male rats have a larger SNB which contains more motoneurons than females (Breedlove & Arnold, 1983).

Functional dimorphisms

The vasopressin and oxytocin containing nucleus (VON) of the pig hypothalamus exhibits a significant sexual dimorphism in adulthood, with females displaying three times the number of VON neurons and two times as large a VON area (Van Eerdenburg & Swaab, 1991). Both sexes show a 2.5 fold increase in neuron number around puberty, but the female VON continues to increase in neuron number between 1 and 2.5 years of age. The function of the VON is yet to be delineated, however, because of the timing in sexual differentiation it is likely involved in reproduction.

Van Eerdenburg also studied the supraoptic nucleus (SON) of the hypothalamus of the pig, an area characterized by large neurons that produce vasotocin or oxytocin. Again a sexual dimorphism was found,

though it did not persist into adulthood. Males showed earlier SON enlargement with a 30% and 50% greater volume than females, at 30 weeks and 1 year respectively (Van Eerdenburg, Lugard-Kok & Swaab, 1992). At two and a half years of age there were no sex differences in SON volume. Unlike the VON, testosterone does not suppress development of the SON and gonadectomy does not lead to an increase in SON volume and cell number.

Sexual differences have also been found in the brain opioid system which has been implicated in the modulation of the hypothalamo-pituitary complex. U-opioid receptors in particular have been implicated in the control of gonadotropin and prolactin release since the density of hypothalamo-pituitary u-opioid receptors in the female rat is higher than that of mature male rat (Limonta, Dondi, Maggi & Piva, 1991). Further it appears that the hormonal environment at birth may dictate the development of this sexual dimorphism. Administration of testosterone to female rats soon after birth leads to an ontogenetic pattern of u-receptors similar to that of males, while early orchidectomy in males presents a maturational process similar to that of normal female rats (Limonta, Dondi, Maggi & Piva, 1991).

Brain-behavior relationships

Sex difference in the mammalian brain arise from different steroid hormones issued from the gonads early in development. Developing males secrete testosterone which enters the blood stream and travels to the brain. Centrally testosterone may be metabolized to estradiol or reduced to dihydrotestosterone and can bind receptors, which in turn bind specific segments of DNA to increase or decrease the expression of genes. These DNA domains may dictate the pattern of development for neural circuits necessary for the production of masculine structures and behavior. Feminine behavior and neural structure results when the masculinizing actions of testosterone do not occur. These developmental processes occur during early critical periods in development (Arnold & Schlinger, 1993).

Many experiments have been done in which orchidectomy and hormonal manipulations have been used to test the reversibility and stability of sexually dimorphic neural structures, and their relationship to sex-specific behaviors. For example gonadectomized adult male rats, with AOB bilateral lesions, will perform the female receptive position lordosis when primed with estradiol-progesterone; whereas, gonadectomized estradiol primed males with intact AOBs will not

(Segovia & Guillamon, 1993). This finding suggests that the sexually dimorphic AOB is related to the inhibition of feminine sexual behavior. Similarly bilateral lesions to the sexually dimorphic BAOT facilitates maternal behavior in both female and male rats. Male orchidectomy and female androgenization performed on day 1 can reverse the sexual dimorphism of the BAOT (Segovia & Guillamon, 1993).

There is significant evidence that the GABAa/benzodiazepine receptor Cl- channel complex is involved in the sexual differentiation of vomeronasal structures, such as the AOB, which contain GABA neurons and fibers. It was found that diazepam administered rat pups on post day 0 through 16 lead to decreases in the volume of the AOB mitral cell layer and the number of mitral cells in male rats, while female rats were unaffected (Perez-Laso et al. 1994). These results mimic the effects produced by gonadectomizing the male rat. Diazepam may act by increasing endogenous GABAergic activity, altering Cl- flux and inducing cellular loss in the genetic male (Perez-Laso, et al., 1994). Male rats are more susceptible to diazepam effects; it may be that females have a natural increase in GABAergic activity, regulated by sex steroids, which leads to normal feminization or diazepam might suppress the neurotrophic activity of gonadal steroids on the male AOB (Perez-Laso et al., 1994).

The medial preoptic area (MPA) is involved in reproduction, for example in the display of maternal behavior. Lesions to the MPA after parturition abolishes maternal behavior, while estrogen benzoate implants facilitate it (Numan, 1988). Transplants of MPA tissue from male rats to females induces increases in both masculine and feminine sexual behavior. This at first dichotomous role for the MPA is explained by the fact the MPA has both androgen and estrogen receptors (Segovia & Guillamon, 1993). The sexually dimorphic nucleus of the preoptic area (SDN-POA) also seems to be involved in the control of sexual behavior. In sexually naive males, bilateral lesions to the SDN-POA decreased the number of animals ejaculating, and/or increased latencies to the first mount, intromission, and ejaculation (Segovia and Guillamon, 1993).

The volume of the SDN-POA can be influenced by the hormonal environment perinatally. Castration of a 1 day old male rat will significantly reduce the adult size of the SDN-POA (Gorski, Gordon, Shryne & Southam, 1978). Administration of testosterone propionate on day 2 to the feminized male restores the SDN-POA to normal male volume (Jacobson, Csernus, Shryne & Gorski, 1981). Testosterone propionate administered to intact female pups on day 2 or 4 increases

the volume of the SDN-POA in the adult (Gorski et al., 1978). It is believed that testosterone masculinizes the SDN-POA through aromatization of testosterone to estradiol (Rhees, Shryne & Gorski, 1990). Perinatal treatment of females with diethylstilbestrol (DES), a synthetic estrogen, increases the volume of the SDN-POA to that of males (Tarttelin & Gorski, 1988). There is a definite critical period for this masculinization by estradiol; onset of the hormone-sensitive period begins on day 18 of gestation and terminates on post-natal day 5 (Rhees et al., 1990).

The preoptic area-anterior hypothalamus (POA/AH) is a sexually dimorphic region which has been implicated in masculine and feminine sexual behavior. Lesions of this area lead to deficits in masculine sexual performance while implantation of testosterone into this area activates male coital behavior after castration (Cherry & Baum, 1990). Similarly, electrical stimulation of the POA/AH activates sexual response in male rats and opossums. Furthermore, bilateral lesions of the male nucleus of the POA/AH in ferrets are associated with decrements in male sexual behavior, and enhancement of female proceptive behaviors in response to estradiol benzoate (Cherry & Baum, 1990). It appears that the MN-POA/AH functions normally to inhibit female sexual displays.

The amount of social play, a sexually dimorphic behavior in many species, also appears to result from differences in early hormonal environment between the sexes that leads to the differentiation of neural tissue (Meaney, 1988). Adult male Norway rats, *Rattus norvegicus*, engage in more frequent play-fights than females of this species. Exposure to androgens neonatally influences the amount of play-fighting; testosterone given to female neonates leads to masculinization of social play (Goy & Goldfoot, 1974). Given that the sex-difference in play fighting occurs prior to hormonal surges accompanying puberty, it is most likely that testosterone has an organizing effect on the central nervous system. It also appears that the sex difference in social play is a testosterone or DHT effect rather than testosterone-derived estradiol; neonatal administration of flutamide, an anti-androgen, prevents masculinization of play-fighting (Meaney, 1988). The amygdala has been suggested as the sexually dimorphic neural locus for social play. Lesions to the amygdaloid complex on days 21 or 22 reduce the male level of play-fighting to female levels while having no effect on female play-fighting (Meaney, 1988).

The sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) is also very dependent on the neonatal hormonal environment.

Early in fetal development females possess the group of muscles which control penile erection. As these muscles atrophy, however, the SNB regresses in the female. Females treated with androgen are spared the muscle atrophy and secondarily the SNB (Fishman, Chism, Firestone & Breedlove, 1990). It is the muscle cells, not the motoneurons, that contain the androgen receptors which trigger the development of this sexual dimorphism.

Important research has shown that naturally occurring exogenous events may also contribute to the masculinization of the neural system and sexual behavior. For example, male rats receive more perineal stimulation from maternal licking than do female rats. This maternal bias appears to result from the difference in hormonal status of rat pups; Moore, (1982) in an experiment where injections of testosterone, estradiol or dihydrotestosterone were administered on the day of birth to females, found that all three manipulations lead to equivalent amount of maternal licking to males. Later research by Moore (1992) demonstrated that this difference in maternal stimulation leads to some of the dimorphisms between the sexes in nervous system morphology and behavior. Moore(1984) varied maternal licking by interfering with the mothers' olfaction and then gonadectomized and subsequently administered testosterone to both males and females. Male rats which received less maternal licking had longer ejaculatory latencies, longer post-ejaculatory intromission latencies, and longer inter-intromission intervals than controls. Females showed commensurate deficiencies in copulatory timing. This model exemplifies how variations in hormone levels between the sexes may act indirectly to affect behavioral displays rather than directly on the central nervous system.

Related research in rats has shown that infantile handling has an interactive effect with hormone levels in determining the size of the sexually dimorphic corpus callosum. There is marked sexual dimorphism of the rat corpus callosum, with the male corpus callosum being larger than the female and having greater width of the genu and splenium (Berrebi, Fitch, Ralphe, Denenberg, Friedrich & Denenberg, 1988). Handling of rats in infancy enhances these sex differences (Denenberg, Fitch, Schrott, Cowell & Waters, 1991). Female rats handled in infancy and given an injection of testosterone propionate (TP) at Day 4 will have callosa equivalent in size to males while females administered TP without infantile handling, do not develop a larger callosum (Denenberg, et al. 1991). These authors suggest that corticosterone, released during handling, interacts with the organizational effects of TP on the central nervous system. Denenberg

(1981) further hypothesized, in comparing handled and nonhandled male rats, that handling leads to a more lateralized brain observable in a variety of behavioral tests.

There are sexually dimorphic areas in the brain of mammals. These sex differences in neural structure are correlated with differences in hormonal environments during ontogeny, and these dimorphisms can be reversed with appropriate hormonal manipulations early in development. The brain areas with the greatest amount of sex differences have been shown, through lesion studies and hormonal implants, to be intimately involved in sexual and maternal behavior. However, in order to understand the extent to which these dimorphisms influence behavior we must ensure the focus is on the correct behaviors, avoiding the possibility that the neurological structure in question is more tightly tied to some other behavioral display.

CONCLUSIONS

There are undeniably areas in the brain whose structure varies according to the gonadal status or the behavior of the possessor. This is the case in fish, amphibians, reptiles, birds, and mammals. Whether this is also the case in primates is a separate, debatable issue, which we do not address here.

Most of the studies we reviewed left the causal relations unexplored: do particular brain structures dictate particular aspects of sexual behavior, or can the behavior activate feedback processes that lead to changes in brain structure? Examples of both patterns have been found in certain species of fish and birds. In some taxa there is an obvious change in brain structure preceding the demonstration of sex-specific behavior. Other taxa demonstrate behavioral change triggered by environmental events and changes in brain structure followed. Examples were explored where the perinatal hormonal environment, which is ever important in the development of neural structures, acts differently upon genetic males and females, with one sex or the other being more resistant to its effects.

No single phyletic trend is obvious, though this could easily be the consequence of the small number of taxa that have been examined, as well as by the rather different aims of the studies. Bird studies focused on the neural control of the song of males, while fish experiments examined cues that led to behavioral and gonadal changes in sex. Amphibian and reptile studies were concerned primarily with seasonal

changes in behavior and neural structure. Mammalian studies have often had as their primary goal the elucidation of the role of prenatal hormones in the shaping of gonadal and neural structures, with the interactive role of behavior often ignored. However, it is also possible that there is simply no single trend.

While no singular trend was discovered, there were common themes surrounding the nature of sexual dimorphisms throughout many taxa which are a useful focus for further study. The hypothalamus, including areas such as the preoptic, sexually dimorphic and suprachiasmatic nuclei, was the structure most cited as being sexually dimorphic including examples in amphibians, reptiles, birds and mammals. Central and peripheral structures intimately involved in communication were significantly different between males and females in size and/or composition in fish, amphibians, birds, and mammals. While between taxa the communication system may be as varied as the EODs in fish, song in birds, or odor detection in mammals, all represent ways to send signals between the sexes, often critical for the preservation of reproduction. Similar differences in production or accumulation of analogous hormones involved in the development and maintenance of sex differences in structure or behavior were also found across taxa, including the complicated feedback loop controlling gonadotropin release.

At least two conclusions can be provisionally drawn. First, as with other indicators of sex, such as gonads, karyotype, or genitals, specific brain structures are but one element of the many that define sex. The studies we have reviewed reinforce the view that sex is a multifaceted and complex attribute. Secondly, sexuality has not necessarily evolved linearly from a particular primitive vertebrate ancestor: sexuality is variously manifested in the different classes of vertebrates (cf Short & Balaban, 1994). Thus it is likely that central control mechanisms have multiple origins, reflecting the varied evolutionary strategies required by different species for their reproduction and survival. This is a lesson well known to comparative biologists and psychologists, but perhaps one that others who would use comparative data need to be taught (cf Klopfer, 1996).

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