1976

Similarities in Analgesia Produced by Cervical Probing and Intracranial Stimulation to the Mesencephalic Grey Matter

Kathleen Casey Westlake
College of William & Mary - Arts & Sciences

SIMILARITIES IN ANALGESIA PRODUCED BY CERVICAL PROBING AND INTRACRANIAL STIMULATION TO THE MESENCEPHALIC GREY MATTER

A Thesis

Presented to

The Faculty of the Department of Psychology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

---

by

Kathleen C. Westlake

1976

APPROVAL SHEET

This thesis is submitted in partial fulfillment of

the requirements for the degree of

Master of Arts

Kathleen C. Westlake

Approved, August 1976

Herbert Friedman

E. Rae Harcum

Linda C. Petty

Ellen F. Rosen, Chairperson

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The purpose of this study was to compare the analgesia produced by intracranial stimulation of the central grey matter of the mesencephalon and analgesia produced by cervical probing. Previous research has found that anlgesia was produced by cervical probing and by intracranial stimulation. If this analgesia was produced by changing activity in the central grey, interactions should occur between estrogen presence and intracranial stimulation, thus enhancing analgesia between cervical probing and intracranial stimulation in enhancing analgesia, and between cervical probing and intracranial stimulation to the central grey in producing analgesia. The predicted interactions were significant, indicating that analgesia produced by intracranial stimulation directly to the central grey cause changes there that were similar to ones produced naturally by cervical probing. The effects of palpation following cervical probing were also examined. Palpation following cervical probing was found not to enhance analgesia, thus palpation does not appear to activate changes in the central grey that are the same as cervical probing with palpation.

SIMILARITIES IN ANALGESIA PRODUCED BY CERVICAL PROBING AND INTRACRANIAL STIMULATION TO THE MESENCEPHALIC GREY MATTER

INTRODUCTION

Certain areas of the brain function in more than one mode of behavior. The mesencephalic central grey may serve a dual role in the reception of sexual stimulation and nociceptive stimulation. This area of the brain has been shown to concentrate radioactively labled estradial benzoate in female rats (Pfaff, 1972). Intracranial stimulation to the lateral borders of this area produce analgesia of the lower two thirds of the rat's body (Mayer, 1971). Recently, cervical probing has been shown to inhibit firing rate of single units in this area of the brain in female rats (Petty, 1975).

The rationale behind the present study is to examine the effects of intracranial stimulation of the mesencephalic central grey, and the effect of cervical probing both before and paired with palpation, the prediction being that these two types of stimulation work by causing changes in the central grey--intracranial stimulation as a direct non-physiological stimulus and cervical probing as a direct physiological one. By this hypothesis the analgesia results from changes in activity levels in the central grey of the mesencephalon.

Effects of Estrogen

Pfaff (1972) administered radioactively labled estradial benzoate and found that it became highly concentrated in specific parts of the limbic system, including the central grey, within two hours of its administration (Pfaff, 1972, Pfaff and Keiner, 1972). Pfaff believed that estrogen-concentrating structures in the limbic system, preoptic area

and the hypothalamus were involved in the mechanism of estrogen's action on female sexual behavior. Pfaff has shown that established neuroanatomical pathways linked the above mentioned structures with connections from the olfactory system, basal forebrain, and such longitudinal associative pathways as the periventricular fiber system and medial forebrain bundle (1972). Whether or not these pathways participate in the mediating the effects of estrogen has not yet been determined.

Pfaff (1972) has attempted to localize supraspinal pathways controlling mating actions by using bipolar stimulating electrodes to stimulate those areas of the brain which picked up estrogen. Electrode positions for stimulating tail and rump movements similar to the lordosis posture were isolated at the junction of the mesencephalon and the diencephalon. Many of these areas of stimulation were found to be in the central grey (Pfaff, 1972). The distribution for these points in the mesencephalon coincided with anatomically defined distributions of ascending fibers from the spinal cord. These results also showed an overlap between the distribution of points leading to the lordosis posture, and regions containing the greatest number of estradial-concentrating neurons, specifically the central and preoptic areas of the mesencephalon.

Focusing upon the central grey, Pfaff (1972) hypothesized that neurons from the spinoreticular pathway enter the region of the lateral borders of the central grey, and produce a change in neural activity there. These neurons have been shown to be connected with descending pathways that innervate back and rear leg muscles which are responsible for the lordosis response (Pfaff, 1972).

Lordosis Posture

Mating activity in the female rat is a very complex activity. Nevertheless, the lordosis posture, which is a whole body reflex, can easily be elicitated under certain conditions and thus is convenient to study. The lordosis posture is characterized by an extension of the legs, an arching of the back such that the head, rump, and tail are raised while the thoracic region is lowered with the tail deflected to one side. It is necessary for the female to assume this posture to allow the male to achieve intromission and ultimately ejaculation (Pfaff, 1972). The lordosis reflex is facilitated by the presence of estrogen, although estrogen is not necessary for the lordosis posture to be achieved (Komisaruk & Diakow, 1973). Ovariectomized female rats with no hormone replacement therapy will lordose about 50% of the time to cervical probing with perineal palpation, and slightly less often to perineal palpation following cervical probing (Komisaruk, 1974; Komisaruk & Diakow, 1973).

In a natural courtship situation, the male rat begins the mating sequence with pressure applied by his forepaws to the female's hindquarters. This pressure is thought to initiate the lordosis posture. The male continues the mating sequence with a series of mounts accompanied by pelvic thrusts. Several intromissions, separated by dismounts, are achieved until the male ejaculates. As a part of this sequence, the female attains and holds the lordosis posture longer after each successive intromission until the final ejaculation (Kuehan & Beach, 1963; Diakow, 1974).

Komisaruk et al. (1971) has shown that lordosis to cervical probing with palpation is a more sensitive measure of estrogen levels than the

vaginal smear. Komisaruk (1974) has shown that estrogen enlarges the genital sensory field. Recordings from the pudendal nerve, which receives input from the perineal region, show that rats treated with estrogen increased the sensory field of the genital region 22.0% in length; 26.3% in width; 31.9% in area; and 75% in the clitoral region. The sensory field was measured by determining the limits of the body surface which generated action potentials in the pudential nerve when the body was brushed or scratched. This field extension persisted even after the pudendal nerve was severed. Komisaruk (1974) hypothesized that estrogen modified sensory activity by acting directly on the peripheral nervous system perhaps to assist the female rat in orienting to copulatory thrusts of the male.

Rodriguez-Sierra et al. (1974) demonstrated that lordosis in response to flank-perineum palpation alone could be induced by previous probing of the cervix. Thus, prior cervical probing enabled a previously ineffective stimulus (flank-perineal palpation alone) to become effective.

Komisaruk (1974) believed that cervical probing used in this way acts as a trigger to responsivity to a previously ignored stimulus, and showed that cervical probing also can improve lordosis encounters between the male and female. Ovariectomized, estrogen primed female rats which would not lordose to mating attempts of vigorous males were cervically probed with perineal palpation. Following this treatment, a significant number of the probed females showed responses to mating attempts of males. Thus, it is clear that cervical probing with perineal palpation, and perineal palpation following cervical probing may be causing similar changes in neural activity in the female rat.

Cervical Probing and Nociceptive Stimulation

Komisaruk and Wallman (1973) found that constant pressure applied to the cervix along with palpation produced a lordosis posture and an inhibition of responses to nociceptive stimulation which reached the analgesic level. Apparently this stimulation blocks neuro-transmissions of pain to the central grey rather than immobilizing the animal so that it cannot respond to painful stimuli. Evidence for this comes from Petty (1975). Recording from chronically implanted electrodes in the mesencephalic central grey, she found that when cells were inhibited by cervical probing they tended also to be inhibited by sexually relevant stimuli, but were facilitated by nociceptive stimuli. Units facilitated by cervical probing and sexually relevant stimuli were inhibited by nociceptive stimulation. Also, units that were facilitated by nociceptive stimulation were inhibited by cervical probing. Inhibition occured in units that had been facilitated by nociceptive stimulation alone. Petty hypothesized that cervical probing blocked responses to painful stimulation, not by reducing overall activity levels, but by selectively altering responses of neurons in the mesencephalic central grey to nociceptive stimuli. Rosen, Petty and Westlake (1974) have shown that cervical probing blocked an escape response (tail-flick) to nociceptive stimulation (radiant heat).

Komisaruk et al. (1967) and Ramirez et al. (1967) observed the "sleep-arousal" state of the EEG changed to a sleep pattern during cervicle probing. Neurons in the cortex showed an increase in firing rate when the EEG changes from a sleep-like to an arousal state. This occured either spontaneously or when painful stimulation was applied. However, cervical probing tended to prevent the EEG from changing the

sleep-like state to the arousal state during painful stimulation. Komisaruk (1974) interpreted these findings as evidence that cervical probing altered neural activity to produce a blockage of incoming nociceptive stimulation, but did not depress brain activity in general or overall activity of the body.

Komisaruk & Wallman (1973) has also reported that during cervical probing the animals capacity to vocalize was not impaired, and that the subject would be able to vocalize in pain if it was feeling apin. Rats that had previously vocalized to painful stimulation stopped vocalizing when the painful stimulation was paired with cervical probing. This would indicate that an inhibition of pain occured, not that an overall depression of the subject's body responses prevented the subject from responding.

Komisaruk (1974) postulated that inhibition of responses to painful stimulation by cervical probing may have an adaptive significance in reducing the painful aspects of copulation and parturation. Evidence for this comes from Pierce and Nuttal (1961) who discovered that if female rats were given an opportunity to escape from males into a separate chamber, they stayed there for shorter periods of time after intromission than before intromission, indicating that some adversive components of mating may be dropping out. Bermant (1966) allowed rats the opportunity to press a bar to allow a male to enter the cage. Female rats waited shorter periods of time after each intromission to allow the male to enter than they did during earlier periods. If pain were present, the female would presumably find the mating experience adversive and avoid the male. However, as the courtship continues, any painful aspects of mating apparently diminishes, and the female becomes less responsive

to aversive components of the mating ritual. This would indicate that if mating had aversive components, these components are reduced through cervical probing.

Intracranial Stimulation and Analgesia

Turning now to central mechanisms, analgesia has been produced by intracranial stimulation to the mesencephalic central grey that shows reduced withdrawal from nociceptive stimulation. Mayer, Wolfle, Akil, Carter, and Liebeskind (1971) found that analgesia can be produced by intracranial stimulation to the mesencephalic central grey matter of such a magnitude that the animal becomes totally non-responsive to painful stimulation. This analgesia persisted after stimulation had ceased for up to 3 hours.

Critics have suggested that intracranial stimulation of the mesencephalic central grey does not really produce analgesia, but depresses and inhibits the ability of the animal to make motor responses in general. Mayer et al. (1971) holds that the failure of the subject to respond to noxious stimulation was not due to a generalized motor deficit, but rather to selectively reduced responses to the noxious stimulus. The rationale for this was that the subject remains responsive to other stimulation, e.g. visual or auditory, and responds appropriately to other stimulation both during and following the stimulation.

Mayer and Liebeskind (1974) tested seven brain areas to observe analgesic effects of stimulation. The areas under investigation were the mesencephalic central grey, diencephalic perventricular grey, ventral tegmentum, lateral hypothalamus, ventrobasal thalamus, dorsomedial thalamus, and septal area. They found that only intracranial stimulation of

the mesencephalic central grey and perventricular grey abolished all responses to noxious stimulation. Intracranial stimulation in these areas produced analgesia of the same magnitude as that obtained in the same subjects by large dosages of morphine (19mg/kg) (Mayer and Liebeskind, 1974).

Mayer has ruled out the possibility that the analgesia produced by intracranial stimulation is simply due to distraction by pleasurable stimulation, for rats implanted in the mesencephalic central grey that show significant analgesia when stimulated will not bar press to receive self-stimulation. Many animals were found to self-stimulate at a high rate, but later tests indicated that these animals were not analgesic (Mayer et al., 1971; Mayer & Leibeskind, 1974). Mayer and Leibeskind (1974) believed that the analgesia that was produced is caused by intracranial stimulation activating neural systems that inhibit the transmission of nociception at the spinal level.

Hypotheses and Predictions

Thus there exists empirical evidence to support the hypothesis that intracranial stimulation to the central grey of the mesencephalon may be activating a nociceptive blocking system that is usually triggered in the natural setting by cervical probing. The previous evidence indicates that both cervical probing and intracranial stimulation to the mesencephalic central grey may cause similar changes by affecting a neural loop which includes the central grey of the mesencephalon that gates nociceptive stimulation.

The present experiment attempts to replicate the findings of previous researchers that the presence of estrogen enhances the lordosis

response. When estrogen is absent, a substantially lower lordosis response would become obvious. Palpation during and following cervical probing would produce the strongest lordosis response in the presence of estrogen. Thus, the effect of estrogen would be to enhance the lordosis reponse to cervical probing with and following palpation and to enhance the analgesic effects of cervical probing. Intracranial stimulation to the central grey of the mesencephalon will produce analgesia, as will cervical probing paired with and following palpation.

Since the experimenter hypothesized that intracranial stimulation to the mesencephalic central grey activates artifically a neural system that is naturally activated by cervical probing, these two types of stimulation should work together to produce analgesia. Also, since palpation following cervical probing appears to act as a trigger to cause the animal to react to previously ignored sexually relevant stimuli, it is possible that it too may stimulate this neural loop involving the central grey of the mesencephalon the same as cervical probing with palpation, thus analgesia to palpation following cervical probing is expected, and should produce analgesia similar to that caused by cervical probing paired with palpation.

METHOD

Subjects: The subjects of this experiment were 42 female albino Holtzman rats which were ovariectomized one week after their arrival in the lab. The rats were 100 days old on arrival. They were housed in separate cages in an animal room that was maintained at 25° C. with a twelve hour light-twelve hour dark cycle. The rats were fed standard Purina Laboratory Chow, and tap water ad lib. Following surgury the rats were allowed a one week rest. Following the post surgury rest, the rats were stereotaxically implanted with bipolar stainless steel electrodes (Plastic Products, Roanoke, Va.) in the mesencephalic central grey matter using ether as an anesthetic and 0.5 cc of atropine sulfate as a pre-anesthetic. The atlas target coordinates were A.P.: 5.8; Lat.: 0.8; and D. V.: 5.5 (Pelligrino and Cushman, 1967). Two days after surgury the rats were given five minutes of electrical stimulation through the electrode. When stimulation was terminated, analgesia was tested using a tail-pinch device, and applying 2.72 Kg of pressure against the distal 15 mm of the rat's tail. Any movement or vocalization during the tail-pinching procedure was taken to indicate that the rat was not anlgesic. All rats found not to be proven analgesic were eliminated from the experiment. This left 16 analgesic rats.

Apparatus: The stimulation apparatus was a Grass SD9 stimulator in conjunction with a Grass constant current Unit (CCU-1). The current produced was a 50 hz, 12 mamp monopolar square wave of 50 msec. duration.

Nociceptive stimulation was provided by a tail-flick device (modified after that used by Mayer, 1971). This is a radiant heat source of

57° C. emineting from a light bulb (G. E. bhd) with filament focused by a concave mirror on the most distal portion of the rat's tail. The bulb was powered by a 21 V, 5 amp D. C. current. It was shut off automatically after 9 seconds in order to prevent tissue damage. A score on the tail-flick consisted of four trials, the last three of which were averaged together; the intertrial interval for tail-flick was one and one half minutes for cooling of the device after each trial.

The other device for administering noxious stimulation was the tail-pinch device, which placed from 0.45 Kg to 5.44 Kg of pressure against the the tip of the rat's tail. It was a push-pull spring scale mounted on a wooden block. Pressure was applied to the rat's tail by a metal pad with an area of 1/20 sq. cm. up against another metal pad mounted in the wooden back stop. During intracranial stimulation the rats were restrained in a plastic cylinder, but during testing, the rats were held in the experimenter's hand.

Procedure: Fourty to fourty-four hours before testing, subjects were injected with either 1 ugm of estradial benzoate dissolved in 0.1 cc sesame seed oil (Fisher Laboratories) per 100 grams of body weight or a comparable amount of oil alone (0.35 cc) subcutaneously. Each subject was run twice, the first time injected with either oil or estrogen, and tested both with and without intracranial stimulation once, and the second time about 48 hours later injected with the other substance, and again tested with and without intracranial stimulation.

The order of hormone presentation was determined by use of a restricted random number table (Friedman, 1969). In each test situation, the rat was either presented with eight stimulus conditions without intracranial stimulation, or following five minutes in intracranial

stimulation. The eight conditions were: 1) tail-flick paired with cervical probing; 2) tail-flick following cervical probing; 3) tail-flick alone; 4) tail-pinch with cervical probing; 5) tail-pinch with palpation following cervical probing; 6) tail-pinch alone; 7) cervical probing paired with palpation alone; and 8) palpation following cervical probing alone.

Cervical probing consisted of exerting a constant pressure to the cervix of the rat's vaginal tract with a 1 cc glass syringe plunger moistened with water. While probing, the thumb of the other hand restraining the rat comes in contact with one of the rat's flanks, and the ring finger with the other flank. The index and middle fingers lie on either side of the tail and apply intermittent pressure to the perineum (perineal palpation). The heel of the hand rests on the rat's back, but does not apply pressure. To achieve lordosis following cervical probing, one begins with the lordosis with cervical probing as described above, but while maintaining perineal palpation, remove the probe.

Data collected were for the tail-flick, the latency of removing the tail from the heat source, with a ceiling of nine seconds; amount of pressure tolerated from the tail-pinch until some body or tail movement, or a vocalization occurred, with a maximum amount of pressure set at 2.72 Kgs. These upper limits were used because beyond these points permanent damage to the rats might occur, thereby modifying further measurments.

Lordosis postures were judged on a four-point rating scale. A score of four indicated a typical full lordosis with the head, rump, and tail raised, and the thorax lowered. A score of three indicated the head and rump were raised, but the arch in the back was not present. A

score of two indicated the rump was raised, but the head was not, nor did the back arch. A score of one indicated that the head, rump and tail did not become raised, and the back did not arch.

Data were collected between seven and eleven a.m. eastern standard time.

At the end of the experiment, the rats were sacrificed using an overdose of pentabarbatol, and were perfused with a 0.9% saline followed by a 10% formaldehyde in 0.9% saline solutions. After perfusion, the brains were removed, embedded in egg yolk hardened with a formaldehyde solution, and sectioned with a microtome into 50 u sections. These sections were mounted on slides and stained with crystal violet staining solution for verification of electrode placement.

RESULTS

Histology

Histological examination (see Fig. 1) shows that all subjects in this study had electrode placement in the more lateral portions of the central grey of the mesencephalon. This was to be expected since all subjects were analgesic.

Order Effects

A t-test for independent samples (d.f. 14) $t_{(14)} = .05$ was performed on tail-flick scores of subjects given estrogen first as compared to those given estrogen last to look for order effects. No significant differences were found.

Validity of Rating Scale

An unconventional rating evaluation system for the lordosis response was used in this experiment. The Pearson correlation between the present rating scale and the evaluation of the lordosis posture via a photographic technique of Rosen and Petty (1974) was 0.69 (d.f. = 4) which was significant at the 0.01 level.

Lordosis Response

Table 1 summarizes the means for the lordosis response. Examination of this table reveals that rats receiving estrogen showed a greater lordosis score, both with palpation and cervical probing together and palpation following cervical probing. These data were analyzed using an

FIGURE 1

Estimated location of electrode tips. Numbers indicate subjects (Pellegino & Cushman, 1967).

Figure 1

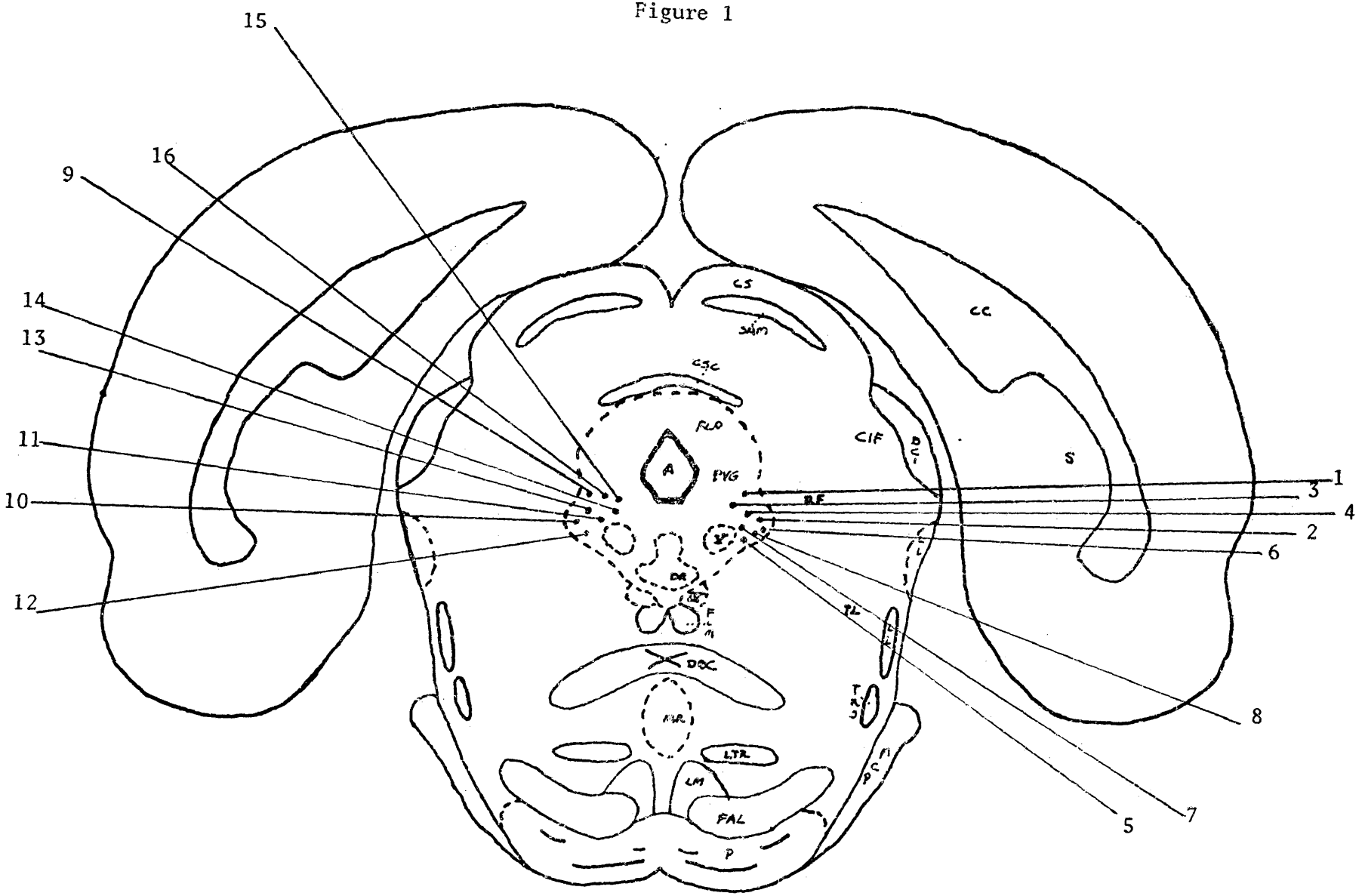

TABLE I

Table of means for lordosis ratings with palpation during and following cervical probing.

	During	Following
Estrogen and Intracranial Stimulation	3.75	3.56
Estrogen and No Intracranial Stimulation	3.8	3.8
No Estrogen and Intracranial Stimulation	3.44	3.19
No Estrogan and No Intracranial Stimulation	2.8	2.69

analysis of variance for repeated measures. There were three independent variables: Palpation during cervical probing, palpation following cervical probing; presence or absence of estrogen; presence or absence of intracranial stimulation. Therefore this is a 2x2x2 design with all subjects receiving all treatments. This analysis is summarized in Table 2. The F values for estrogen, stimulation and the estrogen-by-stimulation interaction were all significant. There was no significant difference between palpation during cervical probing and palpation following cervical probing.

Figure 2 shows the effect of estrogen on the magnitude of the lordosis response. These data indicate that the strength of lordosis was enhanced by administration of estrogen. Also intracranial stimulation to the central grey of the mesencephalon enhanced the lordosis posture in the absence of estrogen. Thus, both these factors affect the rated magnitude of the lordosis response. A significant interaction between intracranial stimulation and the presence of estrogen was shown.

A test for simple main effects was performed to further analyze the estrogen-by-stimulation interaction. This analysis is presented in Table 3. It is clear that estrogen was effective in increasing scores on the lordosis response both when the animal was given electrical stimulation to the mesencephalic central grey, and when intracranial stimulation was not present. In the presence of estrogen, it is difficult to judge the effect of intracranial stimulation. Looking at Figure 2, it is clear that in the presence of estrogen, cervical probing with intracranial stimulation to the central grey and cervical probing without intracranial stimulation produce almost equally high lordosis response scores. Palpation following cervical probing with intracranial stimulation shows a

TABLE 2

Analysis of variance of the lordosis response

Source	dF	MS	F
Blocks	15		
Treatments	7		
A Estrogen	1	15.8	58.73*
B Cervical Probing	1	0.64	2.38
C Intracranial Stimulation	1	1.33	4.94*
AXB	1	0.06	0.22
AXC	1	4.12	15.315*
BXC	1	0.18	0.669
AXBXC	1	0.03	0.11
Residual	105	0.269	
Total	127		

FIGURE 2

Effects of presence of estrogen on magnitude of lordosis response.

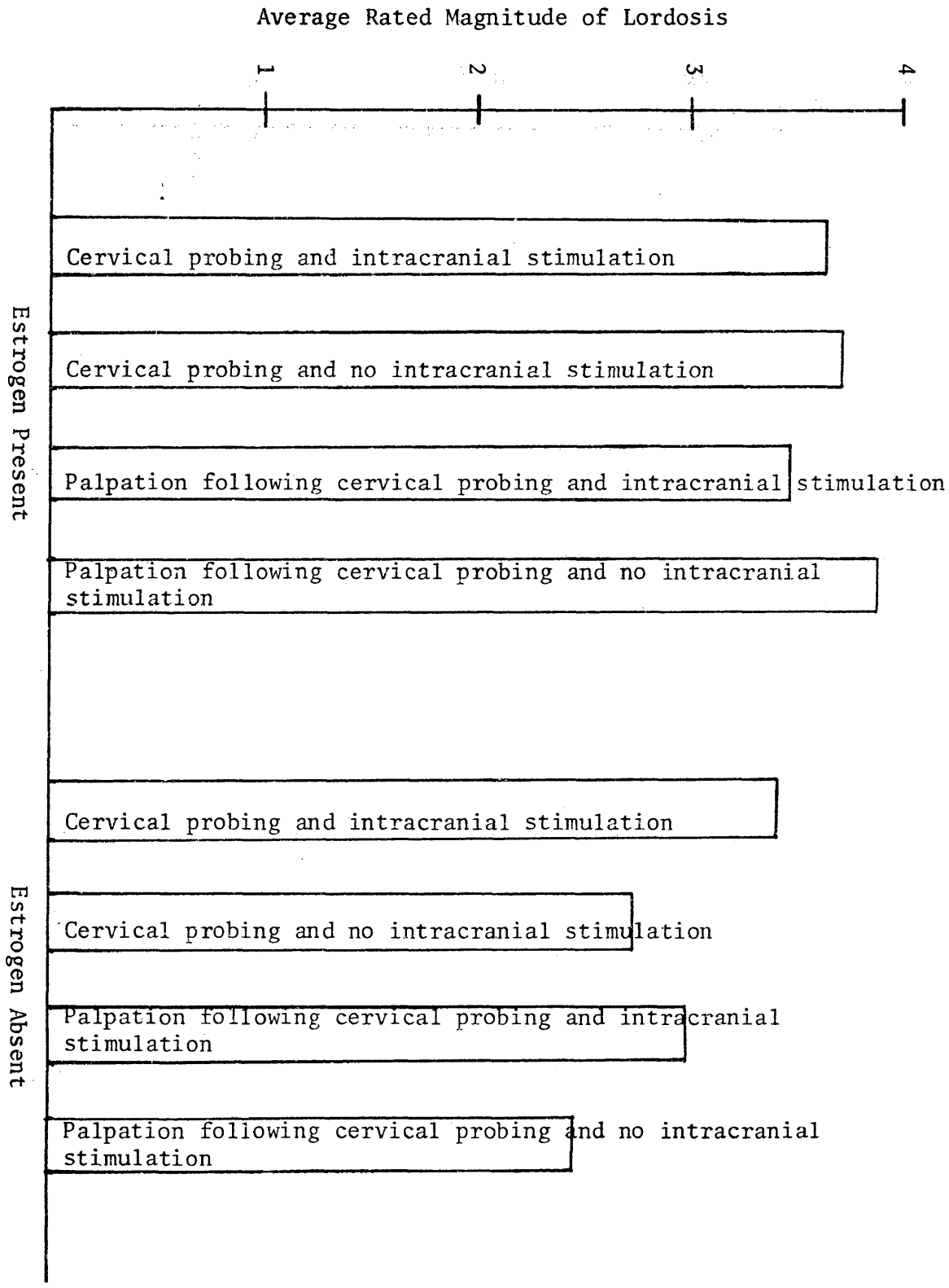
Average Rated Magnitude of Lordosis
1
2
3
4
Estrogen Present
Cervical probing and intracranial stimulation
Cervical probing and no intracranial stimulation
Palpation following cervical probing and intracranial stimulation
Palpation following cervical probing and no intracranial stimulation
Estrogen Absent
Cervical probing and intracranial stimulation
Cervical probing and no intracranial stimulation
Palpation following cervical probing and intracranial stimulation
Palpation following cervical probing and no intracranial stimulation

Figure 2

TABLE 3

Tests of simple effects for the significant estrogen by intracranial stimulation interaction on the lordosis response.

Source	dF	MS	F
A Estrogen	1	15.8	58.74*
A at C_1	1	1.9	7.06*
A at C_2	1	18.65	69.33*
C Intracranial Stimulation	1	1.33	4.94*
C at A_1	1	0.4	1.49*
C at A_2	1	5.065	18.828*
Residual	105		

slightly lower score, and palpation following cervical probing without intracranial stimulation shows the highest mean score of all. However, all these scores were very high due to the presence of estrogen. Without estrogen, the highest scores arise from cervical probing and intracranial stimulation to the mesencephalic central grey presented together. The next highest score results from palpation following cervical probing paired with intracranial stimulation. Therefore, when estrogen was not present, the highest scores arise when intracranial stimulation was present. Thus, estrogen appeared to show the greatest effect on the lordosis score. When estrogen was not present, intracranial stimulation to the mesencephalic central grey had the next greatest effect on producing a strong lordosis score.

Tail-Flick

Table 4 summarizes the means of the tail-flick responses. It is clear from this table that the greatest overall mean latency was for groups receiving cervical probing with palpation. Looking at Figure 3, when tail-flick latency was measured along with cervical probing and palpation, all latency scores were very high, indicating analgesia in all cases. When subjects received palpation following cervical probing the effect was not as strong. When subjects received intracranial stimulation to the central grey and no estrogen, they demonstrated the longest latency. When they received estrogen but no intracranial stimulation, they showed the next shortest latency. When they received estrogen and intracranial stimulation, they showed the next shortest latency, and when no estrogen or intracranial stimulation was given, a very short latency was demonstrated. When subjects were given no palpation and no

TABLE 4

Table of means for tail-flick responses with palpation

	Alone	During/cp	Following/cp
Estrogen and Intracranial Stimulation	5.81	8.38	5.61
Estrogen and No Intracranial Stimulation	4.87	8.32	6.13
No Estrogen and Intracranial Stimulation	5.99	8.64	6.57
No Estrogen and No Intracranial Stimulation	4.31	8.31	4.77

FIGURE 3

The effect of palpation and cervical probing on latency of tail-flick.

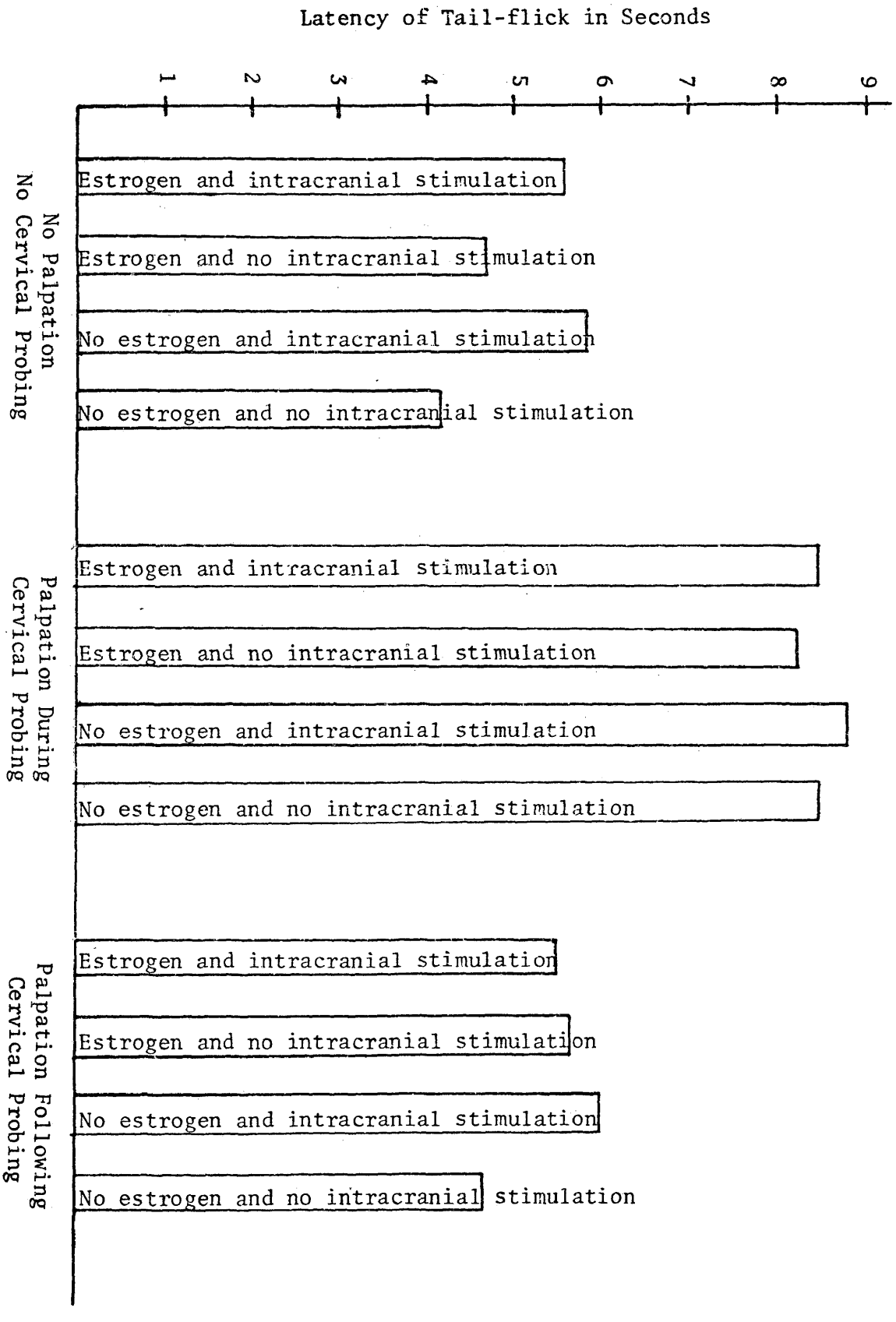
Latency of Tail-flick in Seconds
1
2
3
4
5
6
7
8
9
No Palpation
No Cervical Probing
Estrogen and intracranial stimulation
Estrogen and no intracranial stimulation
No estrogen and intracranial stimulation
No estrogen and no intracranial stimulation
Palpation During
Cervical Probing
Estrogen and intracranial stimulation
Estrogen and no intracranial stimulation
No estrogen and intracranial stimulation
No estrogen and no intracranial stimulation
Palpation Following
Cervical Probing
Estrogen and intracranial stimulation
Estrogen and no intracranial stimulation
No estrogen and intracranial stimulation
No estrogen and no intracranial stimulation

Figure 3

cervical probing, they demonstrated analgesia similar to that when palpation was given following cervical probing. Thus palpation following cervical probing did not produce analgesia.

Figure 4 shows the means of the tail-flick latencies with intracranial stimulation to the mesencephalic central grey. The greatest latency occured when intracranial stimulation was presented with cervical probing and no estrogen. Next highest latencies were estrogen and cervical probing with no intracranial stimulation, and estrogen and cervical probing without estrogen. All these latencies were essentially the same. In the intracranial stimulation conditions, the remaining conditions ordered themselves as follows: no estrogen with palpation following cervical probing; no estrogen and no cervical probing; and finally estrogen and palpation following cervical probing. When no intracranial stimulation was present, the effect of estrogen with palpation following cervicle probing shows a latency greater than the same condition with intracranial stimulation to the mesencephalic central grey. No-estrogen with cervical probing produced the same latency, then followed no-estrogen with palpation following cervical probing, and finally no-estrogen with no cervical probing. Thus, there appears to be three levels of latency: Those receiving cervical probing regardless of the other conditions, and showing the highest latency; those receiving intracranial stimulation to the central grey of the mesencephalon regardless of the other conditions, as well as those receiving no intracranial stimulation but receiving estrogen with palpation following cervical probing and showing moderate latencies, and thus moderate analgesia; and finally those that received no intracranial stimulation and no cervical probing or palpation following cervical probing, showing very short latencies, and thus no analgesia.

FIGURE 4

The effect of intracranial stimulation on tail-flick latency.

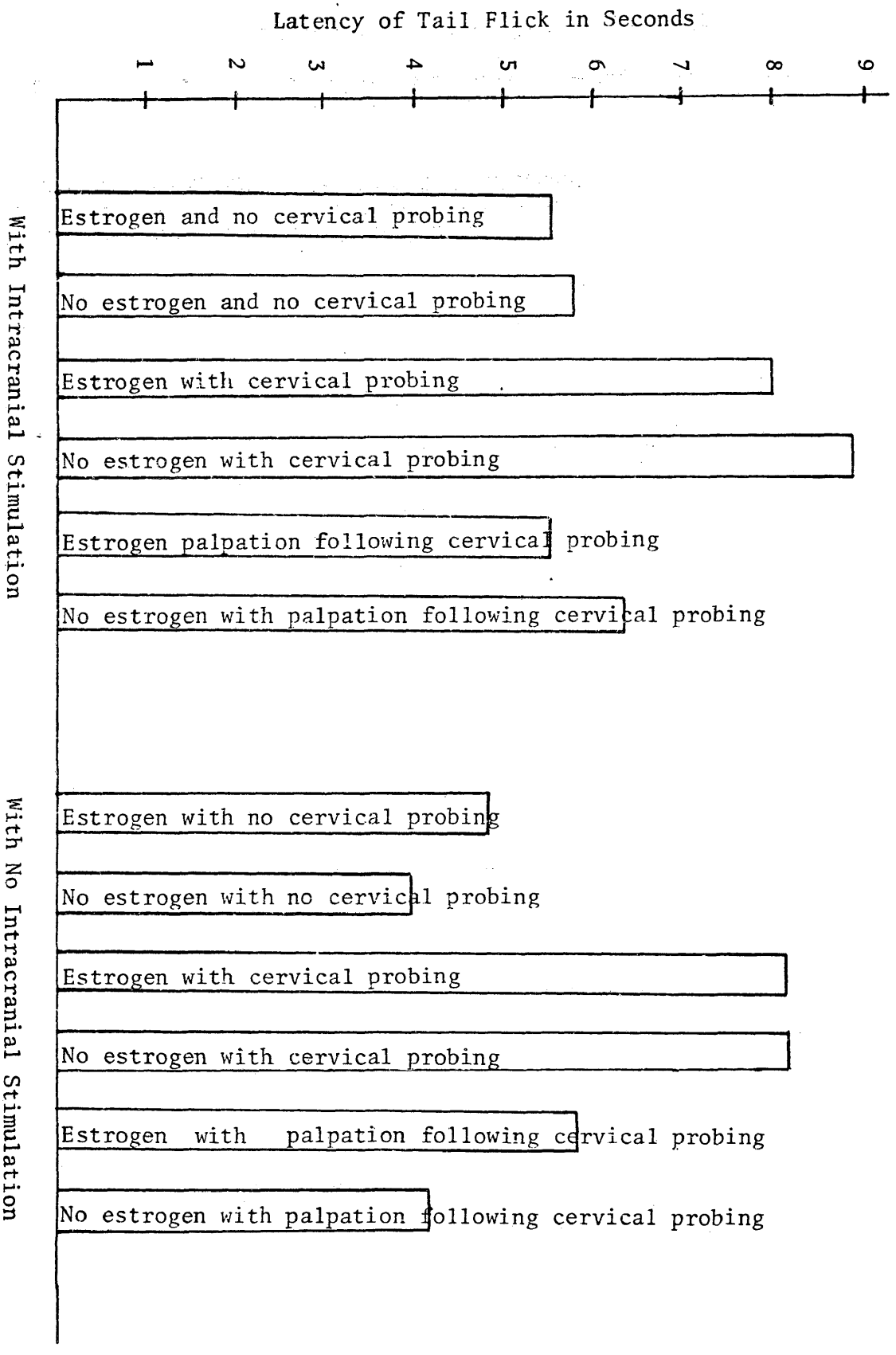
Latency of Tail Flick in Seconds
1
2
3
4
5
6
7
8
9
With Intracranial Stimulation
Estrogen and no cervical probing
No estrogen and no cervical probing
Estrogen with cervical probing
No estrogen with cervical probing
Estrogen palpation following cervical probing
No estrogen with palpation following cervical probing
With No Intracranial Stimulation
Estrogen with no cervical probing
No estrogen with no cervical probing
Estrogen with cervical probing
No estrogen with cervical probing
Estrogen with palpation following cervical probing
No estrogen with palpation following cervical probing

Figure 4

The data were analyzed using the same type of analysis of variance for repeated measures. The independent variables were: presence or absence of estrogen; presence or absence of intracranial stimulation to the mesencephalic central grey; and palpation during cervical probing, palpation following cervical probing, and no palpation or cervical probing.

This data is summarized in Table 5. Estrogen did not have a significant effect in producing analgesia. Cervical probing produced significantly greater analgesia than no cervical probing or palpation, and intracranial stimulation to the central grey produced greater analgesia than no intracranial stimulation.

There was also a significant interaction between estrogen and intracranial stimulation to the central grey. An analysis of simple main effects is presented in Table 6. It shows a significant effect for estrogen in promoting analgesia when intracranial stimulation was not present; a significant effect for intracranial stimulation producing analgesia when estrogen was present, and a significant effect for intracranial stimulation when estrogen was not present. Estrogen did not show a significant enhancement of analgesia in the presence of intracranial stimulation. Intracranial stimulation to the mesencephalic central grey appears to be more effective in the absence of estrogen, but estrogen to be equally effective in both the presence and absence of intracranial stimulation.

A Tukey's test was performed to investigate differences between groups receiving palpation paired with cervical probing, palpation following cervical probing, and no cervical probing or palpation. A summary of this data analysis is presented in Table 7. It shows that there was a significant difference between groups receiving palpation with and without cervical probing. There was a significant difference between

TABLE 5

Analysis of variance for tail flick-response

Source	dF	MS	F
Blocks	11		
Treatments	15		
A Estrogen	1	0.28	0.12
B Cervical Probing	2	184.28	79.43*
C Intracranial Stimulation	1	24.64	10.62*
AXB	2	0.605	0.26
AXC	1	14.84	6.4*
BXC	2	5.13	2.21
AXBXC	2	4.5	1.94
Residual	165		
Total	191		

TABLE 6

Tests of simple effects for the significant interaction of estrogen by intracranial stimulation on analgesia

Source	dF	MS	F
A Estrogen	1	0.28	0.12
A at C_1	1	5.22	2.25
A at C_2	1	9.88	4.26*
C Intracranial Stimulation	1	24.64	10.62*
C at A_1	1	0.64	0.41
C at A_2	1	38.76	16.71
Residual	165		

TABLE 7

Tukey's test for differences between means for groups on cervical probing for tail-flick data

During - After:	Q = 13.9*
During - None:	Q = 16.63*
None - After:	Q = 2.63

groups that received palpation during cervical probing and those receiving palpation following cervical probing. Those subjects that received palpation during cervical probing were significantly more analgesic than those subjects that received palpation following cervical probing. There was no significant difference between subjects receiving no cervical probing or palpation and those receiving palpation following cervical probing. This would indicate that those subjects receiving palpation following cervical probing were by and large not analgesic.

Thus, the data gathered from the tail-flick indicates that cervical probing is the most salient factor in producing analgesia, and is more effective in producing analgesia than intracranial stimulation. When cervical probing was present, intracranial stimulation without estrogen produced the greatest analgesia. Subjects receiving palpation following cervical probing and no intracranial stimulation showed an increase in latency when estrogen was present; otherwise there was no difference in the effects from this and no palpation or cervical probing.

Tail-Pinch

Table 8 summarizes the means for the tail-pinch data. Figure 5 examines the effect of cervical probing on the amount of pressure tolerated. In the cervical probing condition the greatest amount of pressure tolerated was in the presence of estrogen with no intracranial stimulation, and the no intracranial stimulation and no estrogen condition. The pressure tolerated here was relatively high. With palpation following cervical probing, intracranial stimulation shows the greatest analgesia with presence of estrogen showing a moderate amount of pressure. With no cervical probing, intracranial stimulation to the mesencephalic central

TABLE 8

Table of means for tail-pinch responses with palpation

	Alone	During/cp	Following/cp
Estrogen and Intracranial Stimulation	5.4	5.93	5.38
Estrogen and No Intracranial Stimulation	2.72	4.78	3.44
No Estrogen and Intracranial Stimulation	5.44	5.84	5.5
No Estrogen and No Intracranial Stimulation	2.52	4.41	3.13

FIGURE 5

The effect of cervical probing and palpation on amount of pressure tolerated.

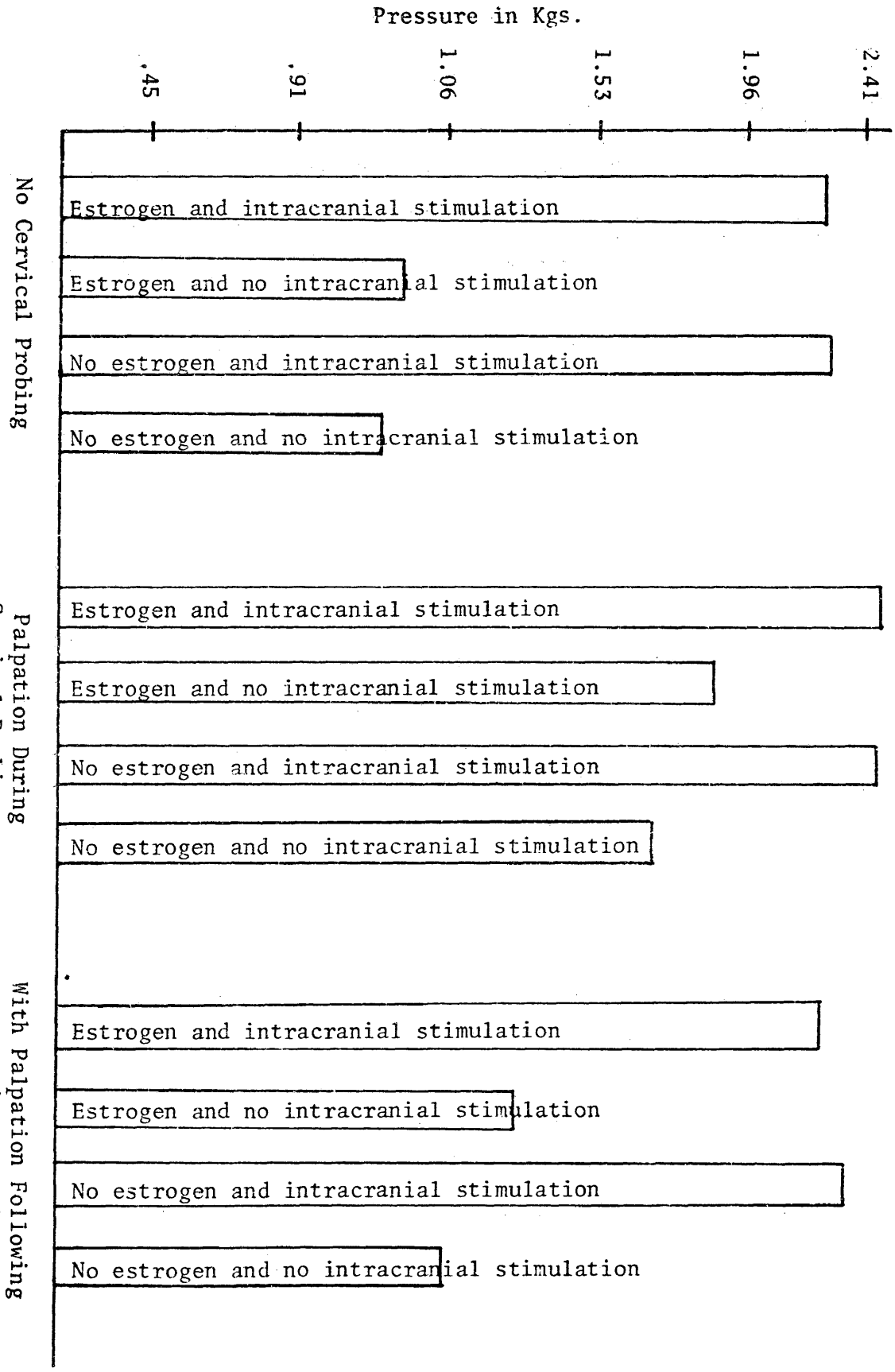
Pressure in Kgs.
.45
.91
1.06
1.53
1.96
2.41
No Cervical Probing
Estrogen and intracranial stimulation
Estrogen and no intracranial stimulation
No estrogen and intracranial stimulation
No estrogen and no intracranial stimulation
Palpation During Cervical Probing
Estrogen and intracranial stimulation
Estrogen and no intracranial stimulation
No estrogen and intracranial stimulation
No estrogen and no intracranial stimulation
With Palpation Following Cervical Probing
Estrogen and intracranial stimulation
Estrogen and no intracranial stimulation
No estrogen and intracranial stimulation
No estrogen and no intracranial stimulation

Figure 5

grey produces the greatest analgesia, while other stimuli in this condition did not produce analgesia.

In Figure 6, the effect of intracranial stimulation is shown as being very strong, and all groups receiving intracranial stimulation to the mesencephalic central grey show analgesia. Therefore, discussion of differences among groups here would be fruitless, since all scores are similar. The reason for this similarity is that subjects for this study were chosen as analgesic by being stimulated intracranially without estrogen, and tested on the tail-pinch. Thus, the effect of intracranial stimulation as being this strong should not be suprising. It must be considered a result of a selection factor, and thus a demonstration of a ceiling effect in this case.

In the absence of intracranial stimulation, cervical probing both with and without estrogen produced the greatest amount of pressure tolerance. Next comes estrogen with palpation following cervical probing, and no estrogen with palpation following cervical probing; these pressure levels are small, however, and do not indicate analgesia. The next greatest amount of pressure tolerance is with estrogen and no cervical probing, the least occurs with no estrogen or cervical probing. Thus, the greatest analgesia is produced by intracranial stimulation to the mesencephalic cental grey regardless of other factors. When intracranial stimulation was not present, cervical probing both with and without estrogen produce analgesia.

The tail-pinch data were analyzed using the same analysis of variance for repeated measures design as used for tail-flick. The independent variables were the same as were employed in the tail-flick design, also making this a 2x2x2 design. In Table 9, these data again show no signi-

FIGURE 6

The effect of intracranial stimulation on amount of pressure tolerated.

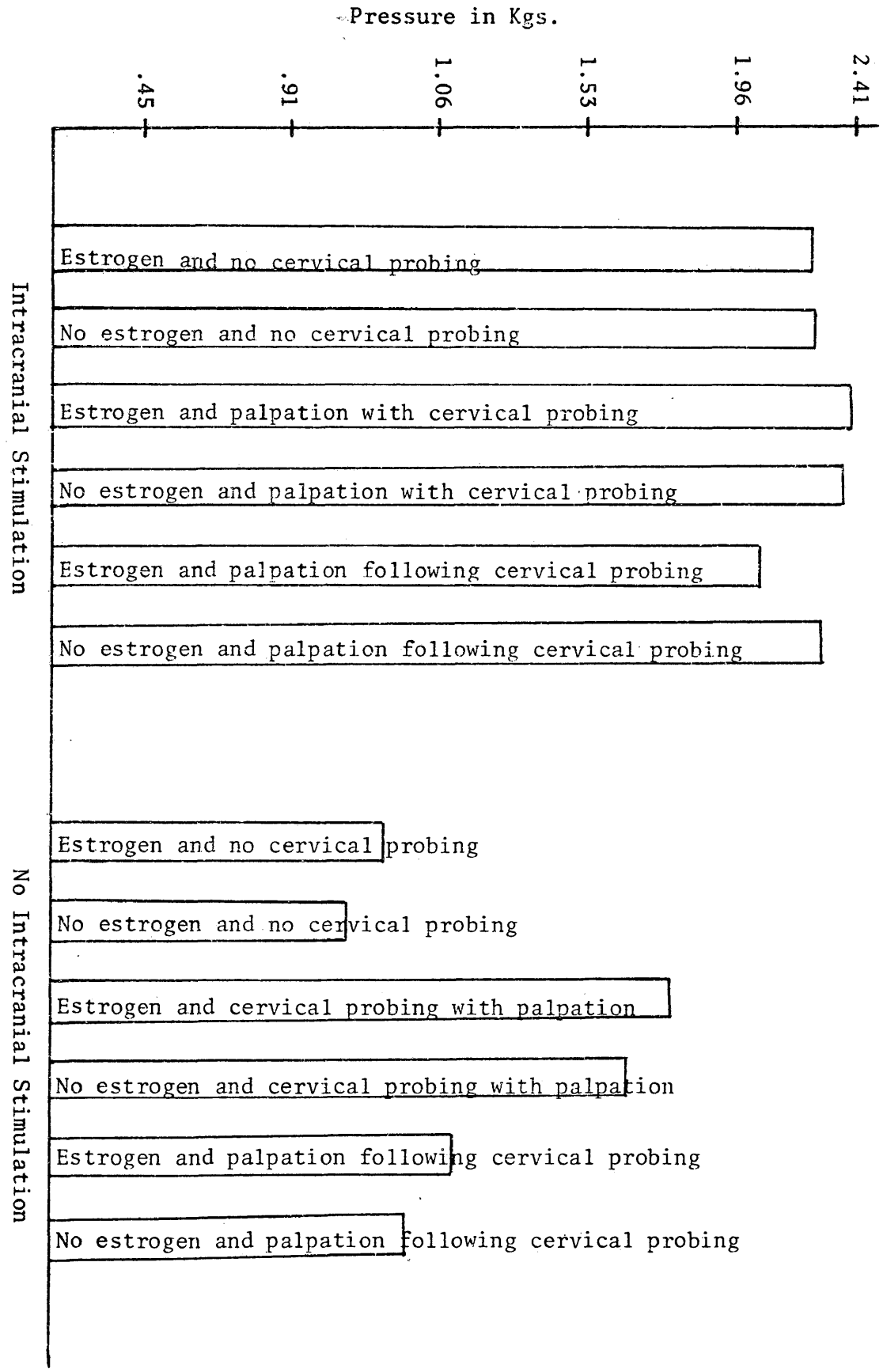
Pressure in Kgs.
.45
.91
1.06
1.53
1.96
2.41
Intracranial Stimulation
Estrogen and no cervical probing
No estrogen and no cervical probing
Estrogen and palpation with cervical probing
No estrogen and palpation with cervical probing
Estrogen and palpation following cervical probing
No estrogen and palpation following cervical probing
No Intracranial Stimulation
Estrogen and no cervical probing
No estrogen and no cervical probing
Estrogen and cervical probing with palpation
No estrogen and cervical probing with palpation
Estrogen and palpation following cervical probing
No estrogen and palpation following cervical probing

Figure 6

TABLE 9

Analysis of variance for tail-pinch response

Source	dF	MS	F
Blocks	15		
Treatments	11		
A (Estrogen)	1	0.79	0.72
B (Cervical Probing)	2	25.18	23.1*
C (Intracranial Stimulation)	1	210.91	193.5*
AXB	2	0.13	0.12
AXC	1	1.33	1.22
BXC	2	9.3	8.5*
AXBXC	2	0.01	0.009
Residual	165	1.09	
Total	191		

ficant effect of estrogen in producing analgesia. Cervical probing produced significant analgesia, as was also the case for intracranial stimulation. Further analysis using a test for simple main effects is presented in Table 10. The effect of intracranial stimulation to the mesencephalic central grey in producing analgesia was effective in both the presence and absence of cervical probing; cervical probing was effective in producing analgesia in the absence of intracranial stimulation. However, in the tail-pinch condition, intracranial stimulation masked all possible effects of cervical probing, producing no visable effect of cervical probing in the presence of intracranial stimulation.

A Tukey's test was performed on differences between palpation during cervical probing, palpation following cervical probing, and no palpation or cervical probing in producing analgesia. A summary of this analysis is presented in Table 11. This table shows that there was a significant difference between subjects receiving palpation during cervical probing and those receiving no cervical probing. There was a significant difference between those subjects receiving palpation during and following cervical probing. There was no significant difference between subjects that received no palpation or cervical probing and those receiving palpation following cervical probing. Subjects receiving palpation following cervical probing were not analgesic.

Thus data gathered from tail-pinch indicates that intracranial stimulation to the mesencephalic central grey produces analgesia, regardless of other conditions, and intracranial stimulation and cervical probing interact to produce analgesia, but it is in part masked by the overpowering effect of intracranial stimulation in producing analgesia in the tail-pinch condition.

TABLE 10

Tests of simple effects for the significant interaction of cervical probing and intracranial stimulation on analgesia

Source	dF	MS	F
B (Cervical Probing)	2	25.18	23.1*
B at C_1	2	2.15	1.97
B at C_2	2	32.335	29.67*
C (Intracranial Stimulation)	1	210.91	193.5*
C at B_1	1	125.72	111.34*
C at B_2	1	27.46	25.19*
C at B_3	1	73.91	67.8*
Residual	165	1.09	

TABLE 11

Tukey's test for differences between means for groups on cervical probing for tail-pinch data

During - After:	Q = 16.92*
During - None:	Q = 20.29*
None - After:	Q = 2.63

DISCUSSION

In summary, estrogen enhanced the lordosis response both in the case of palpation during cervical probing and palpation following cervical probing. Intracranial stimulation to the mesencephalic central grey enhanced the lordosis score when estrogen was absent, but when estrogen was present, the effect of cervical probing and intracranial stimulation on analgesia was essentially the same.

The tail-flick data indicates that cervical probing and intracranial stimulation to the mesencephalic central grey were both effective in producing analgesia but cervical probing produced much longer latencies before the flick than did central grey stimulation. Estrogen was shown to decrease latencies when central gray stimulation was present and increase or not effect latencies without stimulation to the central grey. The effect of palpation in producing analgesia only occured when palpation was paired with cervical probing. Palpation following cervical probing was not effective in producing analgesia.

The tail-pinch data indicated that estrogen alone does not enhance analgesia. Again, the effect of palpation in producing analgesia was only visable when palpation was paired with cervical probing, and analgesia occured to intracranial stimulation. Stimulation of the central grey produced higher pressure tolerance than did cervical probing. The highest scores were obtained from a combination of cervical probing and electrical stimulation to the mesencephalic central grey. Palpation following cervical probing was not effective in producing analgesia.

It was hypothesized that analgesia produced by intracranial stimulation to the mesencephalic central grey, and cervical probing, as well as the effect of the presence of estrogen were causing changes in the same neural nociceptive blocking mechanism that included the mesencephalic central grey as a common part of that mechanism in which these stimuli can work together. If this were the case, the presence of estrogen would be expected to produce higher lordosis scores to cervical probing than if estrogen was not present, and that there would be no difference in scores from subjects receiving palpation with cervical probing than those receiving palpation following cervical probing alone. These results were found, and replicated the work of Pfaff (1971) who found that presence of estrogen increased lordosis response to cervical probing, and of Komisaruk (1974) who found that presence of estrogen increased lordosis to cervical probing, and of Komisaruk (1974) who found that palpation following cervical probing produced lordosis responses similar to those achieved by palpation during direct cervical probing. Analgesia resulted from intracranial stimulation to the mesencephalic central grey, thus replicating the work of Mayer (1974). Cervical probing paired with palpation produced analgesia, thus replicating the work of Komisaruk (1974) and Rosen, Petty, and Westlake (1974).

Evidence for cervical probing and intracranial stimulation to the central grey both effecting the central grey in the same manner comes from the fact that estrogen appears to affect the results of cervical probing and intracranial stimulation in the same way. When estrogen was absent, and cervical probing presented with intracranial stimulation, the resulting analgesia is the greatest. When estrogen is present, and cervical probing presented with and without intracranial stimulation to the

mesencephalic central grey, there is no change in analgesia in the tail-flick condition. This is further supported by the significant interactions of estrogen with intracranial stimulation to the central grey of the mesencephalon and cervical probing with central grey stimulation. Thus, if estrogen affects intracranial stimulation and cervical probing in the same way, and estrogen pools in the central grey (Pfaff, 1971) then it seems likely that both intracranial stimulation and cervical probing may affect the central grey of the mesencephalon in the same manner, thus supporting the hypothesis.

It was proposed by Komisaruk (1974) that palpation following cervical probing increased the effects of previously ignored sexually relevant stimulation. If this is the case, palpation following cervical probing may also effect the mesencephalic central grey, and show a blocking of nociceptive stimulation, as is the case in palpation paired with cervical probing.

The mesencephalic central grey is the most likely site of a facilitation because Mayer (1974) found that intracranial stimulation to the central grey produced a state of analgesia, while similar stimulation to other areas did not produce analgesia. Pfaff (1971) found that radioactively labled estradial benzoate pooled in the central grey of the mesencephalon. He also noted that ascending spinal pathways may be connected by the presence of estrogen in such a way that estrogen could be instrumental in triggering a neural pathway from the lower back to the mesencephalic central grey. Isolated examples of such pathways exist, and it is highly likely that they may be interconnected by the presence of estrogen into one unit that may be activated by stimulation in mating. Pfaff (1971) hypothesized that estrogen may alter the activity of certain selected neurons in the mesencephalic central grey.

Komisaruk (1974) and Rosen, Petty, and Westlake (1974) have shown that cervical probing produced a state of analgesia. Komisaruk hypothesized that analgesia thus produced is caused by changes in activity in the mesencephalic central grey. Petty (1975) has shown that cervical probing inhibits the firing rate of single cells in the mesencephalic central grey of female rats primed with estrogen. She also found that units that were facilitated by nociceptive stimulation were inhibited by cervical probing, and when cervical probing was presented with nociceptive stimulation, inhibition occured in units that had been facilitated previously by nociceptive stimulation alone.

The findings of the present experiment replicate the findings of Mayer (1974), Komisaruk (1974) and Pfaff (1971). Also the findings of this experiment indicate the expected interactions. Thus, facilitation occured between estrogen and intracranial stimulation and also action between cervical probing and intracranial stimulation. When intracranial stimulation to the mesencephalic central grey was absent, the effect of estrogen on cervical probing produced the strongest lordosis score. When intracranial stimulation was present, the effect of estrogen on cervical probing showed no greater lordosis response than intracranial stimulation without estrogen. Thus it seems possible that intracranial stimulation to the central grey is activating some mechanism in the mesencephalic central grey that is triggered in nature by cervical probing to produce analgesia.

The results indicated that the significant interaction occured, but not necessarily under all stimulus conditions. The interactions between estrogen and intracranial stimulation was not significant in the tail-pinch condition, and the interaction between intracranial stimulation and cervical probing was not significant in the tail-flick condition.

Palpation following cervical probing caused no significant differences between palpation without cervical probing and palpation following cervical probing, indicating that palpation following cervical probing did not produce analgesia. Komisaruk (1974) indicated that palpation following cervical probing acted as a trigger that enhanced the effectiveness of later cervical probing, either by a vigorous male, or by probing with a syringe plunger. The findings of this experiment indicated that it may assist in triggering later mating responses, but that it did not play a role in producing analgesia, and considering this, it may be wise to consider palpation following cervical probing as another totally different neural mechanism.

Thus, the findings of this experiment indicate that the interactions between intracranial stimulation to the mesencephalic central grey and estrogen produce analgesia, and that intracranial stimulation and CP interact to produce analgesia similarly. This supports the hypothesis that these may not be separate mechanisms, but that these two types of stimulation may both cause similar changes in the mesencephalic central grey--intracranial stimulation artificially and cervical probing naturally--which causes a blocking of responses to nociceptive stimulation. These findings also indicate that palpation following cervical probing does not produce analgesia, and that the similar effects of palpation following cervical probing to palpation during cervical probing may be due to another different mechanism.

BIBLIOGRAPHY

Diakow, C. D. Of Physiological Psychology. In press.

Komisaruk, B. R. Neural and hormonal interactions in the reproductive behavior of female rats. In W. Montagna, & W. Sadler (Eds.), Reproductive Behavior. New York: Plenum Publishing Corp., 1974, 97-127.

Komisaruk, B. R. Introduction of lordosis in ovariectomized rats by stimulation of the vaginal cervix: Hormonal and neural interrelationships. In C. H. Sawyer, & R. A. Gorski (Eds.), Steroid Hormones and Brain Function. Berkeley, California: University of California Press, 1971, 127-141.

Komisaruk, B. R., & Diakow, C. J. Lordosis reflex intensity in rats in relation to the estrous cycle, ovariectomy, estrogen administration, and mating behavior. Endocrinology, 1973, 93, 548-557.

Komisaruk, B. R., & Wallman, D. Blockage of pain responses in thalamic neurons by mechanical stimulation of the vagina in rats. Program and Abstracts of the Society for Neuroscience, 3rd Annual Meeting, 1973, 315.

Komisaruk, B. R., McDonald, P. E., Whitmayer, D. I., & Sawyer, C. H. Effects of progesterone and sensory stimulation on EEG and neuronal activity in the rat. Experiments in Neurology, 1971, 19, 494-507.

Kuehan, R. E., & Beach, R. A. Quantitative measurements of sexual receptivity in female rats. Behavior, 1963, 21, 282-299.

Mayer, D. J., & Liebeskind, J. C. Pain reduction by focal electrical stimulation of the brain: An anatomical and behavioral analysis.

Brain Research, 1974, 68, 73-93.

Mayer, D. J., Wolfle, T. L., Akil, H., Carder, B., & Liebeskind, J. C. Analgesia from electrical stimulation in the brain stem of the rat. Science, 1971, 174, 1351-1354.

Pelligrino, L. J., & Cushman, A. J. A Stereotaxic Atlas of the Rat Brain. New York: Appleton-Century-Crofts, 1967.

Petty, L. C. Chronic recording of singal unit responses in the mesencephalic central grey to somatosensory, nociceptive, and sexually relevant stimuli in female rats. Unpublished doctoral dissertation.

Pfaff, D. W. Interactions of steroid sex hormones with brain tissue: Studies of uptake and physiological effects. In S. Sigal (Ed.), The Regulation of Mammalian Reproduction. Springfield, Illinois: Charles C. Thomas Publishers, 1972, 8-22.

Pfaff, D. W., & Reiner, M. M. Estradial-concentrating cells in the rat amygdala as part of a limbic-hypothalamic hormone-sensitive system. In B. Eleftheriou (Ed.), Neurobiology of the Amygdala. New York: Oxford University Press, 1973.

Pierce, J. T., & Nuttal, R. L. Self-paced sexual behavior in the female rat. Journal of Comparative Physiological Psychology, 1961, 54, 310-313.

Ramerey, V. P., Komisaruk, B. R., Whitmayer, D. I., & Sawyer, C. H. Effects of hormones and vaginal stimulation of the EEG and hypothalamic units in rats. American Journal of Physiology, 1967, 212, 1376-1384.

Rodriquez-Sierra, J. F., Crowley, W. R., & Komisaruk, B. R. Vaginal stimulation in rats induces prolonges lordosis responsiveness and sexual receptivity. Journal of Comparative Physiological Psychology, 1974. In press.

Rosen, E. F., Petty, L. C., & Westlake, K. C. Effects of sex hormones and cervical probing in responses to nociceptive stimulation. Paper presented at Psychonometric Society Annual Meeting, Boston, Mass., 1974.

VITA

Kathleen Casey Westlake

Born April 5, 1948 in Detroit, Michigan, the author graduated from Northville High School in 1966. She was a science major at Schoolcraft College in Livonia, Michigan with a concentration in chemistry from 1966-1968. In 1968 she transfered to Michigan State University where she was a psychology major. She graduated in 1971 receiving a Bachlor of Science with Honors degree from the College of Social Sciences.

In September, 1974 the author entered the College of William and Mary as a candidate for the Master of Arts degree in Psychology. From September, 1974 to September, 1975 the author was a psychologist intern at Eastern State Hospital. From February, 1975 to September, 1975, she worked exclusively in the Richmond geographic unit of Eastern State Hospital. From September, 1975 to May, 1976 she was a teaching assistant in psychology at the College of William and Mary.