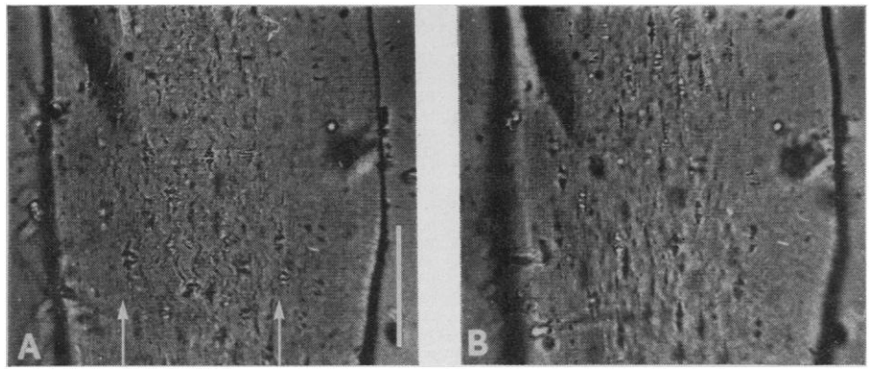


Fig. 2. Selected cine micrographs of an isolated fiber that has shortened to 1.3 $\mu$m during tetanic stimulation. Temperature was 20°C. Calibration mark represents 50 $\mu$m. Exposure time 2 msec. (A) In normal Ringer solution. Wavy myofibrils are visible between the arrows delineating the part that has become inactivated. The force was 0.04 times that at slack length. (B) In Ringer solution containing 3 m*M* caffeine. All the myofibrils appear to be straight. The force was 0.23 times that at slack length.

from the distance between the ends of the fiber. The striation spacings determined by this method differed from the values measured on the photographs by less than 0.05 $\mu$m. Stimulation was transverse by a pair of bright platinum plate electrodes, each tetanus coming at 1-minute intervals. The general techniques have been described previously (*3*, *5*).

Preliminary studies showed that contractures with shortening induced by 5 to 10 m*M* caffeine produced considerably more force than tetani at the same short length. The results, however, were regarded as unreliable, because these contractures had a time course much slower than that of a tetanus, caused the striation spacing along a fiber to be very irregular, and usually ended with at least part of the fiber irreversibly shortened. These difficulties did not arise when we compared cine micrographs of tetani in normal Ringer solution to those in Ringer solution containing caffeine in concentrations that did not themselves produce shortening. At such concentrations, caffeine is believed to lower the threshold for contractions initiated by action potentials, thus making a given action potential more effective in activating contraction, particularly as regards the peak magnitude of force development in a twitch (*4*). We found that the maximum force developed during a tetanus can also be increased by the addition of caffeine. Figure 1 shows the length-force relationships at 20°C for two single fibers. Caffeine, being somewhat more effective at 3 m*M* than at 2 m*M* concentration, increased the force at the plateau of the relationship by 5 to 10 percent. This supports the idea that muscle can reversibly develop force significantly greater than that usually thought to reflect the maximum load-bearing capacity (*6*). But the action of caffeine was particularly obvious at the shorter lengths: at 1.3 $\mu$m, where, in agreement with the findings of Gordon, Huxley, and Julian (*1*), our fibers had ceased to produce force in the control solution, they still generated up to 35 percent of maximum force during tetani in 3 m*M* caffeine. Moreover, they were able to shorten reversibly and produce force at striation spacings as short as 1 $\mu$m. Correspondingly, the wavy myofibrils that appeared in the core of a shortened fiber in the control solution, remained straight during shortening to the same length in the presence of caffeine (Fig. 2). Caffeine, therefore, can sustain the activation of the central myofibrils that usually become inactivated during shortening induced by membrane depolarization. This action is correlated with an increase in the ability to develop force at short lengths and can be explained by the idea that caffeine improves the effectiveness of membrane depolarization in producing activation.

We thus support the conclusion drawn from our previous findings (*3*), namely, that an important factor that contributes to determining the length-force relationship as a muscle shortens is a decreasing degree of activation.

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## EEG Responses in Regularly Menstruating Women and in Amenorrheic Women Treated with Ovarian Hormones

Abstract. *Electroencephalographic driving reponses to photic stimulation vary with the menstrual cycle and with manipulations of ovarian hormones thought to control the menstrual cycle. Estrogens reduce driving responses to photic stimulation, and estrogen plus progesterone enhance these responses. The electroencephalographic changes may reflect the effects of gonadal steroid hormones upon central adrenergic processes.*

The gonadal steroid hormones, estrogen and progesterone, are suspected of playing significant roles in brain functions (*1*, *2*). We report here that driving responses to photic stimulation varied with the menstrual cycles of normal females (study 1) and could be manipulated by administrations of estrogen and progesterone to amenorrheic women (study 2). Fewer electroencephalographic (EEG) driving responses occurred in the preovula-

tory phase of the menstrual cycle, when blood estrogen levels are rising (*3*), than in the postovulatory phase, when blood estrogen and progesterone levels are both elevated (*4*). Similarly, estrogen administered to amenorrheic women decreased EEG driving, and estrogen plus progesterone administration increased EEG driving over that observed when estrogen was administered alone.

It has been suggested that EEG driving is related to central adrenergic functioning, since substances thought to enhance adrenergic functioning (for instance, norepinephrine) suppress the alpha driving response to light, whereas substances known to induce adrenergic blockade (for instance, chlorpromazine) increase alpha driving responses to photic stimulation (*5*).

The present results may, therefore, indicate effects of gonadal steroid hormones on central adrenergic processes.

*Study 1.* Subjects were 20 normal, regularly menstruating women, 18 to 21 years of age, who reported menstrual cycles of 28 to 30 days and who were studied throughout two successive menstrual cycles. Subjects with histories of epilepsy, head tramua, drug use, or who were on regular medication of any kind, were excluded from the study. Time of probable ovulation was determined from daily basal body temperature (BBT) curves, for each of two consecutive menstrual cycles. Ovulation is followed by a rise in BBT, which usually occurs at about midcycle. On the 8th and 20th days of each of the two successive menstrual cycles, EEG's were taken. In 15 of the 20 women, the 8th day preceded and the 20th day succeeded the thermal shift in each of the two cycles. In the remaining five women, no thermal shift could be detected for one or both menstrual cycles; these subjects were therefore dropped from the study. One additional subject who showed an abnormal EEG was also dropped. The study is therefore based on 14 subjects.

The EEG's were taken with a Grass model 5P5 electroencephalograph preamplifier [as described previously (*5*)]. Briefly, bipolar occipital electrodes placed 2.5 cm to either side of the midline and 2.5 cm above the inion were used to record the EEG responses to each of 18 photic stimulation trials. A Grass $PS_2$ photic stimulator was employed. The stimulation trials each lasted 10 seconds, were spaced approximately 20 seconds apart, and were at 5, 10, 15, 20, 25, and 30 *f*/sec, with one stimulation trial at each of steps 2, 4, and 8 of the Grass intensity scale. The EEG "driving" response was defined as evocation of alpha waves for two consecutive seconds, or evocation of the EEG waves at the fundamental or harmonic frequency of the photic stimulation for two consecutive seconds, with no other EEG wave form being visually detectable during that time. The driving response was scored as either present or absent for each stimulation trial.

Table 1. Analysis of variance of EEG driving responses to photic stimulation in 14 normal females, in the pre- and postovulatory phases of two successive menstrual cycles.

| Source of variance | Cycle 1* | | | | Cycle 2† | | | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | Mean square | *F*-Test | *P* | d.f. | Mean square | *F*-Test | *P* |
| Between individuals | 13 | 47.30 | | | 13 | 30.92 | | |
| Between menstrual phases | 1 | 37.00 | 8.29 | $<.02$ | 1 | 108.00 | 12.20 | $<.01$ |
| Error | 13 | 4.46 | | | 13 | 8.85 | | |

* Mean number of EEG driving responses: preovulation phase, $5.6 \pm 3.4$; postovulation phase, $7.9 \pm 5.4$. † Mean number of EEG driving responses: preovulation phase, $5.8 \pm 4.1$; postovulation phase, $9.7 \pm 4.8$.

Table 1 shows the mean number of trials with EEG driving responses for the group for days 8 and 20 of the first and second menstrual cycles. Separate repeated measurements analyses of variance were performed for each cycle. In each of the two menstrual cycles, day 8 showed significantly fewer EEG driving responses than day 20 (first cycle, $F = 8.29$, $P < .02$; second cycle, $F = 12.20$, $P < .01$).

Of the 28 cycles examined, 21 showed more responses to photic stimulation in the postovulatory than in the preovulatory phase of the menstrual cycle ($\chi^2 = 7.00$, $P < .01$).

*Study 2.* Subjects were six women of 24, 25, 32, 31, 39, and 29 years of age, who were treated hormonally for secondary amenorrhea without detectable organic disease which had persisted for 1 year or more. Subject D (see Table 2) had recently been treated for depression in a psychiatric hospital. None of the six had histories of epilepsy or head trauma or were on medication other than that described below.

The number of premedication EEG's recorded on each patient varied from 2 to 5 (Table 2). Thereafter, each patient received 5 mg of oral conjugated estrogens (Premarin) daily for 21 days. On the 17th through 21st days, 10.0 mg of medroxyprogesterone acetate (Provera) was added to the estrogen therapy.

Table 2 shows that all six subjects evidenced significantly less ($P < .01$) EEG driving during estrogen treatment than in the period prior to treatment. The *t*-tests were computed in the following manner. The within-individual sums of squares of error variance of each of the six subjects were computed across all three conditions (no treatment, estrogen, and estrogen plus progesterone). The six estimates of error variance, and their degrees of freedom, were then combined. The degrees of freedom equaled 29. The *t*-tests equaled:

$$\frac{M_1 - M_2}{\left\{\begin{array}{c}\text{(Combined sums of squares of error variance/combined degrees of freedom)} \cdot [(1/n_1) + (1/n_2)]\end{array}\right\}^{1/2}}$$

Four of the six subjects evidenced significantly fewer EEG driving responses during estrogen treatment than during combined estrogen and progesterone treatment (for three of these subjects, $P < .01$; for the fourth, $P < .05$). Moreover, a *t*-test of the difference between the mean for all subjects of EEG driving responses during estrogen treatment and the mean for all subjects of EEG driving responses during estrogen plus progesterone treatment yields a high degree of statistical significance ($t = 6.925$, $P < .001$). There was no difference in driving responses between "estrogen and progesterone" versus "no treatment."

The inhibitory effect of estrogen on EEG driving (see Table 2) suggests that the low levels of EEG driving that were found in the preovulatory phase of the menstrual cycle may be attributed to the rising levels of estrogen that characterize the preovulatory period, whereas the high levels of EEG driving that were found in the postovulatory phase may be attributable to elevated values of estrogen plus progesterone known to characterize the postovulatory period.

The "no treatment" levels of driving that we observed in our six amenorrheic subjects (Table 2) are significantly higher by $F$-test ($P < .001$) than either the pre- or postovulatory driving levels for normal menstruating females (Table 1). Since amenorrheic women evidence low blood and urinary levels of estrogen (*6*), this finding suggests that estrogen deficiency may result in increased levels of EEG driving.

We know of no previous reports of the relationship of the menstrual cycle to EEG driving. However, there have been numerous reports of relationships between the menstrual cycle and shifts in spontaneous cortical rhythms. Abnormality in the EEG and epileptic seizures are often associated with onset of menstruation (*7*). Even in clinically normal persons, cortical activity may shift to markedly slower frequencies during menstruation (*8*). The reasons for these phenomena have been considered obscure. However, it should be noted that blood estrogen levels are relatively low during menstruation, as compared with the peak estrogen levels reached in the immediate preovulatory phase of the cycle (*3*). Abnormalities in the EEG during menstruation may, therefore, be related to relatively low levels of estrogen at that time. Thus, the literature, as well as our own data, suggest an effect of the gonadal steroid hormones upon brain rhythms.

It has been previously suggested that shifts in spontaneous cortical EEG rhythms during the menstrual cycle might be attributable to changes in electrolytic (sodium and potassium) balance; however, this hypothesis has found but little experimental support (*9*). Further, hypopotassemia in man appears to be associated with little or no EEG change (*10*), nor do EEG changes appear to follow intravenous injections of physiologic doses of KCl or NaCl (*11*).

We suggest an alternative explanation of the mechanism by which the gonadal steroids affect the EEG. The EEG driving responses to photic stimulation appear to be sensitive to central adrenergic processes, since these responses are decreased by the peripheral intravenous administration of norepinephrine in cats (*12*). Norepinephrine is believed to facilitate central adrenergic functioning (*13*). Rothballer (*14*) has argued that the effect of epinephrine and norepinephrine upon the cortical EEG is a function of their action upon adrenergic portions of the reticular activating system (RAS), since lesions in the RAS block effects of both drugs on the EEG. The effect of RAS stimulation upon the EEG is well established (*15*).

Estrogens affect the EEG and certain behaviors in ways similar to adrenergic stimulants; for instance, both estrogens and amphetamine, an adrenergic stimulant, lower the arousal threshold to environmental stimuli (*2*) and increase EEG running wheel activity (*1*) in ovariectomized rats. Progesterone, on the other hand, appears to antagonize estrogen effects, since estrogen plus progesterone raised the arousal threshold (*2*) and depressed running wheel activity (*16*). Both estrogens and progesterone penetrate brain tissues (*2, 17*).

A mechanism by which estrogens might affect central adrenergic activity has been suggested by research on changes in activity of the enzyme monoamine oxidase (MAO) in association with feminine reproductive cycles. The MAO inactivates neural supplies of monoamines, such as norepinephrine, which are believed to facilitate neural transmission in the central adrenergic nervous system. Activity of MAO in the posterior hypothalamus has been reported to vary during the estrous cycle of the intact rat (*18*). Ovariectomized rats had elevated levels of hypothalamic MAO activity, whereas ovariectomized rats treated with estradiol benzoate did not differ in their hypothalamic MAO activity from intact control rats (*18*).

Plasma MAO activity in humans has also been reported to vary with the menstrual cycle. Plasma MAO activity was found to be less in the preovulatory phase of the cycle, when blood estradiol levels reach peak values (*3*), than in the postovulatory phase of the cycle, when blood progesterone reaches peak values (*4*). Amenorrheic women with elevated plasma MAO activity showed significant MAO suppression when treated with oral conjugated estrogens. The addition of a progestin to the estrogen treatment resulted in a significant rise in their plasma MAO (*19*).

These reports imply that estrogens tend to inhibit MAO activity, both in rat hypothalami and in human plasma. Since MAO activity is believed to be inversely related to the level of central adrenergic functioning (*11*), it follows that estrogens, which appear to inhibit MAO activity, ought also to enhance central adrenergic processes.

It can therefore be hypothesized that when estrogen levels are elevated, whether due to an exogenous administration or to natural rises during the preovulatory phase of the menstrual cycle, MAO activity in the brain should be depressed, central adrenergic functioning should be enhanced, and EEG driving should be diminished.

Table 2. Electroencephalographic driving responses to photic stimulation in six amenorrheic females before and during 21-day endocrine treatment.

| Subject | Age of subject | No. of responses before treatment, during successive weeks: | | | | | No. of responses at various points during endocrine treatment | | | | | | | t-Test | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Premarin, days: | | | Provera + Premarin, days: | | | | Before treatment, versus Premarin | Premarin treatment, versus Premarin + Povera |
| | | 1 | 2 | 3 | 4 | 5 | 1 and 2 | 8 and 9 | 15 and 16 | 17 | 18 | 19 | 20 | 21 | |
| A | 24 | 12 | | 13 | | | 6 | 10 | 7 | | 11 | 12 | | | 3.17* | 2.52† |
| B | 25 | 18 | 17 | 18 | 14 | 16 | 12 | 12 | 9 | | | 18 | | | 4.59* | 3.63* |
| C | 32 | 5 | 6 | 10 | | | 1 | 0 | 0 | | | | | 10 | 4.88* | 5.01* |
| D | 31 | 18 | 14 | 14 | | | 9 | 7 | 8 | | | 8 | | 10 | 5.37* | 0.66 |
| E | 39 | 11 | 10 | 8 | | | 3 | 7 | 3 | | | | | 12 | 3.90* | 3.97* |
| F | 29 | 18 | 18 | 17 | 18 | 18 | 10 | 13 | 14 | | | | | 16 | 4.47* | 1.90 |

* $P = .01$; d.f. $= 29$. † $P = .05$; d.f. $= 29$.

When progesterone levels are also elevated, owing either to exogenous administration or to natural events during the menstrual cycle, the reverse should occur. Our data conform to this model.

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## Preganglionic Stimulation Increases Calcium Uptake by Sympathetic Ganglia

Abstract. *Isolated superior cervical ganglia of rats accumulate more calcium during preganglionic stimulation than do unstimulated controls; uptake of calcium-45 is approximately doubled by stimulation of 12 impulses per second. The extra uptake is markedly reduced by a high concentration of magnesium, but not by mecamylamine hydrochloride or tetraethylammonium chloride, although all three agents eliminate the postsynaptic action potential. Uptake of calcium-45 is also greatly increased by a high external concentration of potassium.*

In 1940, Harvey and MacIntosh (*1*) observed that calcium was required for the release of acetylcholine from cat superior cervical ganglia as a consequence of presynaptic stimulation. This finding focused attention on the role of $Ca^{2+}$ in transmitter release. These efforts have culminated in the enunciation of a "calcium hypothesis" by Katz and Miledi (*2*). According to this hypothesis, depolarization of a presynaptic terminal causes an increase in its permeability to $Ca^{2+}$; $Ca^{2+}$ diffuses into the terminal, down its electrochemical gradient (*3*), and is then involved in transmitter release. The hypothesis is supported by the observation that presynaptic depolarization of the squid giant synapse (*4, 5*) and the frog neuromuscular junction (*2, 6*), which is dependent on $Ca^{2+}$ and independent of $Na^+$, is a sufficient stimulus to trigger transmitter release. Under appropriate conditions, depolarization of squid presynaptic terminals induces a regenerative response and transmitter release which is dependent on $Ca^{2+}$, even in the absence of $Na^+$ (*5*).

One way to test the calcium hypothesis is to measure directly entry of $Ca^{2+}$ into presynaptic terminals during activity. Although isolated presynaptic nerve terminals from rat brain have an increased rate of $Ca^{2+}$ accumulation when depolarized in media with high concentrations of $K^+$ (*7*), these experiments are open to the criticisms that isolated terminals may not

Table 1. Uptake of $^{45}Ca$ by isolated superior cervical ganglia of rats. Ganglia were incubated in $^{45}Ca$ solutions for 25 to 30 minutes. The member of each pair denoted by a prime (′) was held on the electrodes and stimulated at the frequency shown, for 20 minutes, except where indicated. Uptake of $^{45}Ca$ in ganglia 9, 9′, 11, and 11′ was measured in Ringer with a low $Ca^{2+}$ concentration after exposure to Ringer with a low concentration of $Ca^{2+}$ and a high concentration of $Mg^{2+}$. The concentration of mecamylamine hydrochloride was 50 μmole/liter for ganglia 15 and 15′, and 38 μmole/liter for 16, 16′, 17, and 17′. Uptake of $^{45}Ca$ in ganglia 20 and 20′ was measured after exposure to Ringer with a low $Ca^{2+}$ concentration and to Ringer with a low concentration of $Ca^{2+}$ and a high concentration of $Mg^{2+}$.

| Ganglion pair | Temperature (°C) | Stimulation frequency ($sec^{-1}$) | $Ca^{2+}$ uptake (pmole/mg) | Stimulated $Ca^{2+}$ uptake (pmole $mg^{-1}$ $impulse^{-1}$) |
|---|---|---|---|---|
| *Normal Ringer* | | | | |
| 1 | 34 | | 339 | |
| 1′ | 34 | 10–12* | 484 | 0.0103 |
| 2 | 32 | | 357 | |
| 2′ | 32 | 12 | 773 | .0289 |
| 3 | 32 | | 426 | |
| 3′ | 32 | 12 | 777 | .0244 |
| 4 | 25 | | 258 | |
| 4′ | 25 | 12 | 762 | .0350 |
| 5 | 26 | | 398 | |
| 5′ | 26 | 12† | 675 | .0167 |
| *Normal Ringer, unstimulated pairs* | | | | |
| 6 | 22 | | 201 | |
| 6′ | 22 | | 136 | |
| 7 | 23 | | 374 | |
| 7′ | 23 | | 236 | |
| 8 | 23 | | 269 | |
| 8′ | 23 | | 237 | |
| *Ringer with low $Ca^{2+}$ concentration* | | | | |
| 9 | 25 | | 90 | |
| 9′ | 25 | 12 | 257 | 0.0116 |
| 10 | 25 | | 143 | |
| 10′ | 25 | 12 | 320 | .0124 |
| 11 | 32 | | 133 | |
| 11′ | 32 | 6–12‡ | 363 | .0168 |
| *Ringer with low $Ca^{2+}$, high $Mg^{2+}$ concentration* | | | | |
| 12 | 25 | | 101 | |
| 12′ | 25 | 12 | 81 | −0.0014 |
| 13 | 25 | | 101 | |
| 13′ | 25 | 12 | 108 | .0005 |
| 14 | 32 | | 134 | |
| 14′ | 32 | 12 | 138 | .0003 |
| *Treated with mecamylamine* | | | | |
| 15 | 33 | | 261 | |
| 15′ | 33 | 6 | 345 | 0.0117 |
| 16 | 33 | | 212 | |
| 16′ | 33 | 12 | 444 | .0161 |
| 17 | 31 | | 252 | |
| 17′ | 31 | 12 | 404 | .0105 |
| *Treated with 3 mM TEA* | | | | |
| 18 | 32 | | 264 | |
| 18′ | 32 | 6§ | 688 | 0.0470 |
| 19 | 32 | | 264 | |
| 19′ | 32 | 12 | 638 | .0260 |
| 20 | 33 | | 211 | |
| 20′ | 33 | 12 | 677 | .0324 |

* Ten pulses per second for 2.5 minutes, then 12 per second for 17.5 minutes. † Twelve pulses per second for 23 minutes. ‡ Six pulses per second for 2 minutes, then 12 per second for 18 minutes. § Six pulses per second for 25 minutes.