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SEX DIFFERENCES IN EEG IN ADULT GONADECTOMIZED RATS BEFORE AND AFTER HORMONAL TREATMENT

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SUMMARY

EEG activity was recorded from the left and right parietal cortex in adult male and female Wistar rats that were gonadectomized (GNX) after puberty during 2 days without and 3 days with hormonal treatment (either testosterone propionate, 5α-DHT or vehicle in males and progesterone, estradiol benzoate or vehicle in females). In contrast to EEG characteristics reported for intact rats, GNX abolished right over left parietal activation in both sexes and, sex differences in EEG interhemispheric correlation and in theta and delta relative power in the right parietal; additionally GNX males showed higher absolute power than females. Hormonal treatment reestablished interparietal asymmetry in both sexes and a lack of sex differences in absolute power, however, it was not enough to reestablish sex differences in delta and theta proportion in the right parietal nor in interhemispheric correlation. Differential effects were obtained with testosterone propionate and 5α-DHT in males suggesting that activational effects of testosterone on EEG are probably exerted through testosterone or its aromatized metabolites. The results of our study indicate that the activational effects of gonadal steroids after puberty are necessary for maintaining sex differences in the EEG of the adult rat.

Keywords—EEG; Interhemispheric correlation; Sex differences; Lateralization; Gonadectomy; Sex hormones.

INTRODUCTION

Previous studies have demonstrated that electroencephalographic activity (EEG) is significantly different in male and female adults, and reflects a different functional organization of the brain in humans (Beaumont et al., 1978; Corsi-Cabrera et al., 1993; Flor-Henry et al., 1987; Matsuura et al., 1985) as well as in rats. Male, compared to female rats, showed higher interhemispheric coupling of the EEG activity as assessed by interhemispheric correlation between parietals (IPC), and higher hippocampal and cortical activation as assessed by higher theta relative power (RP) and lower delta RP (Juárez and Corsi-Cabrera, 1995). Sex differences in IPC and, theta and delta RP in the adult rat are dependent

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on the organizational action of testosterone during the prenatal critical period of brain sexual differentiation. Administration of testosterone propionate (TP) during days 14–19 of gestation resulted in masculinization of the EEG of adult females, abolished the differences between TP-treated females and males, and induced significant differences between the TP-treated females and vehicle-treated females. The TP-treated females showed similar values of interparietal correlation, and of theta and delta RP as males, and of higher interhemispheric coupling and theta RP and lower delta RP than vehicle-treated females. TP treatment also induced higher absolute power in both sexes (Juárez et al., 1995).

On the other hand, EEG activity oscillates as a function of the menstrual cycle in women (Becker et al., 1980, 1982; Creutzfeldt et al., 1976; Solís-Ortiz et al., 1994). In the estrous cycle of rats, interhemispheric coupling is significantly higher during the periovulatory phase, indicating that sex steroids not only have organizational actions on cortical EEG but also activational influences, and it may be hypothesized that activational actions of sex steroids are also needed to maintain sex differences in the cortical EEG of the adult rat (Corsi-Cabrera et al., 1992). However, to the best of our knowledge there are no studies on the actions of sex steroids in maintaining sex differences in EEG activity; therefore, one of the aims of this experiment was to investigate whether sex differences in the EEG are present in adult gonadectomized rats that were exposed to the normal organizational effects during the critical perinatal period of sex differentiation of the brain. Since organizational actions have been ascribed to sex steroids in the brain during perinatal (Goy and McEwen, 1980) and during peripubertal periods of development (Arnold and Breedlove, 1985; Clough and Rodríguez-Sierra, 1983) and on sexually dimorphic non-reproductive behaviors (Beatty, 1979; Beatty and Fessler, 1977; Brand and Slob, 1988; Primus and Kellogg, 1989, 1990; Slob et al., 1986; Starkstein et al., 1989), rats were gonadectomized after puberty.

Sex steroids have different effects on neuronal activity and brain excitability. Progesterone and some A-ring reduced metabolites, allopregananolone and pregnanolone, have anxiolytic (Beyer and González-Mariscal, 1991; Bitran et al., 1991; Fernández-Guasti and Picazo, 1992; Rodríguez-Sierra et al., 1984; Wieland et al., 1991), hypnogenic (Heuser et al., 1967; Komisaruk et al., 1967; Lancel et al., 1996) anesthetic (Gyermek and Stoyka, 1975; Mok and Krieger, 1990; Selye, 1942) and inhibitory effects on the EEG arousal threshold and single unit discharges in the cortex, thalamus and hypothalamus (Kawakami and Sawyer, 1959; Komisaruk et al., 1967) as well as anticonvulsant effects (Bäckström, 1976; Landgren et al., 1978). More recently evidence has been accumulating on the direct neuromodulatory action of allopregnanolone and pregnanolone on $GABA_A$ receptors, in a similar way as barbiturates but at different recognition sites (Gee et al., 1987, 1988; Harrison et al., 1987; Korneyev and Costa, 1996; Majewska et al., 1986; Mok et al., 1991, 1992; Morrow et al., 1990; Picazo and Fernández-Guasti, 1995; Paul and Purdy, 1992; Peters et al., 1988; Puia et al., 1990; Turner et al., 1989). Whereas, certain steroids like pregnanolone sulfate and dehydroepiandrosterone have convulsant and proconvulsant effects (Heuser et al., 1965) and they have been reported to antagonize GABA receptor-mediated chloride uptake (Majewska and Schwartz, 1987) and to reduce channel opening frequency (Mienville and Vicini, 1989). Their effects are not selective for GABA: glycine mediated currents are also blocked (Wu et al., 1990) and pregnanolone sulfate potentiates glutamate mediated excitation (Wu et al., 1991).

Estrogens, on the other hand, exert modulatory effects on neuronal excitability. They increase brain excitability (Alcaraz et al., 1969) and the reactivity of olfactory structures (Cartas-Heredia et al., 1978), have antidepressant effects (Itil and Herrmann, 1982; Klaiber

et al., 1979; Oppenheim, 1984) and increase convulsiveness (Bäckström, 1976; Logothetis et al., 1959; Stitt and Kinnard, 1968; Wolley and Timiras, 1962). The excitatory effects of estrogens have been reported in CA1 pyramidal neurons of the hippocampus (Teyler et al., 1980; Wong and Moss, 1991) and in cerebellar Purkinje cells (Smith et al., 1987, 1988).

The major biologically active metabolites of androgens in peripheral tissues, such as the prostate and seminal vesicles are, 5α reduced androgens like 5α-dihydrotestosterone; however, brain tissue also converts androgens through the aromatization of testosterone to 17β-estradiol or of androstenedione to estrone (Martini, 1982). There is much evidence that androgen actions in the brain occur mainly through its aromatization to estrogens; for example, when aromatization of testosterone is pharmacologically blocked, testosterone induced male copulatory behavior is prevented (Christensen and Clemmens, 1975) and interaction with the female increases not only plasma testosterone in the male, but also increases hypothalamic aromatase activity (Dessi-Fulgheri and Lupo, 1982). There is also evidence that the masculinizing effects of testosterone during the perinatal critical period are mediated through its aromatization in the brain to estradiol (McEwen, 1980). In fact, prenatal administration of an antiandrogen, ciproterone acetate, prevents masculinization of the EEG in adult males (unpublished results). However, 5α-dihydrotestosterone (5α-DHT) may also have primary effects on the brain (Hutchison and Steimer, 1984; McEwen, 1980), therefore it is important to elucidate the activational role of testosterone and its metabolite 5α-DHT on the EEG activity of males.

Since estrogens, progesterone, and aromatized and reduced androgens have differential effects on the brain, and since estrogens and progesterone have activational influences on the EEG activity of adult females during spontaneous estrous cycle and there are sex differences in intact adult rats, it is important to explore the activational effects of these sex steroids employing doses that have been found to induce estrous in females and restore sexual activity in males. Therefore, in the present investigation the effect on EEG activity of progesterone or estrogens in gonadectomized females, and of testosterone propionate or 5α-dihydrotestosterone in gonadectomized males were explored.

There is considerable evidence of brain and behavioral asymmetries in the rat and asymmetries by sex interactions. These findings include asymmetries in the cortex (Diamond et al., 1981), hippocampus (Diamond et al., 1983), hypothalamus (Nordeen and Yahr, 1982), nigrostriatal system (Robinson et al., 1980), spatial preference (Sherman et al., 1980), effect of early experience (Camp et al., 1984; Denenberg et al., 1978) and effect of frontal cortex lesions (Lipsey and Robinson, 1986; Robinson, 1979). Sex differences in ultrastructure of the corpus callosum (Juraska and Kopcik, 1988) and of right over left parietal EEG activation has also been reported for intact rats (Juárez and Corsi-Cabrera, 1995), therefore, interparietal EEG asymmetry was also assessed.

In the present experiment we recorded the EEG activity at left and right parietals in both male and female rats that were gonadectomized after puberty, before and after hormonal treatment.

SUBJECTS AND METHODS

Animals

A total of 60 Wistar rats: 30 males, mean weight 338.75 g and 30 females, mean weight 249 g. The subjects were housed under standard laboratory conditions with food and water ad lib, and were maintained under a 12–12-h light–dark cycle with light beginning at 0800h.

Gonadectomy

At 80 days of age males and females were gonadectomized (GNX) under sodium pentobarbital anesthesia (40 mg/kg) and were allowed 10 days to recover from surgery.

Electrode Implantation

Under sodium pentobarbital anesthesia the 60 rats were implanted at 90 days of age, 10 days after GNX. Two electrodes were symmetrically placed at the surface of the dura matter at 3 mm from midline and 3 mm posterior to bregma, and two reference electrodes symmetrically at 10 mm anterior to bregma and 2 mm from midline. After the surgical procedure penicillin was administered. Ten days were allowed for recovery from surgery.

Hormonal Treatment

At 100 days of age, the rats were randomly divided into six groups according to the following hormonal treatment: (1) 10 males received 1 mg/0.10 ml of testosterone propionate (TP); (2) 10 males received 1.3 mg/0.13 ml of 5α-dihydrotestosterone (DHT); (3) 10 females received 2 mg/0.20 ml of progesterone (P); (4) 10 females received 5 μg/0.10 ml of estradiol benzoate (E); and (5 and 6) 10 males (MC) and 10 females (FC) received 0.10 ml of the vehicle and served as controls. All substances were dissolved in corn oil and were intra-muscularly injected daily during 3 consecutive days at 1900h.

EEG Recording

EEG activity was monopolarly recorded from the left and right parietal cortex and was referred to the ipsilateral electrode on a Grass model 8-16E polygraph with filter settings at 1–35 Hz. Animals were individually recorded on 7 days in their home cage inside a sound-attenuating chamber. Recordings were taken between 1700 and 1900h. Before each recording session 10 min were allowed for adaptation. The first two sessions were allowed for adaptation to recording procedures, the following two sessions were recorded before hormonal treatment and served as baseline (BL1, BL2) and the last three consecutive sessions were recorded under hormonal treatment (R1, R2, R3). Hormones or vehicle were injected 24 h before R1, R2 and R3. Epochs of EEG, 2 s each, were captured on a PC through an analogue-to-digital converter, 12-bit resolution, at a sampling rate of 128 Hz.

EEG Analysis

The first 20 artifact-free epochs of EEG from BL1, BL2, R1, R2 and R3 sessions were Fourier transformed and absolute power densities (AP) were obtained for the following broad bands: delta (1.5–3.5), slow theta (4.0–7.5), fast theta (8.0–9.5), alpha (10.0–12.5), beta1 (13.0–17.5) and beta2 (18.0–25.0 Hz) and for the total band (TB) from 1.5 to 25.0 Hz. Relative power (RP) was calculated for the same bands to obtain information on the proportional contribution of each band expressed as a percentage of total power between 1.5 and 25.9 Hz. After the bands were digitally filtered, interparietal correlation (IPC) was obtained in the time domain for the same bands, by means of Pearson product–moment correlation coefficients between successive amplitude values of EEG epochs of left (Lp) and right (Rp) parietal regions (Guevara and Corsi-Cabrera, 1996; Shaw, 1984).

Statistical Analysis

1. To explore sex differences in GNX rats and interparietal asymmetry, the EEG activity of the 2 baseline days before hormone administration was compared between males and

females. Mixed design ANOVAs were done separately for AP, RP and IPC for each band. Sex was the between-subjects variable, baseline sessions and parietal EEG were the within-subjects variables for AP and RP; sex was the between-subjects variable, and baseline sessions was the within-subjects variable for IPC.

2. To explore the effects of hormones on the EEG, mixed ANOVAs were done with the six groups (MC, TP, DHT, FC, E and P) as the between-subjects variable and the recording days (arithmetic mean of BL1 and BL2 and the three treatment days: R1, R2 and R3); and the left and right parietals as the within-subjects variables for AP and RP with groups. Groups was the between-subjects variable and recording days was the within-subjects variable for IPC.

For statistical purposes, power was log-transformed and correlation was transformed to Fisher's Z-scores (Gasser et al., 1982; John et al., 1980). The significant level was set at $p < .05$. For post-hoc pairwise comparisons, Tukey's Studentized t-tests were used.

RESULTS

Sex Differences in Gonadectomized Rats

Although separate ANOVAs were performed for AP, RP and IPC, for sake of clarity, significant results for each main effect, sex and asymmetry, will be described together for the three EEG parameters.

Sex: Main Effects. Figure 1 illustrates EEG AP of the two baseline sessions. AP of delta ($F(1,58) = 6.76$; $p < .01$), slow theta ($F = 5.07$, $p < .02$), alpha ($F = 4.02$, $p < .04$) and the TB ($F = 5.43$, $p < .02$) was significantly higher in females than in males, and the same trend,

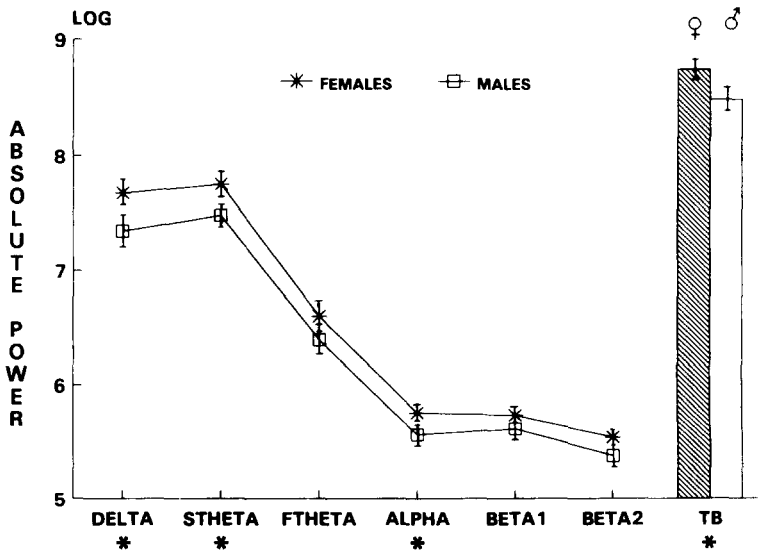

Fig. 1. Mean and standard error of absolute power (log transformed) of delta, slow theta (STHETA) fast theta (FTHETA), alpha, beta1 and beta2, and the total band from 1.5 to 25 Hz (TB) of males and females after gonadectomy and before hormonal treatment. *Indicates significant sex differences.

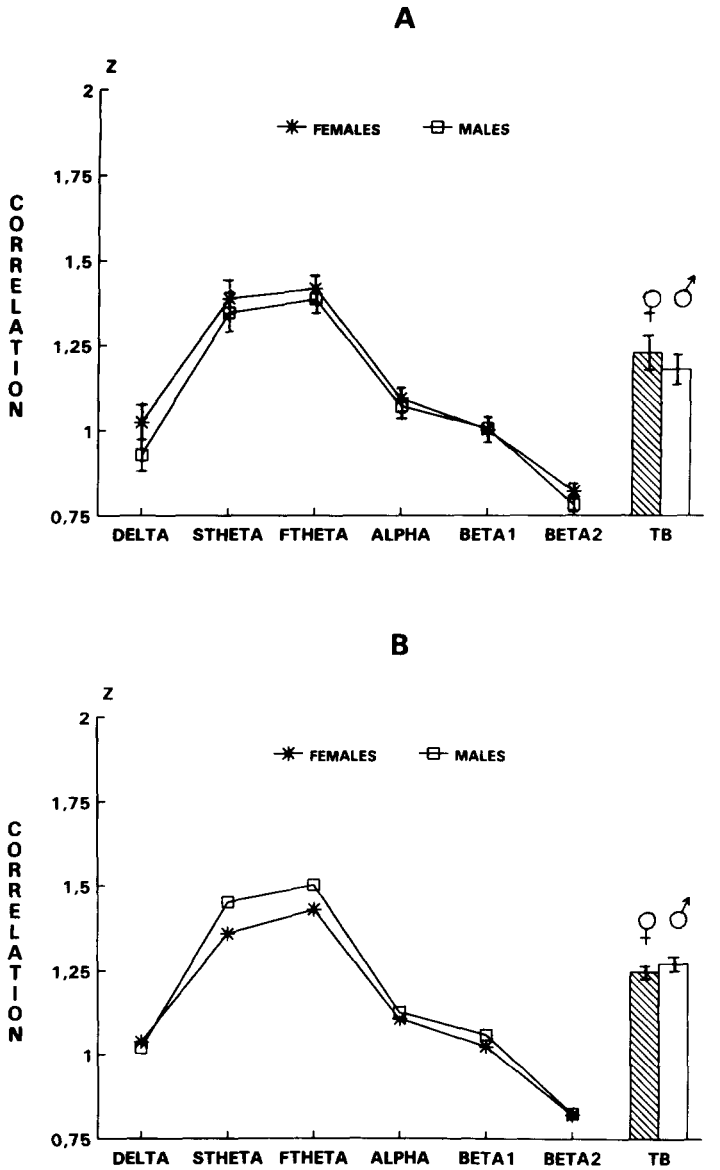

Fig. 2. Mean and standard error of interparietal correlation, transformed to Fisher's Z-scores, for delta, slow theta (STHETA) fast theta (FTHETA), alpha, beta1 and beta2 and the total band from 1.5 to 25 Hz (TB) for males and females. (A) After gonadectomy and before hormonal treatment; (B) after hormonal treatment. Standard errors in (B) are too small for graphic display.

higher AP in females than males, was observed for the rest of the bands. There were no main sex effects for IPC (Fig. 2) nor for RP.

Asymmetry: Main Effects. There were no significant main effects of asymmetry for AP, but there was a significant sex × days × parietals interaction for delta AP ($F(1,58) = 6.82$;

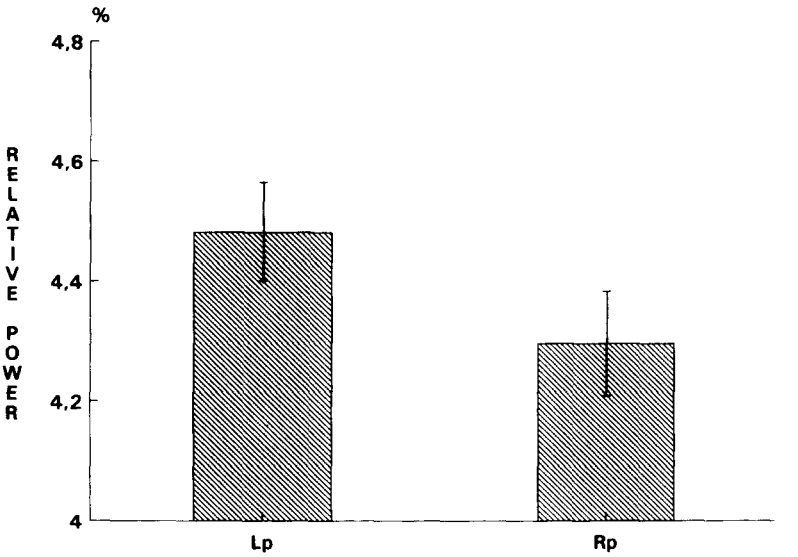

Fig. 3. Mean and standard error of Beta2 relative power in left (Lp) and right parietal (Rp) in gonadectomized rats before hormonal treatment.

$p < .01$). Only males showed asymmetry with lower delta AP in the left than in the right parietal during BL1. Females did not show any asymmetry, but they did show higher delta AP than males in both parietals and in both BL sessions.

RP of alpha ($F(1,58) = 5.47$, $p < .02$) and beta2 ($F = 4.32$, $p < .04$) (Fig. 3) showed significant parietal main effects. Both bands showed higher RP in the left than in the right parietal. Delta and fast theta RP showed significant sex × parietal interactions ($F(1,58) = 4.35$, $p < .03$; $F = 8.12$, $p < .006$; respectively) which indicated that delta RP was lower in males than in females, and that fast theta RP was higher in males than in females, but only in the left parietal (Fig. 4).

Effect of Hormonal Treatment

Group: Main Effects. The sex differences that were observed in AP before hormonal treatment disappeared and there were no significant differences between groups ($p > .05$) for any band. AP of the total band during R3, shown in Fig. 5, was similar between treated males and females. Hormonal treatment decreased AP in females and increased AP in males to abolish the differences observed before hormonal treatment. Untreated groups preserved the same pattern, higher AP in FC and lower AP in MC, although the differences did not reach significance level. There were no significant group main effects for RP nor for IPC of any band.

Group × Treatment Day Interactions. AP of delta ($F(15,162) = 2.56$, $p < .002$), beta1 ($F = 2.08$, $p < .013$), beta2 ($F = 1.93$, $p < .02$) and the TB ($F = 1.81$, $p < .03$) showed significant group × treatment day interactions. The significant results of pairwise comparisons can be summarized as follows:

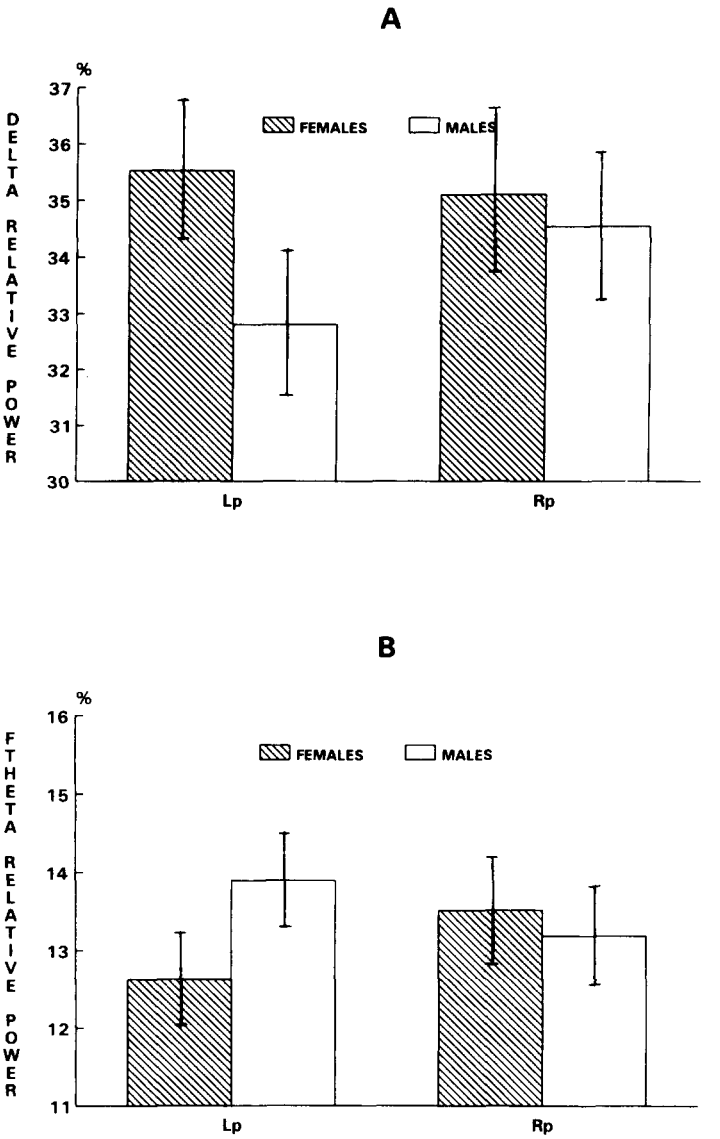

Fig. 4. Mean and standard error of delta (A) and fast theta (B) relative power in left (Lp) and right (Rp) parietals in gonadectomized male and female rats before hormonal treatment.

TP treatment selectively induced an increase in AP of delta, beta1 and TB from BL to R3 (Fig. 6). Beta1 and beta2 AP also increased from BL to R3 in the FC group.

RP of delta also showed a significant group × treatment day × parietal interaction. Significant results of pairwise comparisons for the interaction showed that TP treatment induced a significant increase of delta RP from BL to R1, R2 and R3 only in the left parietal (Fig. 7). In contrast, delta RP in both parietals decreased significantly from BL to R1, R2 and R3 in both male and female control groups and in DHT and P groups. Delta RP did not change in the E-treated group.

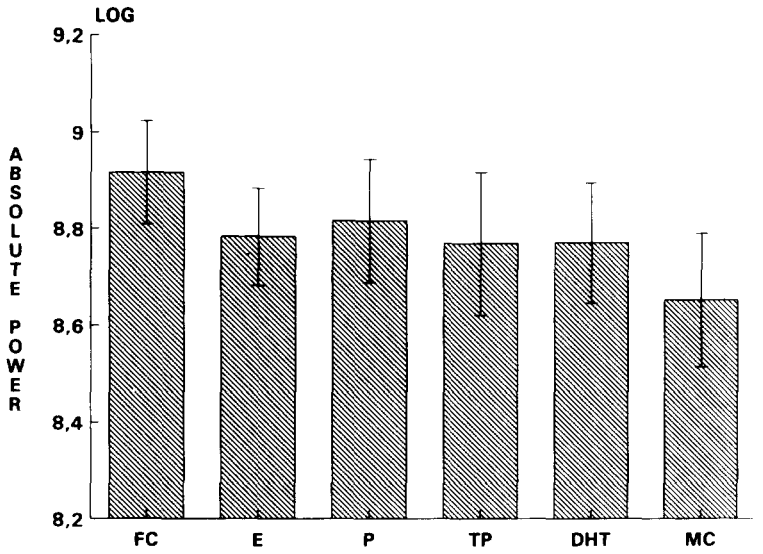

Fig. 5. Mean and standard error of absolute power of the total band (1.5–25 Hz; log transformed) for female control (FC), estradiol- (E) and progesterone (P)-treated females, testosterone propionate (TP) and 5α-dihydrotestosterone (DHT)-treated males and male control groups (MC) after hormonal treatment.

Asymmetry Effects. AP of delta ($F(1,54) = 6.44$, $p < .013$), slow theta ($F = 4.60$, $p < .034$), fast theta ($F = 4.37$, $p < .038$) and TB ($F = 5.17$, $p < .025$) was significantly higher in the right than in the left parietal, and RP of beta2 was higher in the right than in the left parietal.

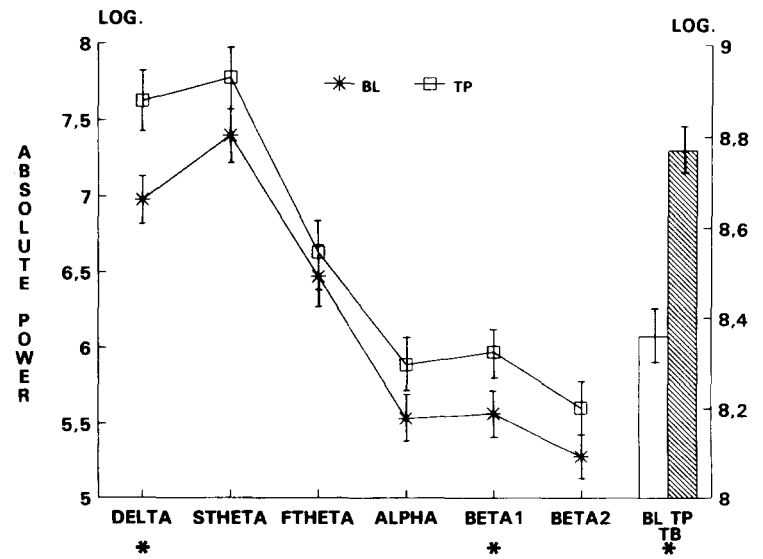

Fig. 6. Mean and standard error of absolute power (log transformed) of delta, slow theta (STHETA); fast theta (FTHETA), alpha, beta1, beta2 and the total band from 1.5 to 25 Hz before (BL) and after testosterone propionate (TP) treatment in gonadectomized males. *Indicates significant differences between BL and TP.

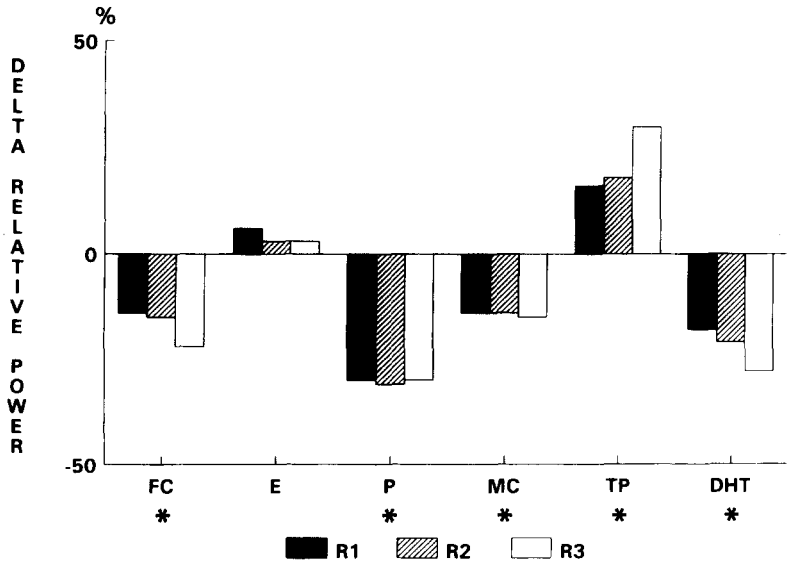

Fig. 7. Relative power of delta expressed as percentage over baseline (= 0) in female control (FC), estradiol- (E) and progesterone (P)-treated females, male control (MC), testosterone propionate (TP) and 5α-dihydrotestosterone (DHT) treated males during the first (R1), second (R2) and third (R3) treatment days.

DISCUSSION

The present results in male and female rats that were gonadectomized after puberty, confirm the hypothesis that most of the sex differences observed in the EEG activity of intact adult rats needs the activational effects of sex steroids. In addition, the results have showed that the low levels of sex steroids induced sex differences in AP, which are absent in intact animals, and modified parietal asymmetry in both sexes.

The main results observed in GNX rats can be summarized as follows: sex differences in IPC and in delta and theta RP in the right parietal were abolished in GNX rats, whereas sex differences in delta and theta RP in the left parietal were preserved. On the other hand, interparietal asymmetry in AP was abolished in both sexes. Despite the absence of interparietal asymmetry in AP shown by GNX rats, the proportional contribution of alpha and beta2 to total power revealed by RP showed a reversed pattern of interparietal asymmetry in both sexes, with higher alpha and beta2 RP in the left parietal instead of higher AP in the right parietal as reported for the controls. Additionally, gonadectomized males showed significantly lower AP of delta, slow theta, alpha and total band than females.

It has been demonstrated that intact adult male rats show higher IPC, higher theta RP and lower delta RP than females, and that both males and females show right over left parietal activation. No sex differences in AP have been reported (Juárez and Corsi-Cabrera, 1995; Juárez et al., 1995). Present results demonstrate that the lack of gonadal steroids after puberty affects the right over left parietal activation in both sexes, it flattens the differences in AP between right and left parietal, even increasing beta2 RP in the left side, and abolishes the sex differences in interhemispheric coupling and in delta and theta proportions, specifically in the right side of the cortex. These results indicate that in addition to the

organizing effects, the activational effects of gonadal steroids after puberty are necessary for the higher activation of the right cortex in both sexes, at least in parietal regions, and for the higher interhemispheric coupling and theta RP in males than in females.

The role of sex steroids for maintaining similar AP in males and females, and the right over left activation, was corroborated by results obtained with hormonal treatment. Hormonal administration re-established the pattern of interparietal asymmetry reported for intact rats, and induced higher AP in the entire spectrum for the right parietal. These results are consistent with the role proposed for sex steroids on brain lateralization (Denenberg et al., 1978). Hormonal treatment also abolished the sex differences observed in AP in GNX rats; females treated with B or P showed an overall decrease in AP, and males treated with TP or DHT showed an overall increase that rendered both sexes similar, whereas untreated rats continued to exhibit the same trend of lower AP in males and higher AP in females. TP showed a stronger effect in this direction than the other hormones, and induced a significantly higher increase in AP than the other hormones, particularly of delta and beta1 AP. The activational effects of testosterone on AP are consistent with the organizing effects of testosterone on EEG. Prenatal treatment with TP during the critical period of sex differentiation of the brain induces higher AP in adult males and females compared with untreated rats (Juárez et al., 1995). In contrast, DHT administration in GNX males did not affect AP and induced a decrease of delta RP.

These results taken together with those observed in prenatally TP-treated rats suggest that the effect of TP on AP is not due to the reduction of testosterone to 5α-DHT, but more probably to a direct effect of testosterone or to the aromatization of testosterone to estrogens. Since delta as well as beta frequencies are of cortical origin (Steriade et al., 1993, 1996) a testosterone or estrogenic effect in males on the balance of cortical activation–deactivation processes may be postulated. Present results also suggest that 5α-reduced testosterone has the opposite effect on cortical inhibitory processes than its precursor, as it decreases delta RP. However, E-treatment in females did not induce the same effects as TP in males. This differential effect of testosterone in males and estrogen in females may be due to the organizing effects of testosterone during the critical perinatal period, thus estrogenic effects in adult rats are acting over previously feminized or masculinized brain tissue. This needs further exploration by administration of E to previously masculinized females during the critical period of sex differentiation and to castrated males during the same period.

Although the doses and the time course of hormonal treatment used in this study are enough to induce estrous in gonadectomized females and to restore sexual activity in castrated males (Hart, 1967; Hawkins et al., 1988; Thornton et al., 1991) they were not able to reestablish all the sex differences in brain activity. The lack of effects may have several explanations. It could be that the higher interhemispheric coupling and the higher theta RP in the right hemisphere that was observed in intact males need higher doses or longer exposure to activational influences of sex hormones than reproductive behavior does. The time interval between IM injection and EEG recordings, 24 h, may also have played a role. For example, in the case of progesterone, there is evidence that hypnogenic effects, which are mediated through bioconvertion into neuroactive steroids, need higher doses and a shorter interval after administration (Lancel et al., 1996). In addition, the effect of sex steroids on EEG activity may depend on specific metabolites or may be even secondary, i.e. mediated by another hormone or neuromodulator, and thus more time may be required for the change to be evident (McEwen and Parsons, 1982). In the case of females, estrous oscillations and feminine characteristics of the EEG reported for intact females may be due to the combined

action of estrogen and progesterone, and the isolated action of each steroid may be ineffective. It is known that the number of progesterone receptors in the brain increases and decreases as a function of estrogen priming (Blaustein and Wade, 1978; MacLusky and McEwen, 1980).

Present results add new information on the activational effects of sex steroids on brain activity. The main new aspects of the present report are: (1) sex steroids do have activational effects on EEG; (2) the activational effects of sex steroids, in addition to their organizing effects, are needed for the higher interhemispheric coupling and higher theta proportion in the right cortex of males, for sex similarity in AP and for right over left parietal asymmetry; (3) the activational effects of testosterone on AP are probably exerted through testosterone or TP aromatized metabolites; and (4) that sex differences in interhemispheric correlation and theta RP probably need longer exposure to sex steroids or to the combined action of E and P in females.

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