Original Paper

Brain, Behavior and Evolution

Brain Behav Evol 2000;55:139–151

Functional Connectivity among Limbic Brain Areas: Differential Effects of Incubation Temperature and Gonadal Sex in the Leopard Gecko, *Eublepharis macularius*

Jon T. Sakata[a] Patricia Coomber[b] F. Gonzalez-Lima[a] David Crews[a,b]

[a]Institute for Neuroscience, University of Texas at Austin, [b]Section of Integrative Biology, Division of Biological Sciences, University of Texas at Austin, Tex., USA

Key Words

Reptile · Temperature-dependent sex determination · Cytochrome oxidase · Aggression · Sexual behavior · Functional connectivity

Abstract

The neural basis of individual differences in behavior has been studied primarily by analyzing the properties of specific neural areas. However, because of the organization of the nervous system, it is also plausible that differences in behavior are mediated by differences in the interactivity or functional connectivity among brain nuclei in particular neural circuits. In the leopard gecko, *Eublepharis macularius,* the temperature of the egg during incubation not only determines gonadal sex, but also shapes the sociosexual behaviors, reproductive physiology, and hormone sensitivity of adult animals. In this study the effects of both incubation temperature and gonadal sex on functional connectivity among limbic brain areas were examined. Functional connectivity was assessed by analyzing covariance patterns in metabolic capacity, as revealed by quantitative cytochrome oxidase histochemistry. It was hypothesized that incubation temperature and gonadal sex affect the propensity to display aggressive or sexual behaviors by altering the functional connectivity within relevant neural circuits. The correlations of metabolic capacity between the anterior hypothalamus and both the septum and preoptic area were significant only in relatively aggressive individuals, suggesting that these circuits may regulate the phenotypic variation in aggressiveness caused by incubation temperature. The correlations between the ventromedial hypothalamus and both the dorsal ventricular ridge and septum were significant only in females, suggesting that these circuits may modulate female-typical sexual behaviors. Correlations among preoptic, hypothalamic and amygdalar areas tended to be distributed across both sexes, suggesting that there may be shared pathways underlying the expression of male-typical and female-typical behaviors.

Introduction

Major contributions to the understanding of individual differences in behavior have come from studies analyzing mean differences in some characteristic of specific brain nuclei. These studies have highlighted parameters such as neuronal structure (e.g. neuronal or nucleus size, dendritic arborization) and gene expression as important in generating behavioral differences. A complementary approach to this endeavor is to focus on functional interactions among brain areas, as the nervous system can be viewed as a complex network of interacting brain nuclei. This approach has

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

Accessible online at:
www.karger.com/journals/bbe

David Crews
Section of Integrative Biology, Division of Biological Sciences
University of Texas at Austin, Austin, TX 78712 (USA)
Tel. 512-471-1113, Fax 512-471-6078
E-Mail crews@mail.utexas.edu

been embraced by electrophysiologists since the late 1970's [Gerstein et al., 1978] and highlighted in neuroimaging studies [e.g. Horwitz et al., 1984; Horwitz, 1991; Friston, 1994]. Anatomical connectivity refers to the patterns of neuroanatomical relationships among brain areas, whereas functional connectivity refers to the pattern of relationships in metabolic activity among brain areas. One way to analyze functional interactions is to assess correlations in brain metabolic activity [Nair and Gonzalez-Lima, 1999]. The underlying theme of this approach is that neural regions that are functionally coupled show coordinated changes in metabolic activity, and the extent of this coordination will be manifested in the strength of the correlation between the areas. For example, if two areas show a large, positive correlation in metabolic activity, an increase in activity in area A will be coupled to an increase in activity in area B. Such covariance studies are important as they help build neural models that complement or challenge models based on means analyses.

In a previous study, the effects of an ecologically relevant parameter, namely incubation temperature, on metabolic capacity in limbic brain areas in the leopard gecko, *Eublepharis macularius*, was investigated [Coomber et al., 1997]. In this species, gonadal sex is determined by incubation temperature (fig. 1), and, interestingly, incubation temperature has profound effects on adult sexual and aggressive behaviors [reviewed in Crews et al., 1998]. For example, males from the male-biased incubation temperature (i.e. 32.5 °C) attack female stimulus animals more frequently than males from the female-biased temperature (i.e. 30 °C) [Flores et al., 1994]. Similarly, females from the male-biased and high (i.e. 34 °C) incubation temperatures are more aggressive than females from the low temperature (i.e. 26 °C) [Flores et al., 1994; Crews et al., 1996]. Males are generally more aggressive than females, and unmanipulated males never display female-typical receptive behaviors. Unmanipulated females never display male-typical courtship behaviors [Flores et al., 1994; Rhen and Crews, 1999, 2000]. Finally, same-sex individuals from different incubation temperatures also differ in their sensitivities to the activational effects of testosterone on male-typical courtship and territorial behaviors [Crews et al., 1996; Rhen and Crews, 1999].

In the analysis of mean differences in metabolic capacity [Coomber et al., 1997], areas such as the anterior hypothalamus (AH), septum, and external nucleus of the amygdala were implicated in mediating differences in aggressiveness among individuals from different incubation temperatures. Areas such as the AH, nucleus sphericus, preoptic area, and ventromedial hypothalamus were implicated in the production of behavioral sex differences. In the current study, we analyzed the covariance patterns in cytochrome oxidase (CO) activity among limbic brain nuclei. Such covariance analyses can be done without anatomical constraints, and this approach is known as functional connectivity [Gerstein et al., 1978]. It provides an overview of functional interactions among brain areas as it reflects both direct and indirect functional connections.

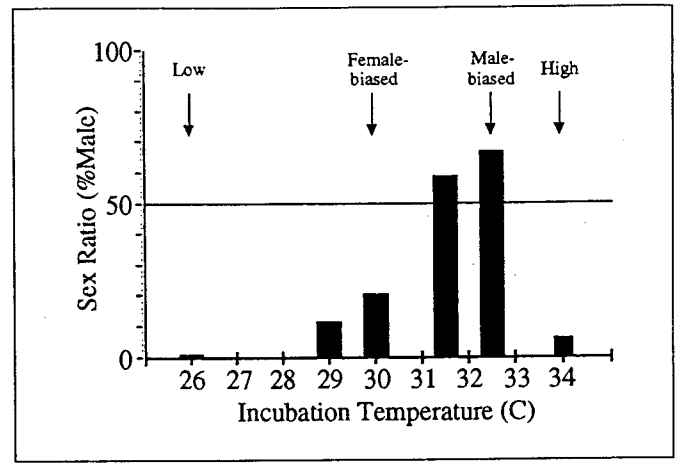


Fig. 1. Sex ratio (percent male) changes as a function of embryonic incubation temperature. Sex ratios are based on data collected over the past nine years (26 °C, n = 178; 29 °C, n = 9; 30 °C, n = 272; 31.5 °C, n = 20; 32.5 °C, n = 467; 34 °C, n = 71).

It was hypothesized that incubation temperature and gonadal sex predispose individuals to express specific behaviors by altering not only the metabolic capacity but also the strength of functional coupling within specific circuits. The strength of the functional coupling between brain areas was assessed by the statistical significance of the correlation in metabolic capacity between brain regions. A major goal of this study was to identify potential neural circuits that could predispose individuals to display particular behaviors. This was done by matching patterns of statistical significance in correlations with patterns of behavioral differences across incubation temperature and gonadal sex. For example, correlations that were significant only in individuals from incubation temperatures that produce aggressive individuals (i.e. male-biased and high temperatures) were highlighted as functional connections that might mediate incubation temperature-induced differences in aggression. Furthermore, correlations that were consistently significant in one sex but not the other were postulated to reflect circuits that underlie sex differences in the propensities to display either aggressive or sexual behaviors. The present

results support predictions based on means analyses pertaining to the neural basis of individual differences in sociosexual behaviors in the leopard gecko and also yield novel hypotheses pertaining to the role of specific brain areas underlying this variation.

Materials and Methods

Interregional covariance patterns in cytochrome oxidase (CO) activity were analyzed among the following brain regions of interest: the anterior hypothalamus (AH), the dorsal ventricular ridge (DVR), the external nucleus of the amygdala (AME), the nucleus sphericus (NS), the preoptic area (POA), the medial portion of the posterior septal nucleus (SEP), the striatum (STR), and the ventromedial hypothalamus (VMH). These areas are hypothalamic or limbic telencephalic regions selected for their roles in sociosexual behaviors or their putative steroid accumulating capacities [Morrell et al., 1979; Young et al., 1994]. The STR was selected as our control region because it is homologous to the mammalian STR and generally regulates motor output rather than specific sexual or aggressive behaviors [Reiner et al., 1984]. Hence, the correlations with the STR served as a control to assess whether incubation temperature and gonadal sex effects on correlations were specific to limbic brain areas implicated in sociosexual behaviors.

Animal care procedures and CO histochemical methods have been reported previously [Coomber et al., 1997; Gonzalez-Lima and Cada, 1998]. Briefly, all animals were housed in isolation in 30×12×6 cm polypropylene containers until adulthood. At one year of age, the animals were killed, and their brains were rapidly frozen in isopentane. All animals were sociosexually naïve upon sacrifice. Brains were sectioned in the coronal plane at 25 μm on a cryostat (Reichert-Jung, Nussioch, Germany), and slides were kept frozen until processing. For quantitative CO histochemistry, slides were first treated in 10% sucrose phosphate buffer (0.1 *M*, pH 7.6) containing 0.5% glutaraldehyde for 5 min. This step facilitates the adherence of sections to slides and does not affect the enzymatic activity of CO as demonstrated empirically in Gonzalez-Lima and Cada [1998]. Slides were then rinsed 4× in 10% sucrose phosphate buffer (5 min each) then incubated for 10 min in Tris buffer (0.05 *M*, pH 7.6) containing 275 mg/l cobalt chloride, 10% sucrose, and 0.5% dimethylsulfoxide. Slides were subsequently rinsed briefly in phosphate buffer then incubated at 37 °C for 60 min in 700 ml of an oxygen-saturated reaction solution containing 350 mg diaminobenzidine tetrahydrochloride, 52.5 mg cytochrome c, 35 g sucrose, 14 mg catalase, and 1.75 ml dimethylsulfoxide in phosphate buffer. Slides were then immersed in 10% sucrose phosphate buffer with 4% formalin (v/v) for 30 min to stop the reaction and fix the tissue, then dehydrated through a series of alcohols (30%, 50%, 70%, 95% 2×, 100% 2×) before clearing with xylene and coverslipping with Permount. This staining protocol results in a linear increase in staining optical density with increased CO activity [Gonzalez-Lima and Cada, 1998].

Slides were analyzed with an image-processing system consisting of a Javelin camera (model JE2362A, Meyers Instruments, Houston, TX) mounted on a Zeiss light microscope, and a Data Translation DT2255 QuickCapture image processor in a Macintosh Quadra computer. For each nucleus, three adjacent sections were measured densitometrically for each animal, and four density measurements were taken on each section for a total of 12 readings/nucleus/animal. All measurements were taken unilaterally for each subject. Optical density values for each nucleus were then averaged and converted into activity units (μmol/min/g tissue wet weight) using a regression based on brain homogenate standards included in each batch [Cada et al., 1995]. Brain homogenate standards served as internal calibration standards to control factors that affected staining intensity and were made by homogenizing whole brains of 12 naïve rats at 4 °C followed by rapid freezing in isopentane. These standards were cut at varying thickness (10, 20, 40, 60, and 80 μm), and the optical densities of these sections were then regressed on the known CO activity of the sections of varying thickness. The CO enzyme activity of the homogenate was spectrophotometrically assessed using the method described in Cada et al. [1995]. This regression derived from activity standards serves to calibrate all values in a batch to a common unit and allows for the aggregation of data from different batches.

For the analysis of functional connectivity, data from sexually naïve, adult leopard geckos that were killed at 50–52 weeks of age were used [females from low incubation temperature (26 °C), n = 15; females from the female-biased temperature (30 °C), n = 12; males from the female-biased temperature (30 °C), n = 9; females from the male-biased temperature (32.5 °C), n = 13; males from the male-biased temperature (32.5 °C), n = 12; females from the high temperature (34 °C), n = 11]. Absolute CO values used in this analysis have previously been reported [Coomber et al., 1997]. Because some data points were missing in the data set (2 values missing for POA and 1 for SEP in data for males from the female-biased temperature; 1 value missing from DVR, STR, and SEP in data for males from the male-biased temperature; 1 value missing from VMH in data for females from the high temperature) significance tests were done specifically on each pair of nuclei. Although CO data were available for males from the high incubation temperature (34 °C), the small sample size (n = 4) would not lead to reliable estimates of functional connections; therefore, covariance patterns in this group were not analyzed. Before investigating interregional correlations, each individual's regional CO values were normalized by dividing the CO value by the whole brain activity estimate for the individual. Whole brain activity estimates were obtained by imaging entire sections from slides mounted on a light box (TrueLite, Meyers Instruments, Houston, Tx., USA) and averaging the activities of all sections. Readings were taken from the most rostral portion of the brain to the level near the midbrain torus semicircularis. This normalization was done to reduce the possibility that significant interregional correlations are artifacts of correlations with whole-brain activity [McIntosh and Gonzalez-Lima, 1992; Cada et al., 1995], and this procedure is analogous to partial correlations factoring out average brain metabolism [Horwitz and Rapoport, 1988]. It should be noted that this normalization is done regardless of whether or not group differences in whole brain activity exist. This is because correlations are done within each group and because the purpose of the normalization is to factor out individual variation in whole brain activity when calculating the correlations.

To ensure the reliability of correlations and to protect against the effects of outliers, all correlations were subjected to a modified jackknife procedure. In this modified procedure, an individual is removed from the data set, and correlations are computed on the data set with n-1 subjects. Significance tests are then done on the correlation using n-1 subjects. The individual is then replaced back into the data set, and another individual is removed. Correlations and significance tests are again computed on the data set with n-1 subjects. This procedure is

Table 1. Interregional correlations ('r') in metabolic activity

Pairs of regions	Female		Female		Male		Female		Male		Female	
	Low temperature (n = 15)		Female-biased temperature (n = 13)		Female-biased temperature (n = 9)		Male-biased temperature (n = 13)		Male-biased temperature (n = 12)		High temperature (n = 11)	
	r	p	r	p	r	p	r	p	r	p	r	p
AH-AME	0.42	0.121	0.24	0.460	0.74	0.021	0.82*	<0.001	0.08	0.814	0.36	0.277
AH-DVR	0.28	0.305	0.71*	0.010	0.76	0.018	0.86*	<0.001	0.79*	0.004	0.88*	<0.001
AH-NS	0.38	0.159	0.77*	0.003	0.90*	0.001	0.87*	<0.001	0.83*	<0.001	0.73	0.010
AH-POA	0.26	0.355	0.31	0.322	0.71	0.075	0.67*	0.013	0.79*	0.002	0.27	0.422
AH-SEP	0.23	0.406	0.52	0.080	0.68	0.065	0.72*	0.006	0.85*	0.001	0.89*	<0.001
AH-STR	0.45	0.091	0.88*	<0.001	0.96*	<0.001	0.83*	<0.001	0.94*	<0.001	0.84*	0.001
AH-VMH	0.04	0.874	0.86*	<0.001	0.77	0.015	0.48	0.095	0.70	0.012	0.80*	0.006
AME-DVR	0.61	0.016	0.32	0.315	0.67	0.048	0.67*	0.012	0.62	0.043	0.35	0.289
AME-NS	0.59	0.020	0.10	0.756	0.77	0.015	0.81*	<0.001	0.46	0.132	0.29	0.394
AME-POA	0.56	0.030	0.41	0.182	0.91	0.004	0.50	0.080	0.38	0.229	0.21	0.537
AME-SEP	0.61	0.016	0.58	0.047	0.54	0.171	0.55	0.053	0.16	0.633	0.31	0.377
AME-STR	0.59	0.020	0.36	0.244	0.69	0.039	0.77*	0.002	0.29	0.388	0.54	0.087
AME-VMH	0.35	0.201	0.41	0.191	0.61	0.083	0.32	0.279	0.25	0.425	0.55	0.103
DVR-NS	0.67*	0.006	0.74*	0.006	0.91*	<0.001	0.83*	<0.001	0.91*	<0.001	0.81*	0.002
DVR-POA	0.86*	<0.000	0.44	0.151	0.66	0.105	0.65	0.016	0.81*	<0.001	0.56	0.072
DVR-SEP	0.71*	0.003	0.80*	0.002	0.76	0.028	0.74*	0.004	0.76	0.011	0.92*	<0.001
DVR-STR	0.62*	<0.000	0.86*	<0.001	0.77	0.017	0.71*	<0.001	0.65*	<0.001	0.83*	<0.001
DVR-VMH	0.80*	0.015	0.84*	<0.001	0.76	0.014	0.91*	0.007	0.89	0.030	0.88*	0.003
NS-POA	0.73*	0.002	0.34	0.278	0.74	0.056	0.58*	0.038	0.89*	<0.001	0.59	0.055
NS-SEP	0.87*	<0.000	0.66	0.020	0.82	0.013	0.64	0.018	0.77	0.006	0.85*	0.002
NS-STR	0.64*	0.010	0.78*	0.003	0.86*	0.003	0.88*	<0.001	0.92*	<0.001	0.83*	0.004
NS-VMH	0.65*	0.009	0.86*	<0.001	0.85*	0.004	0.55	0.054	0.74*	0.006	0.67	0.040
POA-SEP	0.61*	0.016	0.41	0.189	0.44	0.380	0.69*	0.008	0.78*	0.004	0.38	0.282
POA-STR	0.84*	<0.000	0.51	0.091	0.80	0.029	0.67*	0.012	0.92*	<0.001	0.33	0.319
POA-VMH	0.77*	0.001	0.46	0.128	0.37	0.418	0.22	0.463	0.75*	0.005	0.17	0.641
SEP-STR	0.45	0.091	0.76*	0.004	0.61	0.109	0.62	0.023	0.95*	<0.001	0.95*	<0.001
SEP-VMH	0.64*	0.011	0.77*	0.003	0.54	0.168	0.58	0.036	0.60	0.053	0.86*	0.001
STR-VMH	0.57	0.027	0.91*	<0.001	0.69	0.039	0.58	0.036	0.73	0.011	0.94*	<0.001
Whole brain activity#	8.54 ± 0.35		10.03 ± 0.42		9.65 ± 0.47		9.84 ± 0.54		9.00 ± 0.38		9.90 ± 0.54	

Note: The regions of interest are: the anterior hypothalamus (AH), the external nucleus of the amygdala (AME), the dorsal ventricular ridge (DVR), the nucleus sphericus (NS), the preoptic area (POA), the medial portion of the posterior septal nucleus (SEP), the striatum (STR), and the ventromedial hypothalamus (VMH).

* Denotes jackknifed correlations that remain significant at the $p < 0.05$ level for each iteration (i.e. reliably significant).

\# Whole brain metabolic capacity estimates (μmol/min/g tissue wet weight).

repeated until all individuals have been taken out once. Correlations are considered to be 'reliably significant' from zero if they remain significant ($p < 0.05$) throughout each iteration [Cada et al., 1995]. Whereas the standard jackknife procedure essentially calls for the averaging of obtained jackknife correlations to assess statistical significance, this calculation is still affected by the presence of outliers [Sokal and Rohlf, 1995]. Therefore, we selected this modified approach (i.e. multiple significance testing) because it yields more conservative results than the traditional jackknife and is very sensitive to outliers.

To corroborate group differences, correlations that were reliably significant in one group were compared to corresponding correlations that were not reliably significant in another group using Bonferroni-corrected omnibus Student's t tests on paired data. Before comparison, all correlations were transformed to z scores. Three intrasexual comparisons (females: low vs. male-biased temperature and low vs. high temperature; males: female-biased vs. male-biased) and two intersexual (males vs. females at female-biased and male-biased temperatures) were planned based on behavioral differences found in past experiments and on gonadal differences [Flores et al., 1994; Rhen and Crews,

1999, 2000]. Significance was set at $\alpha = 0.05$ after correction for multiple comparisons using a modified Bonferroni correction procedure [Hochberg, 1988].

Finally, whole brain estimates were compared across groups using an analysis of variance (ANOVA). All statistics were done using Version 3.1 of JMP [SAS Institute] for the Apple Macintosh.

Results

Whole brain values were not significantly different among females from different incubation temperatures ($F = 2.54$, $df = 3, 47$, n.s.), among males from different temperatures ($F = 1.18$, $df = 1, 20$, n.s.) or between males and females at the female-biased and male-biased temperatures ($F = 0.33$, $df = 1, 45$, n.s.) (table 1, bottom row).

The jackknife technique successfully highlighted correlations that were very linear and robust to outliers. The numbers of correlations that were significant within each group before the jackknife procedure were 19 for females from the low temperature, 15 for females from the female-biased temperature, 18 for males from the female-biased temperature, 22 for females from the male-biased temperature, 21 for males from the male-biased temperature, and 18 for females from the high temperature. After the jackknife the number of reliably significant correlations were 13, 13, 5, 16, 15, and 13 for the respective groups. An example of two correlations, one that is reliably significant (fig. 2A) and one that is not reliably significant (fig. 2B) is provided. The significant correlations highlighted using the jackknife were highly linear, and most reliably significant correlations were significant at the $\alpha = 0.01$ level. However, three correlations that were significant at the $\alpha = 0.01$ level did not maintain significance throughout the jackknife, and six correlations that were very close to the $\alpha = 0.01$ level remained significant after the jackknife. Therefore, the jackknife method yielded conservative estimates of reliably significant correlations and controlled for type 1 error.

Functional Connections in Individual Groups

All reliably significant correlations among the eight nuclei were positive (table 1; fig. 3), and all correlations listed below are those that remained significant after the jackknife (i.e. reliably significant).

In females from the low incubation temperature, significant correlations were found between the DVR and the NS, POA, SEP, STR and VMH, as well as between the NS and the POA, SEP, STR, and VMH. Metabolic capacity in the POA was also significantly correlated with the CO activity in the SEP, STR, and VMH, and CO activity in the SEP was also correlated with CO activity in the VMH (table 1).

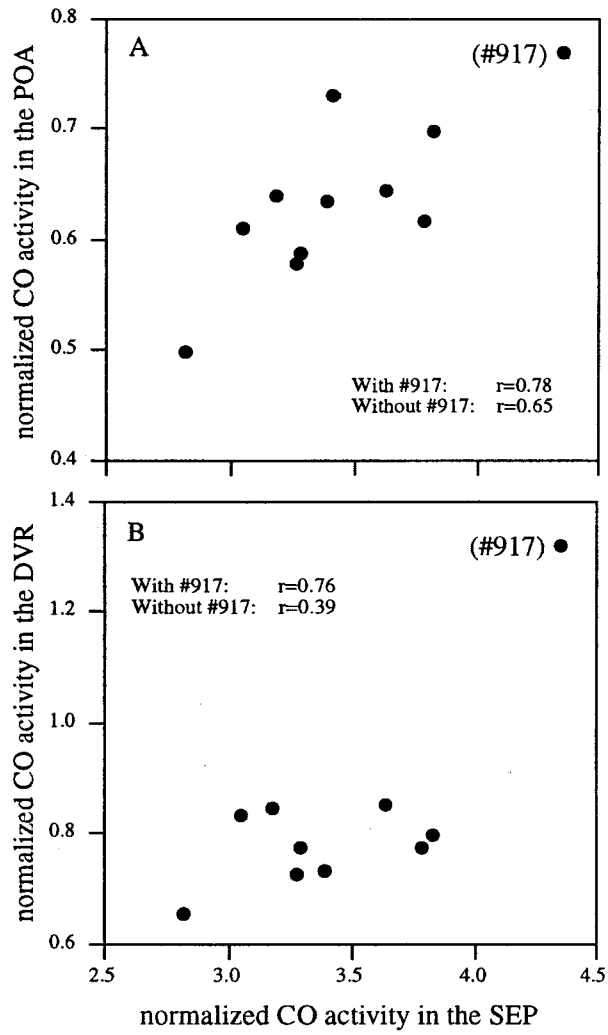


Fig. 2. Scatterplots of normalized cytochrome oxidase (CO) activity in males from the male-biased incubation temperature. **A** The correlation in normalized CO activity between the SEP and the preoptic area (POA) is reliably significant because it remains significant throughout the jackknife iterations. With the inclusion of #917, the correlation is 0.79, and without #917 the correlation is 0.65. **B** The correlation in normalized CO activity between the septum (SEP) and the dorsal ventricular ridge (DVR) is dramatically affected by the outlier (i.e. #917). With the inclusion of #917, the correlation is 0.76, but when the point is removed the correlation drops down to 0.39.

In females from the female-biased temperature, significant correlations were found between the AH and the DVR, NS, STR, and VMH. CO activity in the DVR was also correlated with CO activity in the NS, SEP, STR, and VMH, and activity in the NS was also correlated with activity in the STR and VMH. Significant correlations were also found

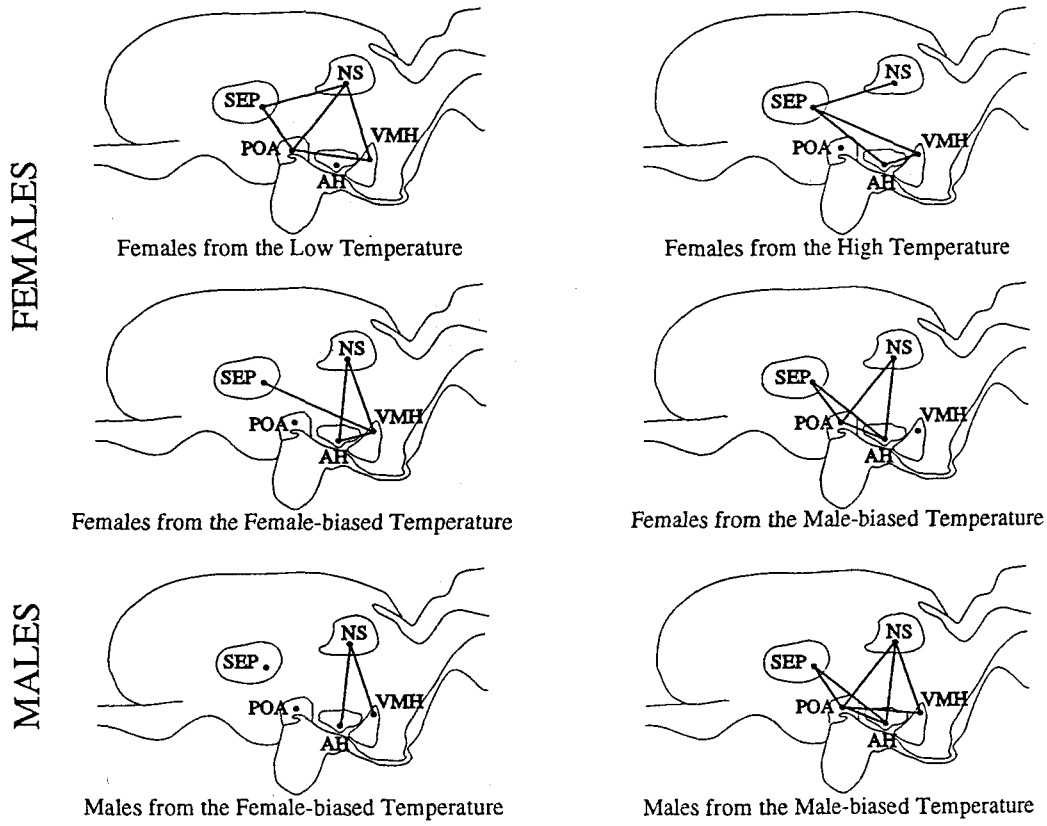


Fig. 3. A diagram of the significant correlations among the anterior hypothalamus (AH), nucleus sphericus (NS), preoptic area (POA), septum (SEP), and ventromedial hypothalamus (VMH). All correlations are positive. Note that the correlation between the AH and SEP is significant only in relatively aggressive individuals.

between the SEP and both the STR and VMH and between the STR and VMH (table 1).

In males from the female-biased incubation temperature, significant correlations were found between the AH and both the NS and STR, and between the DVR and NS. Metabolic capacity in the NS was also correlated with CO activity in the STR and VMH (table 1).

In females from the male-biased temperature, significant correlations were found between the AH and the AME, DVR, NS, POA, SEP and STR. Metabolic capacity in the AME was also correlated with metabolic capacity in the DVR, NS, and STR, and CO activity in the DVR was also correlated with CO activity in the NS, SEP, STR, and VMH. Significant correlations were also found between the NS and both the POA and STR and between the POA and both the SEP and STR (table 1).

In males from the male-biased temperature, significant correlations were found between the AH and the DVR, NS, POA, SEP, and STR. Metabolic capacity in the DVR was also significantly correlated with capacity in the NS, POA, and STR, and CO activity in the NS was also correlated with activity in the POA, STR, and VMH. Finally, there were significant correlations between the POA and the SEP, STR, and VMH and between the SEP and STR (table 1).

In females from the high temperature, significant correlations were found between the AH and the DVR, SEP, STR, and VMH, as well as between the DVR and the NS, SEP, STR, and VMH. CO activity in the NS was correlated with CO activity in the SEP and STR, and activity in the SEP was correlated with activity in the STR and VMH. Finally, there was a significant correlation between the STR and VMH (table 1).

Comparison of Significant Correlations between Groups

Incubation Temperature Effects: Females from the Low Temperature vs. Females from the Male-Biased Temperature. Within this pair, reliably significant correlations were significantly larger than the corresponding nonsignificant correlations ($t = 7.01$, $n = 14$, Bonferroni corrected $p < 0.05$). The correlations between the DVR and POA, between the NS and both the SEP and VMH, between the POA and VMH, and between the SEP and VMH were significant in the females from the low temperature but not in females from the male-biased temperature. The correlations between the AH and the AME, DVR, NS, POA, SEP and STR and between the AME and the DVR, NS, and STR were significant in females from the male-biased temperature but not in females from the low temperature.

Incubation Temperature Effects: Females from the Low Temperature vs. Females from the High Temperature. Within this pair, reliably significant correlations were significantly larger than the corresponding nonsignificant correlations ($t = 6.44$, $n = 12$, Bonferroni corrected $p < 0.05$). The correlations between the DVR and POA, between the NS and both the POA and VMH, and between the POA and the SEP, STR, and VMH were significant in females from the low temperature but not in females from the high temperature. The correlations between the AH and the DVR, SEP, STR, and VMH, between the SEP and STR, and between the STR and VMH were significant in females from the high temperature but not in females from the low temperature.

Incubation Temperature Effects: Males from the Female-Biased Temperature vs. Males from the Male-Biased Temperature. Within this pair, reliably significant correlations were significantly larger than the corresponding nonsignificant correlations ($t = 3.49$, $n = 10$, Bonferroni corrected $p < 0.05$). The correlations between the AH and the DVR, POA, and SEP, between the DVR and both the POA and STR, between the NS and POA, and between the POA and the SEP, STR and VMH, and between the SEP and STR were significant in males from the male-biased temperature but not in males from the female-biased temperature. All the significant correlations for males from the female-biased temperature were also significant for the males in the male-biased temperature.

Gonadal Sex Effects: Males vs. Females at the Female-Biased Temperature. Within this pair, reliably significant correlations were significantly larger than the corresponding nonsignificant correlations ($t = 3.31$, $n = 8$, Bonferroni corrected $p < 0.05$). The correlations between the AH and both the DVR and VMH, between the DVR and the SEP, STR, and VMH, between the SEP and both the STR and VMH, and between the STR and VMH were significant in females but not in males. All significant correlations for these males were also significant for females from the same incubation temperature.

Gonadal Sex Effects: Males vs. Females at the Male-Biased Temperature. Within this pair, reliably significant correlations were significantly larger than the corresponding nonsignificant correlations ($t = 4.02$, $n = 10$, Bonferroni corrected $p < 0.05$). The correlations between the AH and AME, between the AME and the DVR, NS, and STR, and between the DVR and both the SEP and VMH were significant only in females from this temperature. The correlations between the DVR and POA, NS and VMH, between the POA and VMH, and between the SEP and STR were significant only in males at this temperature.

General Effects

Functional connections that were specific to a sex or to individuals from a specific incubation temperature were found, as well as correlations that were consistently significant across most or all groups. The correlations between the DVR and both the SEP and VMH were reliably significant only in females and were not affected by incubation temperature. On the other hand, the correlation between the AH and POA was reliably significant only in males and females from the male-biased temperature, and the correlation between the AH and NS was reliably significant in males and females from both the female-biased and male-biased temperatures. The correlation between the NS and SEP was reliably significant only in females from temperatures that produced sex ratios heavily skewed toward females (i.e. the low and high temperatures), and the correlation between the SEP and VMH was significant among females from all three temperatures that produced female-biased sex ratios. The correlation between the DVR and NS and between the NS and STR were reliably significant across all groups, whereas the correlation between the DVR and both the STR and AH were reliably significant in all but one group.

Discussion

Covariance Analysis of Regional Metabolic Activity

Cytochrome oxidase is a rate-limiting enzyme in aerobic cellular respiration, the brain's primary means of energy production, and an important marker of metabolic capacity [Gonzalez-Lima, 1992; Wong-Riley et al., 1998]. Information on CO activity provides insight into the metabolic history of brain regions, as brain areas that are continually more active will show elevated CO activity relative to less

active regions [Wong-Riley, 1989]. This type of information is very different from other metabolic information such as 2-deoxyglucose uptake or FOS protein expression, both of which reflect evoked or immediate activity. Differences in mean CO activity reflect differences in the baseline activity of particular brain areas, whereas differences in correlations in CO activity are postulated to reflect differences in baseline functional coupling among brain areas. In other words, correlations in CO activity represent functional 'traits' rather than an acute 'state' during behavioral performance (e.g. correlations of 2-deoxyglucose uptake) [Nair and Gonzalez-Lima, 1999]. Moreover, differences in the magnitude of functional relationships (i.e. correlations in metabolic activity) within specific neural circuits are hypothesized to reflect differences in the predisposition to display particular behaviors. Finally, though functional connectivity is intimately related to anatomical connectivity, the two are not synonymous. For example, two areas may be similarly connected anatomically in two groups but due to differences in synaptic activity could have different strengths of function coupling.

In the current study, correlations in metabolic capacity among limbic brain areas were calculated within groups of males and females from different incubation temperatures, and within each group these correlations were tested for statistical significance. The jackknife technique was used to highlight 'reliably significant' correlations (i.e. correlations that were very linear). The patterns of statistical significance in correlations across incubation temperature and gonadal sex were then related to established patterns of behavioral differences across the groups. For example, a correlation that is reliably significant only in females was identified as a functional connection that might modulate sex differences, and correlations that were reliably significant only in relatively aggressive individuals were identified as functional connections that might produce heightened aggressiveness. Patterns in statistical significance were highlighted because a statistically significant correlation can be considered a linear association whose strength is greater than that due to biological noise. Furthermore, patterns of significance levels after the jackknife were emphasized instead of correlation coefficients because correlation coefficients can be heavily influenced by outliers.

It should be noted that the lack of a reliably significant correlation should not be interpreted as a lack of functional coupling between two brain areas. By definition, a correlation is significant if the degree of linearity in the relationship between the two variables is greater than that expected from random chance or biological noise. Therefore, correlations not found to be reliably significant do not necessarily represent functionally inactive circuits; they could represent relatively weak functional connections. In this respect, a correlation that is significant in group A but not group B should be interpreted as denoting a stronger relationship between the two brain areas in group A than in group B. Similarly, the lack of significance in a correlation might be the result of insufficient statistical power. Sample sizes ranged from 9–15 in the current study, and the group with the fewest reliably significant correlations was the group with the smallest sample size (males from the female-biased temperature).

It should also be noted that in this analysis structural constraints were not imposed on the covariance analysis; therefore, functional connectivity, as opposed to effective connectivity [Aertsen et al., 1989], was studied. This method was selected because the addition of neuroanatomical constraints (e.g. structural equations modeling) would be statistically cumbersome and difficult to interpret given the highly complex network of connections within the limbic system. However, because of this approach, significant correlations in CO activity between brain areas could represent indirect relationships. For example, a correlation between area X and Y could be due to the fact that X is directly coupled to area Z and that area Z is directly coupled to area Y, and, therefore, the correlation between X and Y is significant.

Functional Connectivity and Intrasexual Differences in Aggressiveness

Males from the male-biased incubation temperature are more aggressive than males from the female-biased temperature, and females from the male-biased and high incubation temperatures are more aggressive than females from the low temperature [Flores et al., 1994]. Although the heightened aggression of males from the male-biased temperature toward females could reflect a change in courtship behavior, this probably indicates increased aggressiveness because males rarely attack sexually receptive females [Rhen and Crews, 2000]. The correlation between the AH and SEP (fig. 3) was significant only in males from the male-biased incubation temperature and in females from the male-biased and high temperatures. The parallel between the pattern of significant correlations and the pattern of aggressive tendencies across incubation temperatures suggests that the relationship between the AH and SEP might underlie the variation in aggressiveness caused by incubation temperature. Such a prediction is very plausible as both the AH and SEP have been implicated in the display of aggression in a number of species [reviewed in Albert et al., 1992; Kollack-Walker and Newman, 1995; Ferris et al., 1997; Goodson et al., 1999]. For example, in golden hamsters, the integrity of the connections between the lateral SEP and the AH is crit-

ical for the expression of agonistic flank marking [Ferris et al., 1990].

The correlation between the AH and POA was significant only in males and females from the male-biased incubation temperature (fig. 3), and this functional connection might also help explain the aggressive tendencies of individuals from this temperature. The POA plays an important role in the expression of aggression in rodents [Albert et al., 1986; Compaan et al., 1994], and lesions of the AH-POA continuum abolish agonistic behaviors in male green anole lizards [Wheeler and Crews, 1978]. In all lizards studied to date, both areas also either express the genes for sex steroid receptors [Young et al., 1994] or concentrate sex steroids [Morrell et al., 1979].

Because lesions of the AME significantly decrease aggression in some lizards [Tarr, 1977; Greenberg et al., 1984] and because average metabolic capacity in the AME of female leopard geckos across incubation temperature parallels female aggressiveness across incubation temperature [Coomber et al., 1997], it was anticipated that the pattern of statistical significance in correlations between the AME and other areas such as the POA, AH, and SEP would parallel the pattern in aggressiveness. However, such a trend in statistical significance was not found, suggesting that the AME does not play a substantial role in the heightened aggressiveness of individuals from warmer incubation temperatures. This is also suggested from neuroanatomical data as the AME is associated more with the main olfactory system than the accessory olfactory system in other reptiles [Martinez-Garcia et al., 1991; Lanuza and Halpern, 1997; Martinez-Marcos et al., 1999]. Alternatively, it is possible that the AME could influence aggressiveness but does so by interacting with brain areas not included in this analysis.

Functional Connections and Sexual Behavior

The correlations among the POA, AH, VMH, and amygdalar areas such as the NS did not show a pattern of statistical significance that was sex- or incubation temperature-specific (fig. 3; table 1). Although the POA and VMH, respectively, have traditionally been highlighted as neural areas that specifically regulate male-typical and female-typical sexual behaviors [reviewed in Meisel and Sachs, 1994; Pfaff et al., 1994; Godwin and Crews, 1997], this analysis suggests that each of these areas could modulate both male-typical and female-typical sexual behaviors. Indeed, other studies have found similar patterns of neuronal activation in males and females displaying homotypical sexual behaviors. Using the immediate early gene cFOS as a marker of neuronal activation, it was found that in rats and hamsters cFOS expression is elevated in the medial amygdala, bed nucleus of the stria terminalis, medial POA, and VMH in both males and females displaying homotypical sexual behaviors [Wersinger et al., 1993; Flanagan-Cato and McEwen, 1995; Kollack-Walker and Newman, 1995].

Lesion as well as neuroanatomical and pharmacological studies support the notion that there is a common neural circuit that modulates both male-typical and female-typical behaviors. It is well established that lesions of the POA-AH continuum abolish male sexual behavior in a number of species [reviewed in Meisel and Sachs, 1994; Godwin and Crews, 1997]. However, lesions of the medial POA have also been found to inhibit the expression of lordosis behavior in ovariectomized female rats with estradiol implants in the VMH [Bast et al., 1987], and lesions of the AH disrupt the expression of female sexual behavior as well [Law and Meagher, 1958; Herndon and Neill, 1973]. Conversely, it is well established that the VMH is integral in the expression of female sexual behavior [reviewed in Pfaff et al., 1994]. However, lesions of the basal hypothalamus that include the VMH inhibit the expression of courtship behaviors in male green anole lizards [Farragher and Crews, 1979], red-sided garter snakes [Friedman and Crews, 1985], and ring doves [Bernstein et al., 1993]. In male hamsters VMH lesions alter the regulation of postcopulatory ultrasonic vocalizations [Floody, 1989], and in male rats the blockage of androgen receptors in the VMH leads to severe decrements in copulatory behavior [McGinnis et al., 1996]. Furthermore, androgen-accumulating cells that project to midbrain areas regulating male sexual behavior are present not only in the POA but also in the VMH [Lisciotto and Morrell, 1990]. Finally, in the leopard gecko, males with extensive sociosexual experience have elevated CO levels in the VMH relative to age-matched, sexually naive males from the same incubation temperature [Crews et al., 1997]. Altogether, these studies suggest that the same circuit regulates the expression of both types of sexual behavior.

The correlation between the DVR and VMH was significant only in females, and it is possible that a functional connection between the DVR and VMH modulates female sexual behavior. Because the DVR expresses genes primarily for the androgen receptor in whiptail lizards [Young et al., 1994] and because short-term testosterone treatment activates female-typical receptive behaviors in the leopard gecko [Rhen et al., 1999], the transduction of this hormonal signal in the DVR could be important in the display of female sexual behavior.

Although the AME expresses genes for sex steroid receptors in whiptail lizards [Young et al., 1994], only in females from the male-biased temperature was the metabolic capacity in the AME significantly correlated with

metabolic capacity in other preoptic or hypothalamic areas. On the other hand, the NS is relatively devoid of gene expression for sex steroid receptors [Young et al., 1994], but CO activity in the NS was significantly correlated with CO activity in the POA and/or VMH in many groups. Although the analysis of sex steroid receptor gene expression suggests that the AME is the more important amygdalar nucleus regulating sociosexual behaviors, this covariance analysis suggests that the NS might be more functionally relevant. Interestingly, a similar discrepancy between sex steroid receptor expression and functionality is found in hamsters. In male hamsters the distribution of androgen receptors is much higher in the posterior portion of the medial amygdala (MeP) relative to the anterior portion of the medial amygdala (MeA) [Wood and Newman, 1993]. In this respect, the MeP is similar to the AME, whereas the MeA is similar to the NS. Lesions of the MeA eliminate copulatory behavior in male hamsters, whereas MeP lesions only alter the temporal pattern of copulation [Lehman and Winans, 1982; Lehman et al., 1983]; this suggests that the MeA is more critical in the expression of the male sexual behavior than the MeP. If the AME is functionally analogous to the MeP and the NS is analogous to the MeA, then NS lesions should lead to greater decrements in sexual behavior than AME lesions. This is supported by the facts that there exists, overall, more significant correlations in CO activity between the NS and other hypothalamic and preoptic nuclei than between the AME and hypothalamic and preoptic nuclei, that glucose consumption is elevated in the NS in male red-sided garter snakes during courtship [Allen and Crews, 1992], and that AME lesions do not significantly affect the expression of courtship behaviors in male anole lizards [Greenberg et al., 1984].

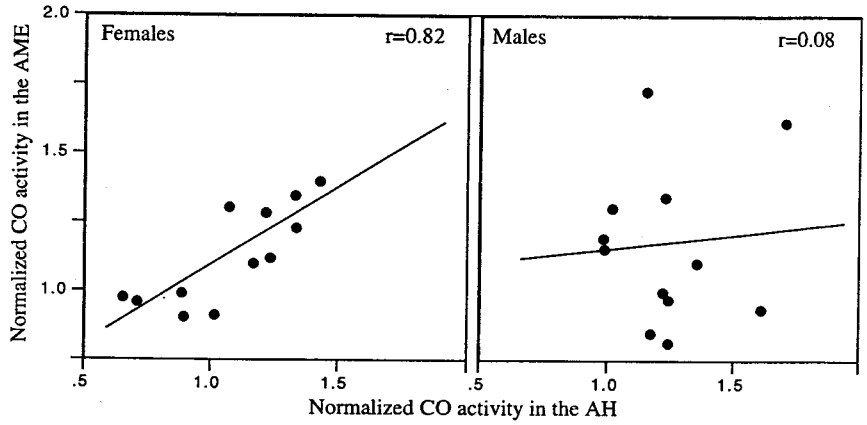
Comparison of the Means and Covariance Approaches

The results from this analysis support some predictions based on a prior means analysis [Coomber et al., 1997] about the neural basis of sex-dependent and incubation temperature-dependent differences in behavior, but also generate a few novel hypotheses about the role of brain areas. The means analysis highlighted the AME and the SEP as potential areas underlying female aggressiveness and the SEP and AH as potential areas regulating incubation temperature-induced differences in male aggressiveness. Furthermore, the AH, NS, and VMH were areas implicated in modulating sex differences in behavior. The current covariance analysis supports the hypotheses that the AH and SEP are important in generating individual differences in aggressiveness and that the VMH modulates female-typical sociosexual behavior. In this respect, the two analyses are complementary. However, this analysis did not identify either the AME as an area that might modulate aggression or the AH and NS as areas that might modulate sex differences in behavior. Novel hypotheses stemming from this analysis are that the dorsal ventricular ridge (DVR) might influence female sexual behavior and that the NS could play a functionally more important role in the display of sexual behavior than the AME.

Differences between the two approaches are not surprising as they lend insight into two different aspects of the nervous system. A means analysis of CO activity focuses on how the baseline metabolic activity of particular brain nuclei change with certain manipulations, whereas the covariance analysis focuses on how baseline interactions among brain areas change with manipulations. Two areas can have similar means across groups but different functional relationships across groups [Nair and Gonzalez-Lima, 1999]. For example, the metabolic capacity in the AH and AME are not different between males and females from the male-biased temperature but the functional relationship between males and females is substantially different (fig. 4). On the other hand, the fact that this covariance analysis supports some earlier predictions gives greater weight to such hypotheses and adds validity to the covariance approach.

The results pertaining to the roles of the SEP and AH in the generation of incubation temperature-dependent differences in aggressiveness speaks to both of these points. The trends in the metabolic capacity across incubation temperature in the SEP paralleled trends in aggressiveness among males and females. The trend in metabolic capacity in the AH, however, did not accurately parallel trends in aggressiveness among females. This covariance analysis suggests that the functional interaction between the SEP and AH is important in the differences in aggressiveness observed across incubation temperature within both males and females. In this respect, the prediction from the means analysis about the SEP is bolstered whereas the prediction regarding the AH is challenged. Future studies will address these hypotheses.

Regarding the differences in predictions dealing with the DVR, the means analysis suggests that the DVR could play a role in intersexual and intrasexual variation in sensitivity to the activational effects of testosterone on courtship behavior. Males in general have elevated CO activity in the DVR relative to females [Coomber et al., 1997], and more males display courtship behaviors with testosterone treatment than females [Rhen and Crews, 1999]. Metabolic capacity in the DVR is elevated in females from the male-biased temperature relative to females from the low temperature [Coomber et al., 1997], and testosterone treatment is



	AH	AME
Females:	1.09 ± 0.07	1.15 ± 0.05
Males:	1.24 ± 0.07	1.18 ± 0.09

Fig. 4. An example of how two brain areas [anterior hypothalamus (AH) and external nucleus of the amygdala (AME)] may possess similar means across two groups (males and females from male-biased incubation temperature) but different functional relationships.

more likely to elicit male-typical courtship behaviors in ovariectomized females from the male-biased temperature than identically-treated females from the low temperature [Crews et al., 1996]. On the other hand, because the correlation between the DVR and the VMH was significant only in females, this analysis suggests that the functional connection between these areas modulates female sexual behavior. Indeed, this difference in functional connectivity could underlie the sex differences in capacity of testosterone or estradiol to elicit female-typical receptive behaviors [Rhen and Crews, 1999; Rhen et al., 1999]. The two hypotheses, however, are not mutually exclusive (i.e. the DVR could modulate both male-typical and female-typical sexual behaviors), and studies using intracranial steroid implants into or lesions of the DVR should be informative.

Concluding Remarks

The present analysis focuses on differences in the patterns of relationships in metabolic activity among brain areas in male and female leopard geckos from different incubation temperatures. This approach was motivated by the hypothesis that differences in the interactivity within the nervous system could be important in producing differences in behavior. By relating patterns of statistical significance in correlations among brain areas across groups to documented patterns of behavioral differences in aggressiveness and sexual behavior across groups, functional connections that might underlie these differences were highlighted. Functional connections between limbic brain areas that were heavily influenced by incubation temperature and gonadal sex were identified, as were correlations that were not dramatically affected by these variables (e.g. between the STR and other brain areas). The results from this analysis make predictions that are consistent with earlier predictions based on an analysis of mean differences in CO activity among individuals from different incubation temperatures and, consequently, gives greater weight to such predictions. This analysis also makes novel hypotheses about the roles of particular brain areas that could assist in the understanding of the neurobiological basis of incubation temperature- and sex-dependent differences in behavior.

The covariance approach has been found to yield hypotheses that are consistent with lesion and metabolic mapping studies [e.g. Nair and Gonzalez-Lima, 1999]. One prediction derived from this analysis is that disruptions of the functional relationship between the AH and SEP or between the AH and POA will significantly reduce the variation in aggressiveness across individuals from different incubation temperatures. Other hypotheses are that disruptions of the functional relationship between the DVR and VMH will lead to decrements in the expression of female-typical sexual behaviors and that lesions of the NS should lead to significantly greater deficits in sexual behavior than lesions of the AME. It is possible that the reported patterns in functional connectivity might underlie behaviors not emphasized in this paper, and future studies manipulating behavior or brain mechanisms would be important to test this possibility.

Finally, a covariance approach is informative because one of the mechanisms by which gonadal steroids affect the propensity to display particular behaviors could be by altering the strength of functional connections among brain areas. Indeed, it has been shown that testosterone affects the strength of synaptic transmission between neurons in the bed nucleus of the stria terminalis and medial POA [Kendrick and Drewett, 1979]. Because neurons are coupled to other neurons and because CO activity is intimately related to synaptic activity [Gonzalez-Lima, 1992], the functional connectivity between brain areas might be affected by the levels of or sensitivity to gonadal steroids as well as by other experiential and environmental parameters.

Acknowledgments

We thank Turk Rhen and Steve Phelps for their statistical and intellectual contributions throughout this project, and Hemu Nair and Dirk Jones for their assistance in imaging and analytical procedures. We also thank Dr. Walt Wilczynski, Ajay Gupta, and Eun-Jin Yang for their helpful comments. This project was funded by NSF DGE-9616181 (JTS), NIMH 057874 (DC), NSF IBN9222075 (FGL), and ATP 361 (FGL).

References

Aertsen, A.M., G.L. Gerstein, M.K. Habib, and G. Palm (1989) Dynamics of neuronal firing correlation: modulation of 'effective connectivity'. J. Neurophysiol., *61:* 900–917.

Albert, D.J., M.L. Walsh, B.B. Gorzalka, S. Mendelson, and C. Zalys (1986) Intermale social aggression: suppression by medial preoptic lesions. Physiol. Behav., *38:* 169–173.

Albert, D.J., R.H. Jonik, and M.L. Walsh (1992) Hormone-dependent aggression in male and female rats: experiential, hormonal, and neural foundations. Neurosci. Biobehav. Rev., *16:* 177–192.

Allen, E.E., and D. Crews (1992) Sexual behavior and 2-deoxyglucose uptake in male red-sided garter snakes *(Thamnophis sirtalis parietalis).* Brain Behav. Evol., *40:* 17–24.

Bast, J.D., C. Hunts, K.J. Renner, R.K. Morris, and D.M. Quandagno (1987) Lesions in the preoptic area suppressed sexual receptivity in ovariectomized rats with estrogen implants in the ventromedial hypothalamus. Brain Res. Bull., *18:* 153–158.

Bernstein, P.L., M. Zuo, and M.-F. Cheng (1993) Social condition affects the courtship behavior of male ring doves with posterior medial hypothalamic lesions. Behav. Neural Biol., *59:* 120–125.

Cada, A., F. Gonzalez-Lima, G.M. Rose, and M.C. Bennett (1995) Regional brain effects of sodium azide treatment on cytochrome oxidase activity: a quantitative histochemical study. Metab. Brain Disease, *10:* 303–320.

Compaan, J.C., A. Wozniak, A.J.H. de Ruiter, J.M. Koolhaas, and J.B. Hutchison (1994) Aromatase activity in the preoptic area differs between aggressive and nonaggressive male house mice. Brain Res. Bull., *35:* 1–7.

Coomber, P., D. Crews, and F. Gonzalez-Lima (1997) Independent effects of incubation temperature and gonadal sex on the volume and metabolic capacity of brain nuclei in the leopard gecko *(Eublepharis macularius),* a lizard with temperature-dependent sex determination. J. Comp. Neurol., *380:* 409–421.

Crews, D., P. Coomber, R. Baldwin, N. Azad, and F. Gonzalez-Lima (1996) Brain organization in a reptile lacking sex chromosomes: effects of gonadectomy and exogenous testosterone. Horm. Behav., *30:* 474–486.

Crews, D., P. Coomber, and F. Gonzalez-Lima (1997) Effects of age and sociosexual experience on the morphology and metabolic capacity of brain nuclei in the leopard gecko *(Eublepharis macularius),* a lizard with temperature-dependent sex determination. Brain Res., *758:* 169–179.

Crews, D., J. Sakata, and T. Rhen (1998) Developmental effects on intersexual and intrasexual variation in growth and reproduction in a lizard with temperature-dependent sex determination. J. Comp. Biochem. Physiol. C, *119:* 229–241.

Farragher, K., and D. Crews (1979) The role of the basal hypothalamus in the regulation of reproductive behavior in the lizard, *Anolis carolinensis:* lesion studies. Horm. Behav., *13:* 185–206.

Ferris, C.F., L. Gold, G.J. de Vries, and M. Potegal (1990) Evidence for a functional and anatomical relationship between the lateral septum and the hypothalamus in the control of flank marking behavior in golden hamsters. J. Comp. Neurol., *293:* 476–485.

Ferris, C.F., R.H. Melloni, Jr., G. Koppel, K.W. Perry, R.W. Fuller, and Y. Delville (1997) Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. J. Neurosci., *17:* 4331–4340.

Flanagan-Cato, L.M., and B.S. McEwen (1995) Pattern of Fos and Jun expression in the female rat forebrain after sexual behavior. Brain Res., *673:* 53–60.

Floody, O.R. (1989) Dissociation of hypothalamic effects on ultrasonic production and copulation. Physiol. Behav., *46:* 299–307.

Flores, D.L., A. Tousignant, and D. Crews (1994) Incubation temperature affects the behavior of adult leopard geckos. Physiol. Behav., *55:* 1067–1072.

Friedman, D., and D. Crews (1985) Role of the anterior hypothalamus-preoptic area in the regulation of courtship behavior in the male Canadian red-sided garter snake *(Thamnophis sirtalis parietalis):* lesion experiments. Behav. Neurosci., *99:* 942–949.

Friston, K.J. (1994) Functional and effective connectivity in neuroimaging: a synthesis. Hum. Brain Mapping, *2:* 56–78.

Gerstein, G.L., D.H. Perkel, and K.N. Subramanian (1978) Identification of functionally related neural assemblies. Brain Res., *140:* 43–62.

Godwin, J., and D. Crews (1997) Sex differences in the nervous system of reptiles. Cell. Molec. Neurobiol., *17:* 649–669.

Gonzalez-Lima, F. (1992) Brain imaging of auditory learning functions in rats: studies with fluorodeoxyglucose autoradiography and cytochrome oxidase histochemistry. *In* Advances in Metabolic Mapping Techniques for Brain Imaging of Behavioral and Learning Functions (ed. by F. Gonzalez-Lima), Kluwer Academic Publishers, Dordrecht, pp. 39–109.

Gonzalez-Lima, F., and A. Cada (1998) Quantitative histochemistry of cytochrome oxidase activity: theory, methods, and regional brain vulnerability. *In* Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease (ed. by F. Gonzalez-Lima), Plenum Press, New York, pp. 55–91.

Goodson, J.L., R. Eibach, J. Sakata, and E. Adkins-Regan (1999) Effect of septal lesions on male song and aggression in the colonial zebra finch *(Taeniopygia guttata)* and the territorial field sparrow *(Spizella pusilla).* Behav. Brain. Res., *101:* 115–128.

Greenberg, N., M. Scott, and D. Crews (1984) Role of the amygdala in the reproductive and aggressive behavior of the lizard, *Anolis carolinensis.* Physiol. Behav., *32:* 147–151.

Herndon, J.G., and D.B. Neill (1973) Amphetamine reversal of sexual impairment following anterior hypothalamic lesions in female rats. Pharmacol. Biochem. Behav., *1:* 285–288.

Hochberg, Y. (1988) A sharper Bonferroni procedure for multiple tests of significance. Biometrics, *75:* 800–802.

Horwitz, B. (1991) Functional interactions in the brain: use of correlations between regional metabolic rates. J. Cereb. Blood Flow Metab., *11:* A114–A120.

Horwitz, B., R. Duara, and S.I. Rapoport (1984) Intercorrelations of glucose metabolic rates between brain regions: application to healthy males in a state of reduced sensory input. J. Cereb. Blood Flow Metab., *4:* 398–409.

Horwitz, B., and S.I. Rapoport (1988) Partial correlation coefficients approximate the real intrasubject correlation pattern in the analysis of interregional relations of cerebral metabolic activity. J. Nucl. Med., *26:* 392–399.

Kendrick, K.M., and R.F. Drewett (1979) Testosterone reduces refractory period of stria terminalis neurons in the rat brain. Science, *204:* 877–879.

Kollack-Walker, S., and S.W. Newman (1995) Mating and agonistic behavior produce different patterns of Fos immunolabeling in the male Syrian hamster brain. Neuroscience, *66:* 721–736.

Lanuza, E., and M. Halpern (1997) Efferents and centrifugal afferents of the main and accessory olfactory bulbs in the snake *Thamnophis sirtalis.* Brain Behav. Evol., *51:* 1–22.

Law, T., and W. Meagher (1958) Hypothalamic lesions and sexual behavior in the female rat. Science, *128:* 1626–1627.

Lehman, M.N., and S.S. Winans (1982) Vomeronasal and olfactory pathways to the amygdala controlling male hamster sexual behavior: autoradiographic and behavioral analyses. Brain Res., *240:* 27–41.

Lehman, M.N., J.B. Powers, and S.S. Winans (1983) Stria terminalis lesions alter the temporal pattern of copulatory behavior in the male golden hamster. Behav. Brain Res., *8:* 109–128.

Lisciotto, C.A., and J.I. Morrell (1990) Androgen-concentrating neurons of the forebrain project to the midbrain in rats. Brain Res., *516:* 107–112.

Martinez-Garcia, F., F.E. Olucha, V. Teruel, M.J. Lorente, and W.K. Schwerdtfeger (1991) Afferent and efferent connections of the olfactory bulbs in the lizard *Podarcis hispanica.* J. Comp. Neurol., *305:* 337–347.

Martinez-Marcos, A., E. Lanuza, and M. Halpern (1999) Organization of the ophidian amygdala: Chemosensory pathways to the hypothalamus. J. Comp. Neurol., *412:* 51–68.

McGinnis, M.Y., G.W. Williams, and A.R. Lumia (1996) Inhibition of male sex behavior by androgen receptor blockade in preoptic area or hypothalamus, but not amygdala or septum. Physiol. Behav., *60:* 783–789.

McIntosh, A.R., and F. Gonzalez-Lima (1992) The application of structural modeling to metabolic mapping of functional neural systems. *In* Advances in Metabolic Mapping Techniques for Brain Imaging of Behavioral and Learning Functions (ed. by F. Gonzalez-Lima), Kluwer Academic Publishers, Dordrecht, pp. 219–255.

Meisel, R.L., and B.D Sachs (1994) The physiology of male sexual behavior. *In* The Physiology of Reproduction. Vol. 2 (ed. by E. Knobil and J.D. Neill), Raven Press Ltd., New York, pp. 3–105.

Morrell, J.I., D. Crews, A. Ballin, A. Morgentaler, and D.W. Pfaff (1979) 3H-Estradiol, ^{3}H-testosterone and ^{3}H-dihydrotestosterone localization in the brain of the lizard, *Anolis carolinensis:* An autoradiographic study. J. Comp. Neurol., *188:* 201–224.

Nair, H.P., and F. Gonzalez-Lima (1999) Extinction of behavior in infant rats: development of functional coupling between septal, hippocampal, and ventral tegmental regions. J. Neurosci., *19:* 8646–8655.

Pfaff, D.W., S. Schwartz-Giblin, M.M. McCarthy, and L. Kow (1994) Cellular and molecular mechanisms of female reproductive behaviors. *In* The Physiology of Reproduction, Vol. 2 (ed. by E. Knobil and J.D. Neill), Raven Press Ltd., New York, pp. 107–220.

Reiner, A., S.E. Brauth, and H.J. Karten (1984) Evolution of the amniote basal ganglia. TINS, *7:* 320–325.

Rhen, T., and D. Crews (1999) Embryonic temperature and gonadal sex organize male-typical sexual and aggressive behavior in a lizard with temperature-dependent sex determination. Endocrinology, *140:* 4501–4508.

Rhen, T., and D. Crews (2000) Effects of gonadal sex and hormone treatment on sexual and agonistic interactions with conspecific males in the leopard gecko, *Eublepharis macularius.* Neuroendocrinology, *71:* 252–261.

Rhen, T., J. Ross, and D. Crews (1999) Effects of testosterone on sexual behavior and morphology in adult female leopard geckos, *Eublepharis macularius.* Horm. Behav., *36:* 119–128.

SAS Institute (1995) JMP User's Guide, version 3.1. SAS Institute, Cary, NC.

Sokal, R.R., and F.J. Rohlf (1995) Biometry. 3rd ed., W.H. Freeman and Co., New York.

Tarr, R.S. (1977) Role of the amygdala in the intraspecies aggressive behavior of the Iguanid lizard, *Sceloporus occidentalis* Physiol. Behav., *18:* 1153–1158.

Wersinger, S.R., M.J. Baum, and M.S. Erskine (1993) Mating-induced FOS-like immunoreactivity in the rat forebrain: a sex comparison and a dimorphic effect of pelvic nerve transection. J. Neuroendocrinol., *5:* 557–568.

Wheeler, J.M., and D. Crews (1978) Role of the anterior hypothalamus-preoptic area in the regulation of reproductive behavior in the lizard, *Anolis carolinensis:* lesion studies. Horm. Behav., *11:* 42–60.

Wong-Riley, M.T.T. (1989) Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. TINS, *12:* 94–101.

Wong-Riley, M.T.T, F. Nie, R.F. Hevner, and S. Liu (1998) Brain cytochrome oxidase: functional significance and bigenomic regulation in the CNS. *In* Cytochrome Oxidase in Neuronal Metabolism and Alzheimer's Disease (ed. by F. Gonzalez-Lima), Plenum Press, New York, pp. 1–53.

Wood, R.I., and S.W. Newman (1993) Mating activates androgen receptor-containing neurons in chemosensory pathways of the male Syrian hamster brain. Brain Res., *614:* 65–77.

Young, L.J., G.F. Lopreato, K. Horan, and D. Crews (1994) Cloning and in situ hybridization analysis of estrogen receptor, progesterone receptor, and androgen receptor expression in the brain of whiptail lizards *(Cnemidophorus uniparens* and *C. inornatus).* J. Comp. Neurol., *247:* 288–300.