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The Effect of Central ß-Adrenergic Receptor Stimulation by Isoproterenol on Sexual Dimorphisms in the Rat

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ABSTRACT

The Effect of Central ß-Adrenergic Receptor Stimulation by Isoproterenol on Sexual Dimorphisms in the Rat

Dena Davidson

Concordia University, 1990.

It has been proposed that neonatal stimulation of central ß-adrenergic receptors could act to block the defeminizing actions of testosterone (T) in female rats, by blocking the aromatization of T to estradiol, the metabolite considered primarily responsible for defeminization in the developing rat.

Experiment 1 tested this hypothesis by comparing the effects of isoproteronal (ISO) in males, females and females given neonatal, subcutaneous injections of testosterone propionate (TP). Measures included adult female sexual behavior, body weight and various organ weights.

Limited support for the hypothesis was seen in the body weight measure. Isoproterenol injections prevented weight increase in TP-treated females, however, a similar effect was not found in males. A sex difference in response to ISO injections on kidney and heart weights was also found. No effects of ISO were seen on gonad weight. No protective effects of ISO were found on the defeminizing effects of T on female sexual behavior in either male or TP-treated females.

Experiment 2, constituted a replication of Experiment 1, except that isoproteronal injections were given into the cistern magnum. A statistically significant effect of ISO female sexual behavior was seen in the direction predicted by the hypothesis. The effect was small, however, and cannot be considered to be behaviorally significant. There was no effect on body weight or on any of the organ weight measures in Experiment 2.

Taken as a whole, these data do not support the hypothesis that neonatal stimulation of central ß-receptors can significantly affect the course of sexual differentiation.

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In 1968, Dörner, Döcke and Moustafa demonstrated that estradiol (E2) placed within the ventral medial nucleus of the hypothalamus (VMH) in ovariectomized adult female rats, was sufficient to activate lordosis in response to male sexual behavior. The VMH was an area of the brain thought to mediate sexual behavior and to be a target for gonadal hormones. By placing hormone directly into this region they hoped to demonstrate that E2 facilitates lordosis by acting on neural circuits mediating some of the components of female sexual behavior. This approach was typical of early research aimed at determining where in the brain gonadal hormones act and how the consequences of their actions lead to female sexual behavior (Barfield, 1976; Barfield and Chen, 1977; Lisk, 1962; Yanse and Gorski, 1976). The results of these experiments were corroborated by others demonstrating that lesions of the VMH decreased lordosis (Dörner, Döcke and Gotz, 1975; Dörner, Döcke and Hinz, 1969) and that electrical stimulation of the VMH facilitated lordosis in estrogen (E) primed rats (Pfaff and Sakuma, 1979). When autoradiographic techniques were used to map the uptake sites in the central nervous system (CNS) for radioactively labeled E2, the hypothalamus, and particularly the VMH, was found to be rich in E-concentrating cells (Pfaff, 1968; Pfaff and Keiner, 1973). Collectively, the evidence demonstrated that adult female sexual behavior is primed by E2 secreted by the gonads, which acts at least in part, on circuits at the level of the VMH.

In addition to the activational effects of gonadal

hormones, there is evidence that certain neurotransmitter systems might mediate this process. In one of the first experiments to suggest that the monoamines participate in the mediation of adult sexual behavior, Meyerson (1964a) reported that lordosis in female rats was attenuated by monoamine oxidase inhibitors, which increased levels of monoamines in the synapse, but not by reserpine or tetrabenazine which brought about monoamine depletion. Meyerson (1964b) went further and examined the effect of monoamine depletion on lordosis induced by replacement of E and progesterone (P) in ovariectomized rats. He found that the lordosis triggered in these animals through the synergism between E and P (Beach, 1948; Young, 1961), could also be mimicked by the combination of E and reserpine, suggesting that monoamine depletion somehow induced estrous behavior in females primed with E alone. Over the next few years the effects of various pharmacological agents, known to alter monoamine activity, were studied on hormone-induced sexual behavior. Although Meyerson and others continued to find a correlation between increased monoamine activity and suppression of females sexual behavior (Meyerson, 1964c, 1966, 1970; Meyerson and Sawyer, 1968; Meyerson and Lewander, 1970) others found the reverse (Crowley, Feder and Morin, 1976; Foreman and Moss, 1978). Clearly monoamines were somehow involved in the activational effects of gonadal hormones on adult sexual behavior, but the direction of these effects remained uncertain.

A natural progression from the studies of adult sexual

behavior was the search for potential organizational effects by monoamines in the neonate. It was possible that the effects that gonadal steroids are known to have on differentiation of the mechanisms underlying anatomy and later expression of sexual behavior (Pheonix, Goy, Gerall and Young, 1959; Gorski, Gordon, Shyrne and Southam, 1978) were mediated through their effects on the monoamine systems.

Kikuyama (1961) studied the effects of monoamine depletors on the defeminizing actions of the testicular hormone testosterone (T) and its estrogenic metabolite, estrone (E1) in the postnatal period. He injected female rats from postnatal day 4-9 with either testosterone propionate (TP) or E1 and found that they developed persistent estrus following puberty and did not form corpora lutea as did females allowed to develop normally. [In the normal cycling female rat, FSH from the pituitary results in the primary follicle erupting, releasing the ova. The follicle is then transformed into corpus lutea which in turn produces P and E in preparation for implantation of the ova into the uterine wall. If fertilization does not take place the corpus lutea diminishes in size and hormonal activity. In defeminized females (females exposed to testicular hormones or their estrogenic metabolites, during the perinatal period), this feedback loop is disrupted and corpus lutea are not formed. Furthermore, rather than cycling through the usual 4 stages (proestrus, estrus, dioestrus, metoestrus), these females maintain a state of

persistent estrus which can be readily identified by viewing the cornification of the cells extracted from vaginal smears (Long and Evans, 1922; Grönroos and Kauppila, 1959).] Surprisingly, when females received injections of the monoamine depleting drug reserpine, on days 4 through 8, or on alternate days 4,6, and 8, in addition to either TP or E1, persistent estrus was blocked, and corpora lutea formation proceeded as normal. Reserpine had no effect on these measures when administered alone. Thus, Kikuyama concluded that when monoamines were depleted, females were somehow protected from the defeminizing effects of TP and E1. Not all forthcoming data, however, were consistent with this hypothesis.

For example, Dörner, Hinz, Döcke and Tonjes (1977) treated female rats during the neonatal period with pargyline, a monamine oxidase inhibitor which prolongs the presence of monoamines in the synapse. Pargyline-treated females showed precocious puberty, and following ovariectomy and hormone replacement, no change in female sexual behavior compared to controls. When tested for male sexual behavior, these females showed diminished male mounting behavior after androgen treatment. Conversely, neonatal injections of reserpine delayed puberty in females and disrupted ovarian cycles such that there were reductions in periods of estrus and prolonged periods of dioestrus. These findings suggested that increases in monoamines facilitated female development whereas monoamine reduction interfered with this process even in the absence of T.

Lehtinen, Hyyppä and Lampinen (1972) found effects in a similar direction. Male and female rats were given a single injection of reserpine on the 4th day after birth, and their sexual behavior was tested at maturity. Tests of spontaneous female sexual behavior revealed that compared to normal females, reserpinized females showed a reduction in receptivity towards stud males and, a disruption of their estrous cycles. In tests for spontaneous sexual behavior in males, reserpinized animals needed fewer intromissions to ejaculate than the controls. Contrary to what Kikuyama's hypothesis might predict, reserpine-induced monoamine depletion appeared to defeminize physiological development and sexual behavior in females and hyper-masculinize males.

Although these early studies indicated that monoamines in were involved the organizational actions of gonadal hormones on the brain and the expression of sexual behavior, the reported direction of these effects were often contradictory. Furthermore, they specified neither the neurotransmitter involved nor the nature of this interaction (Dörner, Hinz, Döcke and Tonjes, 1977; Kikuyama, 1962; Kawashima, 1964; Reznikov, Nosenko and Demkiv, 1979). More recent studies, however, utilizing more selective pharmacological tools have found evidence indicating that noradrenergic systems have an important role in the organizing actions of gonadal hormones.

A key experiment by Heritage, Stumpf, Sar and Grant (1980) has provided anatomical evidence that gonadal hormones act directly on catecholaminergic systems. Using a

formaldehyde-induced fluorescence autoradiographic technique that locates catecholamine (CA) and gonadal steroid receptors simultaneously, they demonstrated a morphologic relation between steroid hormone target sites and CA neurons. Such evidence is critical to explaining how and where these physiological interactions take place. Heritage et al studied the uptake of the androgen receptor binding hormone, 5α-dihydrotestosterone and the estrogen receptor binding hormone 17ß-estradiol (E2). They found that the localization patterns of these hormones were similar for both sexes, but varied between the two sex steroids. Nevertheless, they found that sex steroids were concentrated in the nuclei of CA neurons suggesting that steroids could directly influence CA neurons by exerting genomic effects at nuclear sites. Second, they reported that some of the target cells of steroid hormones were surrounded by CA terminals, indicating that CA systems may influence these neurons via their innervation. More specifically, the CA cell bodies which concentrate E2, were found to be noradrenergic (Heritage et al, 1978). It appears, therefore, that norepinephrine (NE) can act on E2 concentrating neurons, and that NE neurons can themselves be the target of E2 actions. This relation between gonadal hormones and NE suggests that the noradrenergic system maybe a system involved in the organizational actions of gonadal hormones. The localization of gonadal hormones in the nuclei of noradrenergic neurons may provide clues as to how NE is involved in the sexual differentiation of the

developing brain.

Before the actions of NE in the neonatal rat brain can be considered, it is necessary to establish the age at which these systems are in place and to verify their presence in brain tissues known to be involved in sexual behavior, i.e., the hypothalamus.

Noradrenergic Systems

The perikarya of the locus coeruleus (LC) and the lateral tegmental nuclei (LT) are located in the general area of the lower brain stem, and give rise to the noradrenergic systems (Moore and Card, 1984). In the rat embryo, these cells are detected by fluorescence microscopy as early as 13 days of gestation and neurons are detected 1-2 days later (Schlumpf, Lichtensteiger, Shoemaker and Bloom, 1980)). Proliferation of these neurons occurs rapidly between 14-16 days gestation.

In the mature rat brain, axons of the LC , which are the largest group of NE-containing neurons and the major source of NE into the CNS, (Dahström and Fuxe, 1964; Grzanna and Molliver, 1980; Swanson, 1976), project to the spinal cord, cerebellum, thalamus, hippocampus, much of the forebrain, the entire cerebral cortex, and to a lesser extent, the hypothalamus (Moore and Card, 1984; Weiner and Molinoff, 1989). The LT system sends fibres to the brainstem, spinal cord, and hypothalamus (Moore and Card, 1984; Weiner and Molinoff, 1989).

The development of NE neurons in the hypothalamus precedes that of all other monoamines neurons in the CNS.

They can be detected as early as the 12th day of gestation and at that time already show signs of cytodifferentiation (Schlumpf, Lichtensteiger, Shoemaker and Bloom, 1980).

Noradrenergic Receptors

Noradrenergic receptors were first identified by Ahlquiest (1948) as consisting of two types: α and ß. Beta-adrenergic receptors have since been subclassified into ß1 and ß2 (Lands, Luduena and Buzzo, 1967). Both are found on postsynaptic cells (Langer, 1974; Berthelsen and Pettinger, 1977) and are largely concentrated in areas innervated by the LC (Moore and Bloom, 1979). This subdivision is based solely on pharmacological data for although ß1-adrenergic receptors predominate in the heart and forebrain, and ß2-adrenergic receptors predominate in the lung and cerebellum, in the brain, they have not been differentiated in terms of their physiological function; biochemically, both subtypes appear to active adenylate cyclase in response to stimulation by NE (Lefkowitz, Caron, Michel and Stadel, 1982; Minnemann, Pittman and Molinoff, 1981; Weiner and Molinoff, 1989).

Beta-adrenergic binding sites in the rat forebrain are detected by gestational day 15. This period coincides with the proliferation of NE neurons mentioned previously, and the time when NE and the necessary enzymes for its production, tyrosine hydroxylase and dopamine ß-hydroxylase, have been measured (Coyle and Axelrod, 1972a; Coyle and Axelrod, 1972b; Coyle and Henry, 1973). In the adult hypothalamus ß-adrenergic receptor density is generally

moderate (Bylund and Snyder, 1976; Palacios and Wamsley, 1984).

Subclassification of α-adrenergic receptors has also taken place, however, the differentiation is less clear. Initially, subtypes were based on the putative anatomical localization of receptors suggesting that α1-adrenergic receptors were always found postsynaptically whereas, α2-adrenergic receptors, which act as autoreceptors, were located presynaptically (Starke, 1977; Stone, 1987). It now seems that although all presynaptic adrenergic receptors are α2 autoreceptors, there are many examples of receptors which are not presynaptic but have the same pharmacological properties (Bylund, 1985).

Alpha 1 and α2-adrenergic receptors have been found in greatest densities in tissues innervated by the LT; exceptions include the neocortex and hippocampus which are innervated by LC neurons yet contain significant densities of α1 and α2-adrenergic receptors (Palacios and Wamsley, 1984). Alpha 1-adrenergic receptors potentiate the effects of NE-induced β-adrenergic receptor stimulation by acting on the biochemical processes that modulate adenylate cyclase activity after the enzyme has been activated (Daly, Padgett, Creveling, Cantacuzene and Kirk, 1980; Leblanc and Ciarenello, 1984).

Postnatal development of the NE system

Guillamón, de Blas and Segovia (1988) reported a sexual dimorphism in the volume and number of neurons in the LC of Wistar rats. The LC of 3 month old female rats was found to

have significantly greater volume and a greater number of neurons than that of normal males. Interestingly, the LC of females given a single injection of TP at birth, did not differ from that of normal males; it too had less volume and fewer neurons than the LC of normal females. Postnatal gonadectomy of the males did not however, alter LC volume, suggesting that prenatal exposure to testicular hormones may be sufficient to determine cell number and size. Another observation linking NE systems to the effects of perinatal testicular hormones is that of Vaccari, (1980) who found that NE inhibits aromatase in hypothalamic cell cultures Aromatase is the enzyme that converts T to E2, the metabolite considered responsible for many of the defeminizing actions of T in the rat brain. Furthermore, Raum and Swerdloff (1981) reported that ß-adrenergic receptor stimulation interferes with the action of T in defeminizing the hypothalamus. Therefore, it is not surprising to find that the hypothalamus of the neonatal female rat brain should be rich in noradrenergic innervation, which, in theory could protect it from defeminization by circulating aromatizable androgens from their own adrenals or from T secreted by their male siblings in utero.

More recently, investigators have exploited new developments in pharmacology to further examine the role of NE systems of the rat brain and T-induced defeminization of the female rat. For example, Raum and Swerdloff (1981) injected agents known to act on the NE system, into the

lateral ventricle of 4 day old female, Wistar rats, some of which were pretreated with 25 µg TP. These drugs led to selective increases or decreases in NE available to stimulate noradrenergic receptors (see Table 1).

Alpha-methyl-tyrosine methyl ester (AMPT) (50 µg) an inhibitor of tyrosine hydroxylase, the rate-limiting enzyme for NE synthesis, results in a rapid and sustained depletion in neuronal NE without affecting the monoamine serotonin. Phentolamine (50 µg) and phenoxybenzamine (25 µg), both nonselective α-receptor antagonists, act at the postsynaptic autoreceptor, resulting in an increase in NE release. Propranolol (50 µg) is a ß-adrenergic receptor antagonist. Tyramine (100 µg) causes a rapid and short term release of NE which would stimulate all adrenergic receptors.

The effect of these drugs on defeminization was measured by monitoring the age of vaginal opening, incidence of persistent estrus, and corpora lutea formation in mature females. In the strain they used, the average age of vaginal opening was 34 days. Neonatal injections with T is known to delay vaginal opening, therefore, if the pharmacological actions of the drugs interfered with defeminization, one would expect the age of vaginal opening to occur as normal.

The authors found that females treated with any of the drugs alone did not differ in development from their vehicle-treated controls. TP-treated females were, as expected, defeminized, whereas females pretreated with TP and either α blocker, were normal on both measures; the

Table 1: The Effects and Consequences of Various Agents Interacting with Adrenergic Systems

Drug	Effect on α & ß-adrenergic receptors
AMPT	NE synthesis inhibitor; reduces NE stimulation at both α & ß receptors
Phenoxybenzamine & Phentolamine	nonselective α receptor blockers; increases the amount of NE in the synapse due to α2 blockade, results in an increased stimulation of ß-receptors
Propranolol	ß-adrenergic receptor antagonist
Tyramine	causes a rapid release of NE which will act at all adrenergic receptors
Isoproterenol	a potent ß-receptor agonist

(Modified from Raum and Swerdloff, 1981)

persistent estrus and the day of vaginal opening. They explain this effect of the α antagonist by suggesting that because the α-adrenergic receptors (both the postsynaptic and the autoreceptors) are blocked there is more NE released and more available to act at the ß-adrenergic receptor. Interestingly, this blocking action of the α-adrenergic receptor antagonists on the defeminizing actions of TP was reversed when TP and either of the α-adrenergic receptor antagonists was administered together with the ß-adrenergic receptor antagonist, propranolol. The authors proposed that the pharmacologically induced NE release resulted in ß-adrenergic receptor stimulation which in turn blocked TP-induced defeminization, an effect that may be due to the prevention of aromatization of T to E2 (Vaccaro, Canick, Livingston, Fox, Ryan and Leeman, 1980). Thus, when ß-adrenergic receptor stimulation is blocked, as is the case with propranolol, defeminization by TP proceeds as expected.

In support of this latter interpretation, tyramine was also found to block the defeminizing action of TP. Tyramine causes a rapid burst of NE transmission which would have the same effect as autoreceptor blockade in that additional stimulation of ß-adrenergic receptors by NE would occur. If Raum and Swerdloff's hypothesis is correct, then it would follow that blockade of NE secretion should not interfere with defeminization by TP. This proved to be the case. When AMPT and TP were administered there was an increase in the number of females in persistent estrus, 81% compared to

69% of the females given TP alone.

To test the hypothesis that ß-adrenergic receptor stimulation prevents the defeminizing actions of T on the brain by reducing the nuclear accumulation of E2 derived from the aromatization of T, a series of experiments was performed (Raum, Marcana and Swerdloff, 1984). At 4 days of age, female pups were injected intracerebrally with various agents known to act on noradrenergic receptors. Animals were killed shortly afterwards and T, and its metabolites were extracted from hypothalamic nuclear pellets and separated from one another with thin-layer chromatography and/or Celite chromatography. It was found that phenoxybenzamine inhibited nuclear accumulation of E2 converted from T. Recall that phenoxybenzamine blocks α1 and α2-adrenergic receptors increasing activity of NE at the ß-adrenergic receptors. The aromatase inhibitor, 1,4,6-androstratrien-3,17 dione (ATD), provided the greatest reduction in conversion. Isoproterenol (ISO), a potent ß-adrenergic receptor agonist, also caused a significant reduction in accumulation of E2 in the nuclear fraction and interestingly, its mode of action was consistent with the actions of ATD (Lieberburg, Wallach, and McEwen, 1977; Christensen and Clemens, 1975). When the ß-adrenergic receptor antagonist hydroxybenzylpindolol (HBP), was given in conjunction with either ISO or the longer lasting ß-adrenergic receptor agonist isoxsurpine (ISX), it was found that HBP reversed the inhibition of E2 nuclear accumulation induced by both the ISO and ISX, suggesting that these

compounds inhibit accumulation through a specific ß-adrenergic mechanism.

Thus, these experiments using ISO and ISX suggest that the mechanism by which the nuclear accumulation of E2 converted from T is prevented, is via the ß-adrenergic receptor. However, it was still unclear how the actions of phenoxybenzamine produced the same effects. Since, the ß-adrenergic receptor antagonist HBP was able to reverse the inhibition of E2 nuclear accumulation by the ß-adrenergic receptor agonists and by phenoxybenzamine, it seems plausible that the phenoxybenzamine acted as previously suggested, to make more NE available at the ß-adrenergic receptor.

Although Raum and Swerdloff provide persuasive, if somewhat indirect, evidence that NE stimulation of ß-adrenergic receptors prevents the androgenization of the female rat brain by E2 aromatized from T, a similar study by Jarzab, Sickmoller, Geerlings and Dohler (1987) produced data conflicting with this interpretation. They injected female, Sprague-Dawley rats on days 1-6 of life, with either the α1-adrenergic receptor antagonist prazosin (25 µg/day), the α2-adrenergic receptor agonist clonidine (.25 µg/day) or the α2-adrenergic receptor antagonist yohimbine (6.25 µg/day). On day 4 through day 6, these doses were doubled, and were again doubled on day 7. Some of the animals in each drug condition also received 20 µg TP during the first 5 days of life. During development vaginal smears were taken to determine whether the females were in persistent

estrus, and the day of vaginal opening was also noted. At 80 days, animals were ovariectomized and their ovaries were inspected for presence or absence of corpora lutea. After recovery, some females were brought into estrus and female sexual behavior was tested by the extent of the lordotic response to a mount by a stimulus male. The remainder of the females received silastic implants of TP and were tested for male sexual behavior with a receptive female.

Females pretreated postnatally with prazosin, clonidine and yohimbine alone, exhibited normal estrous cycles and their ovaries contained corpora lutea. The T-induced anovulatory syndrome was not prevented by any of these adrenergic drugs and in these animals ovaries weighed less than those of normal females and contained no corpora lutea.

In tests of female sexual behavior the administration of either prazosin or yohimbine alone had no effect on adult lordosis behavior. Clonidine, however, decreased the displays of lordosis compared to control animals. In TP-treated groups females showed a reduced capacity for lordosis, that was not attenuated by yohimbine, whereas, prazosin-treated animals were protected from defeminization. Animals which received clonidine and TP showed normal lordosis behavior which was interpreted by the authors as a cancelation effect on the defeminizing actions of these two agents.

Tests of male sexual behavior failed to reveal any differences between the groups with the exception of a slight elongation of latency to mount in females treated

postnatally with prazosin and TP.

The prazosin blockade of defeminization by TP is similar to the affect seen with phenoxybenzamine and phentolamine reported by Raum and Swerdloff (1981). Prazosin acts to increase the amount of NE available to act at the ß-adrenergic receptors through its blockade of the α1-adrenergic receptors. However, unlike phenoxybenzamine and phentolamine, prazosin does not block the autoreceptor. Thus, although there is no increase in the amount of NE in the synapse, whatever was available was free to act at the ß-adrenergic receptors.

Clonidine, which should lead to reduced NE stimulation of both α and ß-adrenergic postsynaptic receptors by acting at the autoreceptor to decrease NE release, was also able to block defeminization in animals that had received TP. This finding is contrary to that of Raum and Swerdloff who found that the blockade of defeminization by TP required ß-adrenergic receptor stimulation. This discrepancy may be explained by the way clonidine is believed to act. Although clonidine is known to be a centrally acting α2-adrenergic receptor agonist, it is only a partial agonist and has been reported to have antagonist properties (Goodman, Gillman, Rall and Murad, 1985) and therefore would not completely block NE release. It is more difficult to explain the finding that clonidine alone resulted in a reduction in the capacity for lordosis behavior. This suggests a possible defeminizing role for decreases in NE. The effect of yohimbine and TP on lordosis behavior is also inconsistent

with the hypothesis of Raum and Swerdloff. The α2-adrenergic receptor antagonist increases NE secretion by its blockade of the autoreceptors but, unlike tyramine, which also increases NE yet blocked androgenization, TP induced defeminization was not prevented.

To interpret their data, the authors propose that the developing rat brain is undifferentiated and for either a male or female brain to develop steroid hormones must be present. They concur with Raum and Swerdloff (1981) that ß-adrenergic receptor activation by NE mediates feminization, however, they propose that it does so by causing a presumably undifferentiated rat brain to develop into a female rat brain (feminization). Furthermore, they postulate that stimulation of the α1-adrenergic receptor contributes to the TP-induced defeminization. This conclusion is based on the findings with clonidine and prazosin in females pretreated with TP. These drugs have the identical effect of preventing stimulation of α1-adrenergic receptors, but although clonidine also prevents NE stimulation of ß-adrenergic receptors, prazosin does not. Further, although NE is able to act on ß-adrenergic receptors in prazosin-treated females, both prazosin and clonidine effectively attenuated the defeminizing effects of TP. They propose in contradiction to Raum and Swerdloff, that it was the interference with α1-adrenergic receptor transmission that protected the brain from defeminization by TP.

Although, the results from the Jarzab et al study and

the Raum and Swerdloff study are not easily reconciled, it is important to note that different dependent measures were used. Raum and Swerdloff did not test their animals for adult sexual behavior. In the Jarzab et al study, only females were tested and an equally useful test of the hypothesis would have been to test males. Thus, the purpose of the present study was to further explore the hypothesis that ß-adrenergic receptor activation plays a role in organizing those parts of the brain which are known to mediate sexual behavior in the mature organism. The effects of the ß-adrenergic receptor agonist ISO on physiology and behavior was examined. If the Raum and Swerdloff hypothesis is correct and the function of ß-adrenergic receptors is to attenuate the effects of T, then one would predict that females neonatally treated with ISO and TP should be more similar to normal females than to normal males. Similarly, neonatal males treated with ISO, should be more like normal females.

The proposed protecting effect of neonatal stimulation of ß-adrenergic receptors against T, was also tested in the open field. It has been reported that normal females are more active in the open field than normal males, and when neonatal injections of TP have been administered to females, the sex difference typically seen in adulthood is abolished (Broadhurst, 1957, 1958; Gray, Lean and Keynes, 1969). Therefore, if ß-adrenergic receptor stimulation protects against the defeminization by T, this effect should be measured in the open field.

Experiment 1

The effective dose of ISO needed to act on the CNS of a rat pup was unknown at the time of this experiment, therefore, four doses of the drug where chosen based on doses used for similar compounds and from doses of ISO which have been administered to adult rats. Due to the large numbers of animals required for this study the experiment was run in two replications. Animals from each dose and group were represented in both experiments. All behavior testing and measurements were taken at the same ages with the exception of male sexual behavior which was conducted in considerably older animals in the first run.

Method

Subjects.

Ninety-five, Sprague-Dawley rats were born in the laboratory, at Concordia University, to females purchased from Charles River (Willmington, Mass.). Dams were individually housed in plastic breeding cages (Allentown Caging Equipment, Allentown, NJ) and were maintained in a temperature and humidity controlled environment on a 12 h light-dark cycle. Dams were fed laboratory rat chow (Charles River, Canada) and tap water ad libitum. At birth, litters were sexed, culled and randomly reassigned to dams to provide a maximum of five male and five females per dam. Pups were assigned to three groups; male, female or TP-female (females injected with testosterone propionate). Animals from each group were then assigned to one of four doses of ISO; saline vehicle only (SAL), 40 µg l-

isoproterenol HCL (Sigma, St Louis, MO) (I40), 80 µg ISO (I80), and 160 µg ISO (I160) Each dam cared for groups assigned to one dose to prevent the transmission of the drug through skin absorption or from the metabolites in the feces. Initially, there were 10 animals assigned to each dose, however during the course of the study some animals died. Due to the low mortality rate and the random pattern, deaths could not be attributed to the ISO-treatments.

Procedure

Neonatal Treatments. At the time of injection, the dam was removed from her litter prior to removal of the pups. Isoproterenol was dissolved in sterile physiological SAL and subcutaneously (s.c.) injected in .05 ml volume. Pups were injected daily during the first 6 days of life with day 1 representing the day of birth. On the 6th day, doses for the I40 and I80 animals were doubled. TP females were injected on days 1, 3, and 5 with 50 µg of TP (Sigma, St Louis, MO) dissolved in a volume of .05 ml peanut oil. Control females were given injections of the oil vehicle only. All injection sites were sealed with collodion to prevent leakage of the materials (Fisher Scientific, Fairlawn, NJ).

Pups were weaned at 21 days of age and were moved to standard wire mesh cages three days later. Food and water were provided ad libitum. The animals were housed 2 or 3 to a cage until they were 30 days old when they were transferred to individual housing. Two weeks prior to tests of sexual behavior, the animals were moved to a reverse

cycle room. Weights were recorded at the ages of 21, 50, and 90 days.

Female Sexual Behavior Tests. At 75 days of age, all animals were gonadectomized while under methoxyflurane anesthetic (Metofane, Pitman-Moore Ltd./M.T.C. Pharmaceuticals, Mississauga, Ont) for approximately 6-10 min. Gonad weight was recorded at that time. Following surgery, all animals received an injection of .05 ml penicillin (Ayercillin, Ayerst, Canada); they were also given 10 µg of estradiol benzoate (EB, Sigma) in preparation for the tests of female sexual behavior.

Two weeks after surgery, animals were tested for female sexual behavior with vigorously mounting stud males. The stud males received three weeks of sexual experience with receptive females prior to their use in this experiment. Sexual receptivity was induced in the test animals by s.c. injections of 10 µg EB / 0.1 ml peanut oil, 24 h before, and 0.5 mg P (Sigma, St Louis, MO) in 0.2 ml peanut oil, 4 h before testing. All tests were conducted between 14:00-17:00 h during the dark phase of the diurnal cycle. Tests were conducted in semicircular mating arenas (61 cm in diameter x 36 cm deep). Each arena had a plywood floor covered with 1-2 cm of bedding, a Plexiglas front and a concave tin back and was dimly lit with a 7 watt red light bulb. A red-light sensitive camera (Panasonic CCTV camera, model WV-1460), and a video cassette recorder (Sony Betamax VCR, model SLO-420 or SL-HFR30) were used to record the sessions for future scoring. The mating arenas and video

equipment were used in subsequent experiments.

A stud male was placed into each of three arenas and allowed 5 min to adapt. Test animals were then introduced and remained in the arena until the stud male attempted to mount the female on five successive occasions. A mount was acknowledged if the stud male mounted the test animal from the rear and displayed a number of rapid pelvic thrusts. If the stud male ceased attempting to mount, as was often the case when control males were being tested for female sexual behavior, it was replaced after 10 min. If a test rat was not successfully mounted by 3 different stimulus males, it was given a score of 0.

Lordosis behavior was ranked on a scale of 0 to 3, where "0" represented no lordosis, "1" a very weak and short lasting arching of the back, "2" back arched with tail to the side, head erect but without the posture being held, and "3" full lordosis with the posture being held for several seconds after dismount. Each animal was tested once during each of 2 consecutive weeks of testing.

Male Sexual Behavior Tests. At 260 days of age, all animals were tested for male sexual behavior in the first run; however, in the second replication, animals were tested at 125 days of age. All animals were tested following injections of 500 µg TP in 0.1 ml peanut oil (Sigma) given every 2nd day for 2 weeks. On the test day the test animal was placed into the arena and allowed 5 min to adapt before a receptive stimulus female was introduced. Sexual receptivity was induced in the stimulus females by

the same injection series of EB and P described in the previous section. The receptive stimulus female was left with the test animal for 30 min. The session was taped for later scoring of the number of mounts, intromissions and ejaculations by the test animal.

Post mortem organ measures. Animals from each dose condition were killed by rapid decapitation after which, hearts and kidneys were removed, trimmed and weighed in pairs.

Results

Statistical Analysis.

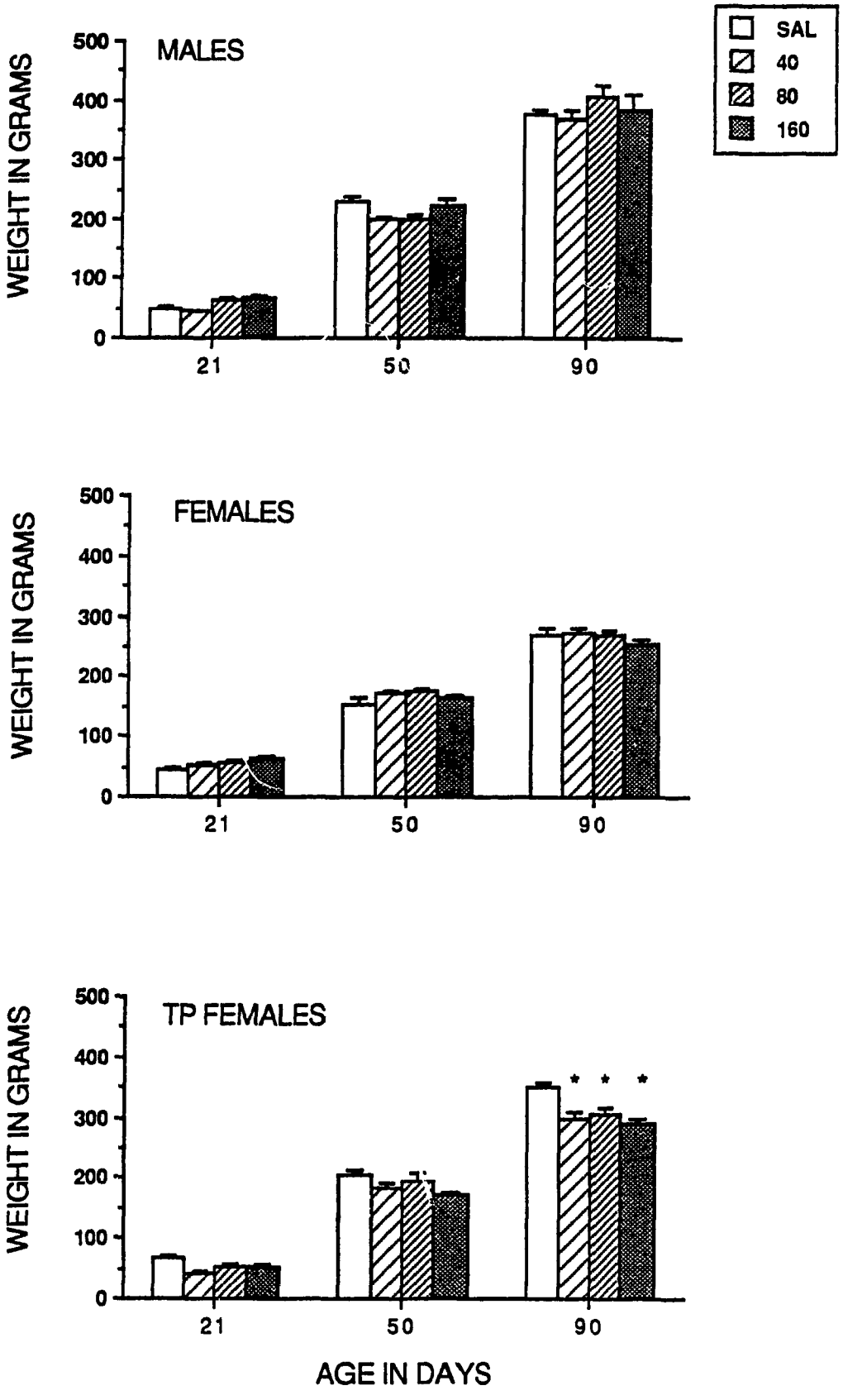
In order to assess the effects of ISO-treatment on body weight and female sexual behavior, 2 three-way split plot analyses of variance were performed; group x dose x age and group x dose x test day, respectively. The effect of ISO-treatment on ovarian and organ weights were analyzed with two-way analyses of variance; group x dose. Post hoc comparisons were made using the Tukey-Kramer procedure (Kirk, 1982).

Body Weight.

The analysis of variance carried out on body weight yielded a group effect, $F(2,85)=72.66, p<.0001$, an age effect, $F(4,170)=3976.8, p<.0001$ and a group x age interaction, $F(4,170)=68.09, p<.0001$. These effects are illustrated in Figure 1, and it can be seen that differences between groups become greater as the animals aged. By 90 days of age, the sex difference in body weight was apparent; females were lightest, males heaviest and TP-females fell

Figure 1. Mean (± 1 S.E.M.) body weight in grams for males, females and TP-females at 21, 50 and 90 days of age. * are significantly lighter than SAL-TP females ($p<.05$).

BODY WEIGHT


MALES
FEMALES
TP FEMALES
WEIGHT IN GRAMS
500
400
300
200
100
0
21
50
90
AGE IN DAYS
SAL
40
80
160
*
*
*

between, showing the expected effects of neonatal TP-treatment on body weight. A significant effect of ISO-treatment was indicated by the group x dose x age interaction, $F(12,170)=2.83, p<.05$. This indicates that over days not all groups were affected in the same way by ISO-treatment. Post hoc comparisons revealed that in TP-females, all three doses of ISO significantly suppressed weight at 90 days of age ($p<.05$).

Gonad Weight.

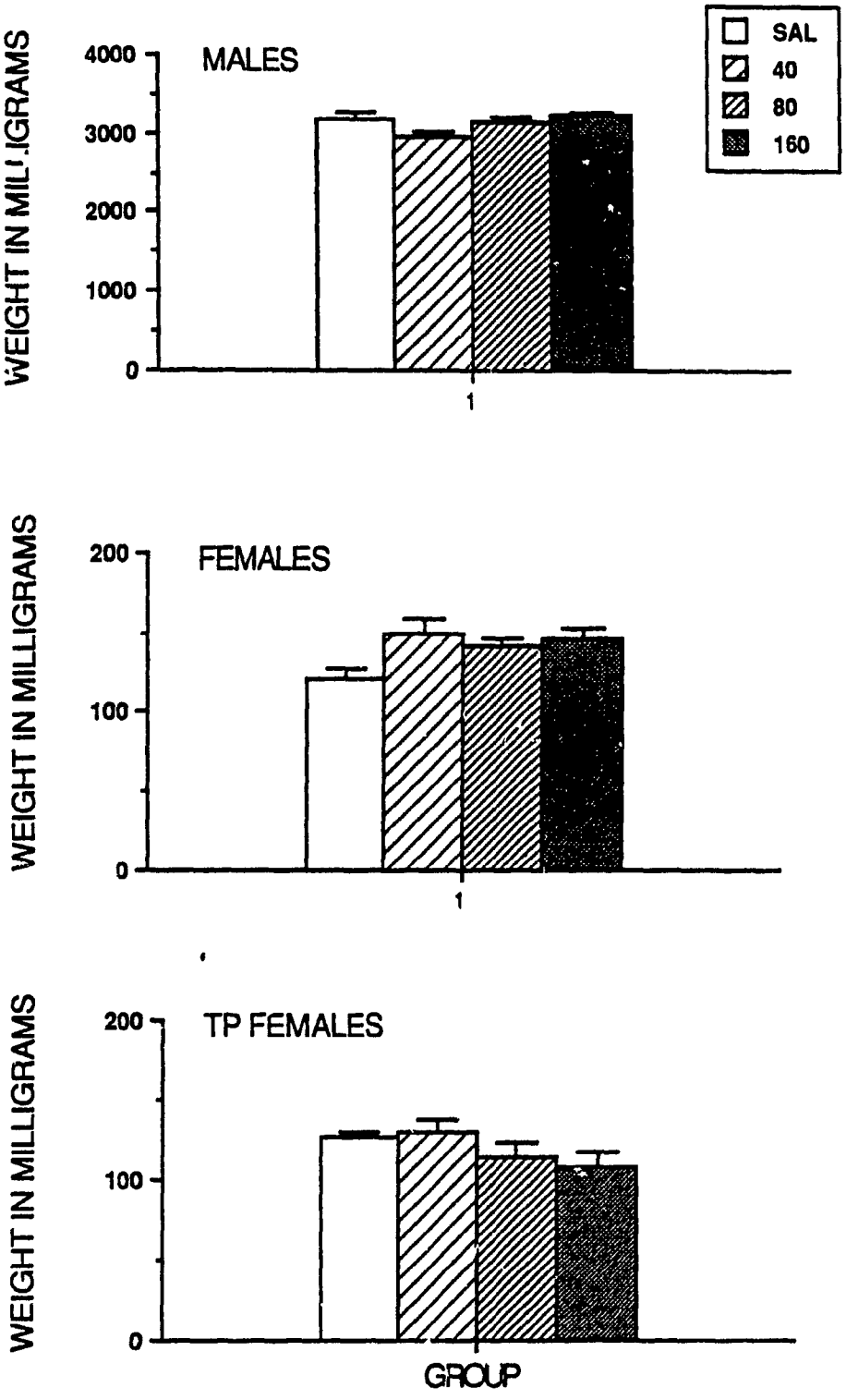
Gonads were trimmed and weighed in pairs. The mean weight for each group are shown in Figure 2. The effects of ISO-treatment on testes weight were analyzed with a one-way analysis of variance. There was a small but significant effect of dose, $F(3,28)=3.07, p<.04$, indicating that weights varied slightly with dose, however, post hoc comparisons were not significant. The effects of ISO-treatment on ovarian weights for females and TP-treated females were analyzed with a two-way analysis of variance. There was a main effect of group, $F(1,58)=14.95, p<.0003$ reflecting the overall lower ovarian weights in TP-females. A group x dose interaction, $F(3,58)=2.99, p<.04$, showed that ISO-treatment increased ovarian weight in females and slightly depressed it in TP-females. Post hoc comparisons were not significant, however.

Heart Weight.

Analysis of variance yielded a significant main effect of group, $F(2,80)=10.47, p<.0001$. The direction of the weights were different in each group and are not easily

Figure 2. Mean (±1 S.E.M.) gonadal weight in milligrams for males, females and TP-females.

GONAD WEIGHT



interpretable (see Figure 3). ISO-treatment had a significant effect on heart weight; dose, $F(3,80)=3.31, p<.02$, and group x dose $F(6,80)=10.72, p<.0001$. Heart weights varied with dose and post hoc analysis showed that I80 and I160-males were significantly lighter than the hearts of SAL-male ($p<.05$). Conversely, ISO-treatment increased heart weight at all doses in females, and at 80 and 160 µg, the increase was significantly different from that of SAL-females ($p<.05$). There was no significant effect of ISO on TP-females, however it is interesting to note that the pattern of ISO-treatment on heart weight was more similar to that seen in males.

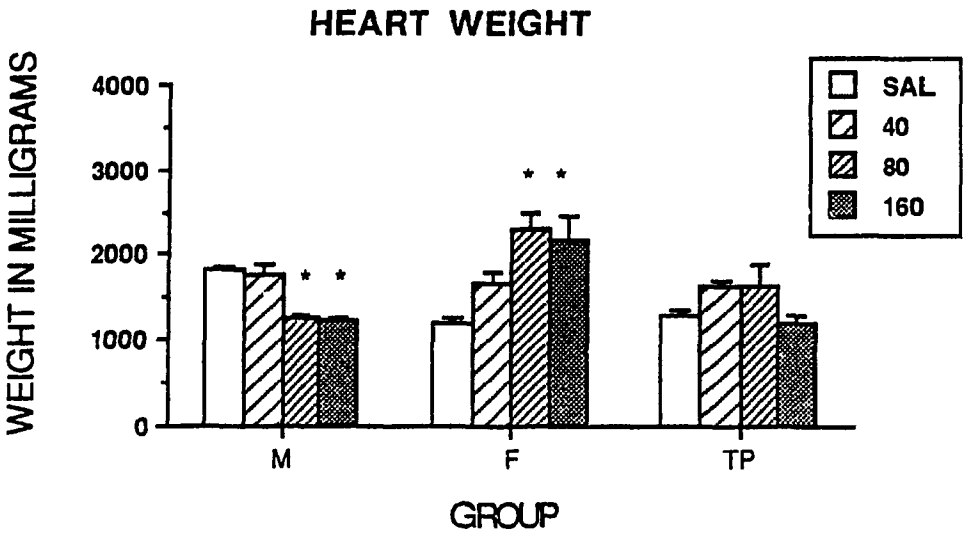
Kidney Weight.

Analysis of variance on kidney weight yielded a main effect of group, $F(2,80)=69.18, p<.0001$. This arises from the fact that kidneys were lighter in females than in males or TP-females. An effect of ISO-treatment on kidney weight was also seen; dose, $F(3,80)=9.97, p<.0001$. It can be seen in Figure 3, that ISO-treatment suppressed kidney weight in a dose-dependent fashion for all three groups and was especially pronounced at the 80 and 160 µg dose in females.

Female Sexual Behavior.

Figure 4 shows the mean total lordosis scores in response to 10 mounts by stud males for each group of animals. Analysis of variance showed a main effect of group, $F(2,82)=231.36, p<.0001$. It can be seen that neonatal TP-treatment completely suppressed lordosis to levels even lower than those found in males and that overall, females

Figure 3. Mean (± 1 S.E.M.) heart and kidney weight in milligrams for males, females and TP-females. * significantly different from SAL animals ($p<.05$).


HEART WEIGHT
WEIGHT IN MILLIGRAMS
4000
3000
2000
1000
0
SAL
40
80
160
* *
* *
M
F
TP
GROUP

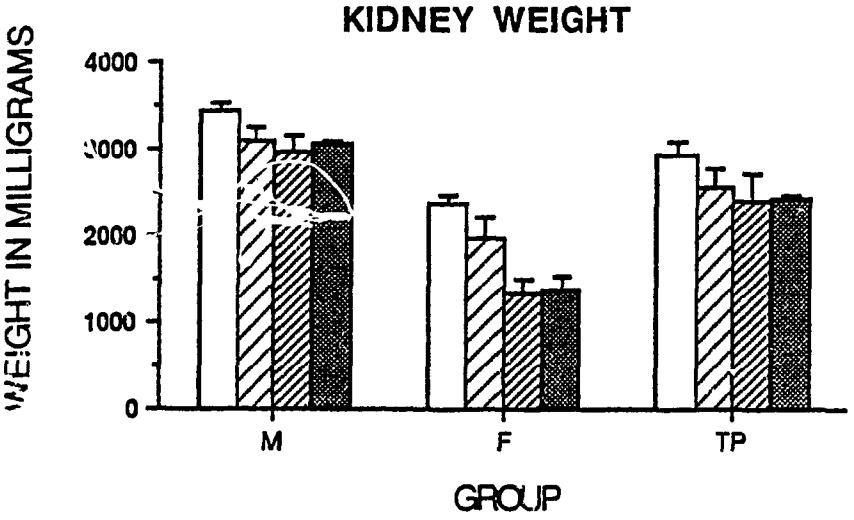
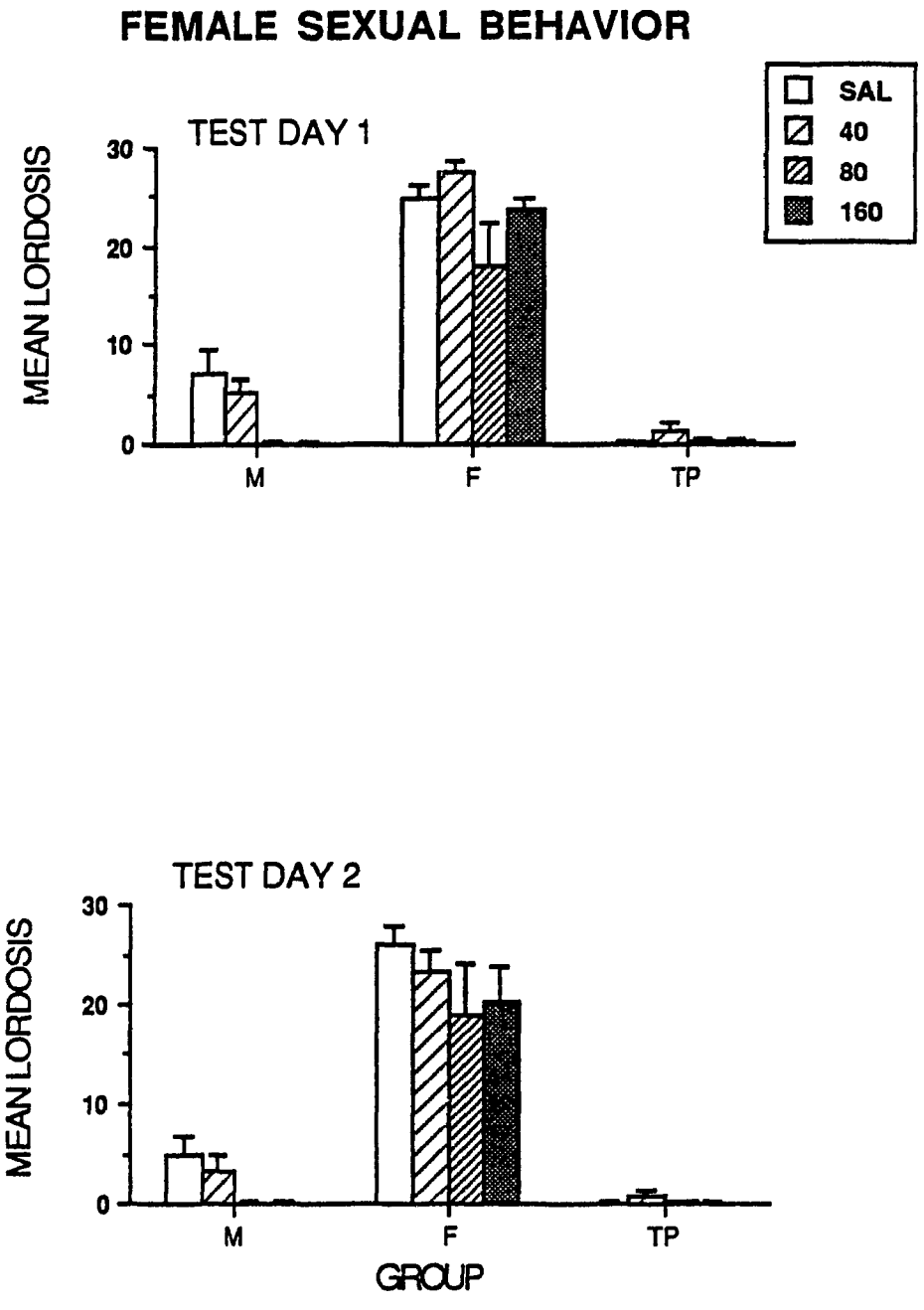

KIDNEY WEIGHT
WEIGHT IN MILLIGRAMS
4000
2000
1000
0
M
F
TP
GROUP

Figure 4. Mean (± 1 S.E.M.) lordosis score after 10 mounts by a stud male for males, females and TP-females on the two tests for female sexual behavior.


FEMALE SEXUAL BEHAVIOR
TEST DAY 1
MEAN LORDOSIS
30
20
10
0
M
F
TP
SAL
40
80
160
TEST DAY 2
MEAN LORDOSIS
30
20
10
0
M
F
TP
GROUP

displayed more lordosis. There was a main effect of dose, $F(3,82)=4.91, p<.003$, reflecting the fact that lordosis was suppressed to different degrees with each dose. The highest doses of ISO produced the largest effects, particularly in males.

Male Sexual Behavior.

Although, all animals were tested for male sexual behavior after TP replacement, sexual behavior was so infrequent that the groups could not be compared. The replacement doses appeared inadequate to restore sexual behavior even among normal males. However, the age at which the animals were tested may have contributed.

Discussion

The present study provided some evidence that ISO protected against the defeminizing effect of TP as determined by the body weight measure. As expected, the body weight of TP-treated females was greater than that of normal females. All ISO-treated TP-females had lower body weights than the TP-SAL females. The protective effect of the ISO could be seen as early as day 21,and by 90 days of age all ISO-TP females were significantly lighter than the SAL-TP females. Isoproterenol-treatment had no significant effect on the body weights of females, and males overall. Interestingly, although ISO had no significant affect overall on males, there is an indication that weight was suppressed temporarily at 50 days of age.

Contrary to the hypothesis that the activation of ß-adrenergic receptors during the period of sexual

differentiation would protect against defeminization of sexual behavior by T, no protection from ISO-treatment was found in either normal males or TP-females. If anything, ISO suppressed lordosis slightly in females and significantly in males on both test days. A suppression could not be assessed in TP-females as lordosis behavior was virtually nonexistent in these animals.

In this experiment, ISO was given systemically making it possible that its effects were mediated via the gonads. There were, however, no significant effects of ISO on gonad weight of either the TP-females or males. Therefore, if ISO had an effect on the gonads, it was not reflected in gonad weight.

There were significant effects of ISO on heart and kidney weights in this experiment confirming previous reports that systemic injections of ISO given neonatally do have actions on peripheral organs (Slotkin, 1987). In males, ISO had a suppressing effect on heart weight compared to the hearts of SAL males which could be seen in I80 and I160-males. For females this effect was reversed with a progressive increase in heart weight in animals given ISO, until I160, where there is a slight decrease in weight. In TP-females heart weight was greater in animals that had received the 40 and 80 µg but not the 160 µg dose.

Interestingly, the pattern of effects of ISO on heart weight was different in males and females; it was suppressed in males and increased in females. Among TP-females, neither the male or the female pattern clearly

emerged. Isoproterenol-treatment appeared to suppressed kidney weight in all groups but clearly females were more effected than either males or TP-females. Although no explanation for the sex difference in response to ISO can be offered here, it is interesting that in each case TP-females responded more like males.

When kidney weights were measured in each of the groups it was found that the weight was suppressed in a dose-dependent fashion, with the weight being lighter for the higher doses. It is interesting, that as in the case of heart weight, females were clearly different from both male and TP-females.

Experiment 2

In Experiment 1, ISO was administered to neonatal rats subcutaneously. One problem with this is that there is no accurate knowledge about the extent to which ISO passes into the brain of the neonatal rat. It is known that ISO given systemically to adults does not reach the brain (Conway, Tejani-Butt and Brunswick, 1987). Although, it is known that the blood brain barrier in the rat fetus is not totally immature (Saunders and Mollgard, 1984), it has been demonstrated that in some species the developing brain allows a greater permeability between blood and cerebral spinal fluid, by lipid insoluble molecules of a wide range of molecular size (Dziegielewska et al, 1979). Even if ISO does reach the brain in the neonatal rat, the amount is uncertain. Therefore, it would be preferable to be able to inject ISO centrally. During the course of the first

experiment a paper published by Slotkin, Windh, Whitmore and Seidler (1988) described an effective dose of ISO that could be administered using a simple method of intercisternal injection. This method was exploited in Experiment 2, which in most other respects constituted a replication of Experiment 1.

Method

Subjects.

Forty-eight Sprague Dawley rats were born to females purchased from (Charles River, Willmington, Mass) and bred in the laboratory. Animals were treated as described in Experiment 1. Pups were assigned to the three groups; M, F, or TP. Animals from each group were then assigned to one of two doses: vehicle only (SAL), 10 µg ISO (I). Initially, there were 8 animals per dose per group, however during the course of the study some animals died. Due to the low mortality rate, and the random pattern, deaths could not be attributed to treatment with ISO.

Procedures

Neonatal Treatments. Intercisternal injections of 10 µg ISO in SAL and 1% ascorbic acid were administered into the cistern magnum with the use of a 10 µl syringe (Hamilton Co., Reno, Nevada). Thirty-one-gauge needles were modified by breaking the tip down to 3 mm following the method used by Slotkin (1988). In hairless rat pups, the translucent skin allows for easy identification of the injection cite. Injections were given on the 1st, 3rd and 5th days of life. In this experiment TP was administered in a dose of 25

µg/.05 ml peanut oil to TP females on days 1, 3 and 5. Control females received the vehicle only.

Pups were weaned at 21 days of age and moved 3 days later to standard wire mesh cages, with food and water ad libitum. They were housed 2 or 3 to a cage until they were 30 days old at which time they were moved to individual housing. From 30 days of age all female animals were inspected daily for vaginal opening. Males were handled at this time to control for effects of daily handling. One week following vaginal opening, daily smears were taken for a period of 10 days. Two weeks prior to behavioral testing, animals were moved to a reverse cycle room. Weights were recorded weekly.

Behavior Tests

Exploratory behavior. Eight, automated open field boxes, were used to measure exploratory behavior. Each 55 cm square plywood unit was dimly lit with a 7 watt red light bulb. The wood was protected from the absorption of odours with black oil based paint and each unit was washed out with soap and water between trials. Exploratory behavior was tested at 70 days of age. Exploratory behavior of the animals, was recorded during 5 min sessions.

Female Sex Testing. At 60 days of age, all animals were gonadectomized as described in Experiment 1. Gonad weight was recorded at that time. At 90 days of age, animals were tested for female sexual behavior following the same methodology described in Experiment 1.

Male Sex Behavior. At 120 days of age, animals were

tested for male sexual behavior following the same methodology described in Experiment 1.

Results

Statistical Analysis.

The effects of ISO-treatment on body weight and behavior were analysed using three-way analyses of variance; group x dose x age and group x dose x test day, respectively. Two-way analyses of variance were used to analyse the effect of ISO on organ and gonad weights (group x dose). Post hoc comparisons were made using the Tukey-Kramer procedure (Kirk, 1982) The effect of ISO on the age of vaginal opening between females was compared with t-tests.

Vaginal Opening.

The effects of neonatal treatment of ISO on the age of vaginal opening was examined in SAL and ISO-females (see Figure 5). There was no difference between ISO and SAL-females as determined by t-test. The vaginas of females injected with TP did not open even in adulthood.

To assess the effects of ISO-treatment on the estrous cycle, vaginal smears were taken for two weeks from the day of vaginal opening for ISO and SAL-females. Both groups of females were found to be cycling normally.

Body Weight.

Analysis of variance carried out on body weight revealed a group effect, $F(2,36)=46.26, p<.0001$, an age, $F(2,72)=1669.56, p<.0001$, a group x age interaction, $F(4,72)=37.93, p<.0001$ and a group x age x dose interaction,

Figure 5. Mean (± 1 S.E.M.) day of vaginal opening for females and ISO-females.

DAY OF VAGINAL OPENING

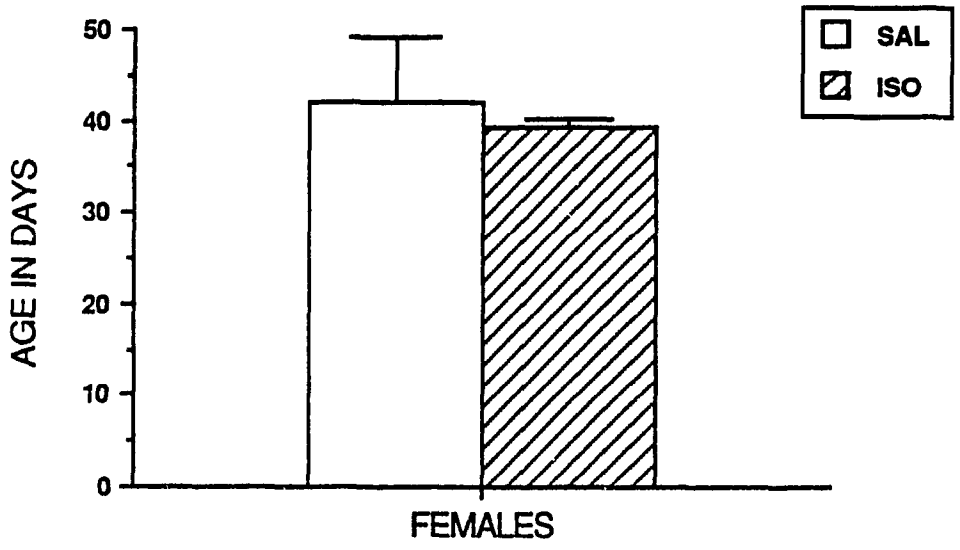
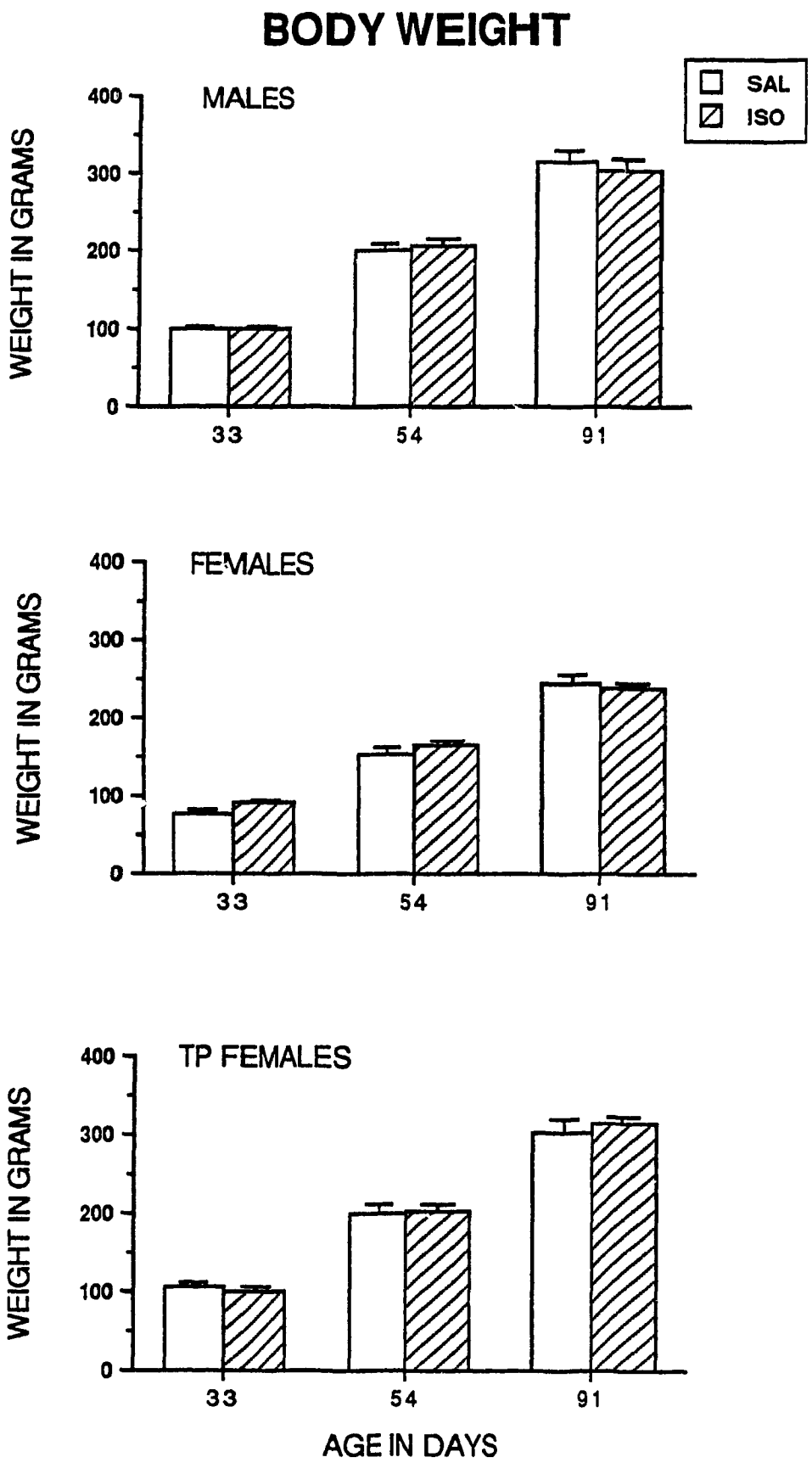


Figure 6. Mean (± 1 S.E.M.) body weight in grams for males, females and TP-females at 33, 54 and 91 days of age.


BODY WEIGHT
SAL
ISO
MALES
FEMALES
TP FEMALES
WEIGHT IN GRAMS
400
300
200
100
0
33
54
91
AGE IN DAYS

$F(4,72)=2.89, p<.03$. These effects are shown in Figure 6, and it can be seen that there are differences in weight between groups, males are heaviest, females are lightest and TP-females fall between, and that these differences change as a function of age and dose. Although weight increased with age, the pattern of weight change with ISO-treatment in the three groups was slight and unpredictable.

Gonadal Weight.

The analysis of variance carried out on the gonad weight revealed that although there were slight variations between the gonads of SAL and ISO-treated animals in all three groups none of the effects was significant. Mean gonadal weight for each of the groups is illustrated in Figure 7.

Organ Weight.

ISO-treatment had no effect on organ weights for any of the three groups. Group means for organ weights are illustrated in Figure 8.

Open Field Behavior.

A three-way analysis of variance showed no effect of ISO-treatment on ambulation. Although the group effect came close to significance, it did not reach significance, $F(2,39)=1.23, p<.09$, a sex difference in activity between normal males and females was found (normal males, $x=192$ females, $x=239$). It can be seen in Figure 9, that TP females did not show the expected reduction in activity compared to normal females.

Female Sexual Behavior.

Figure 7. Mean (± 1 S.E.M.) gonadal weight in milligrams for males, females and TP-females.

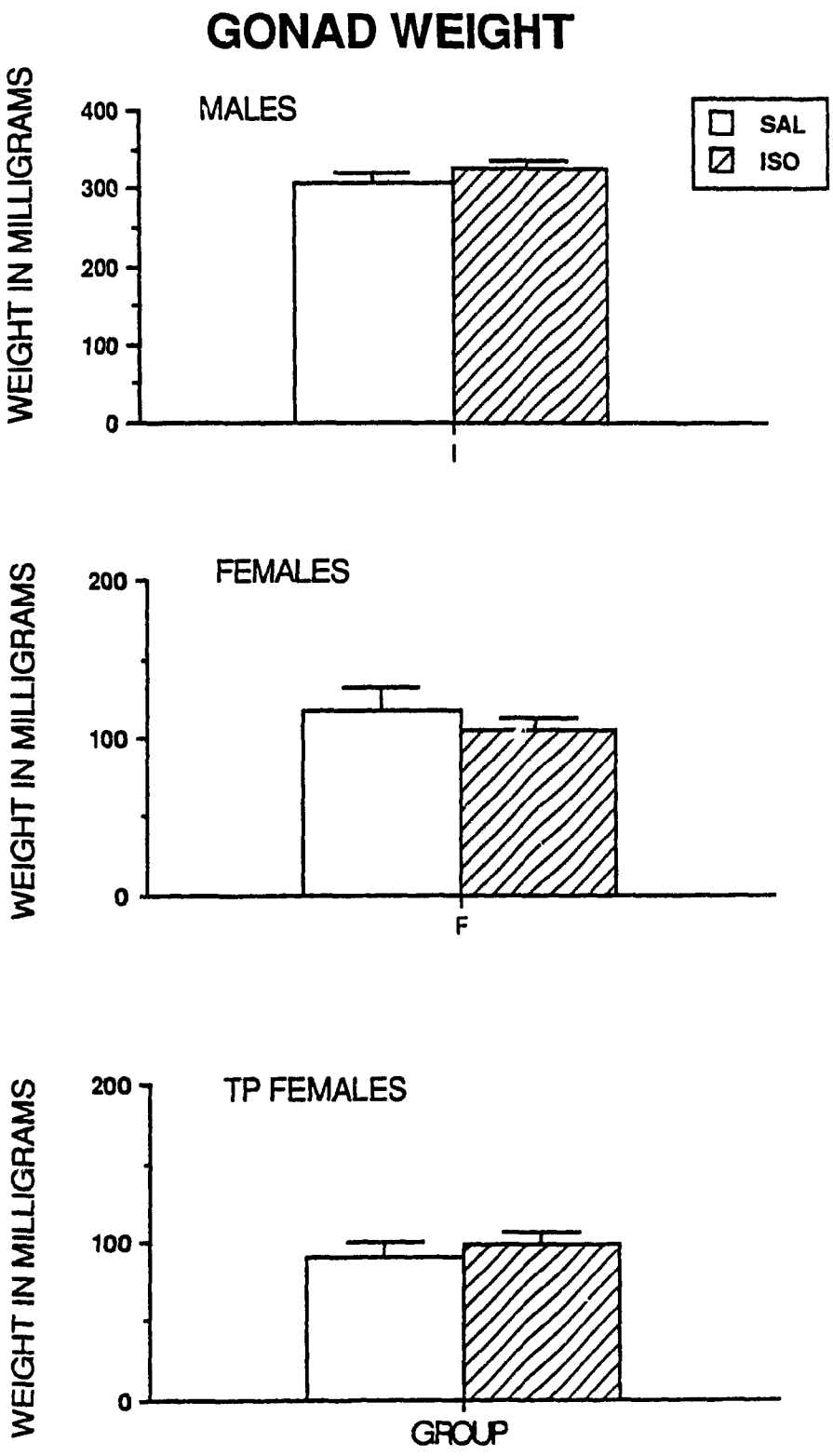
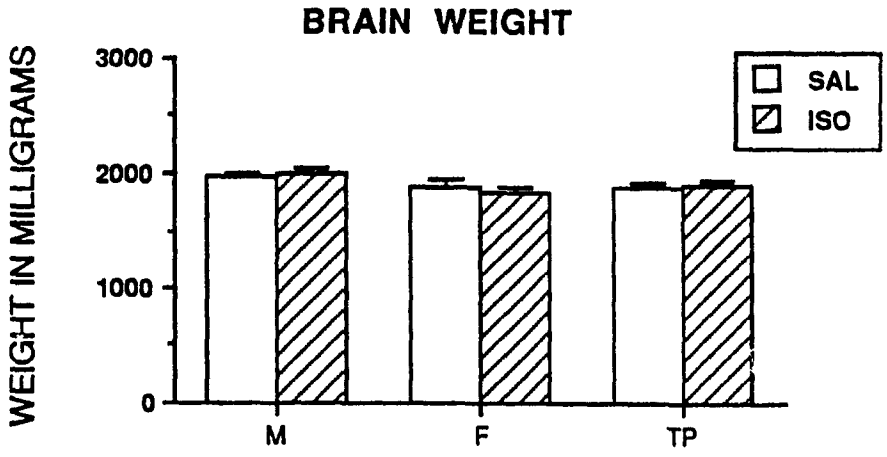
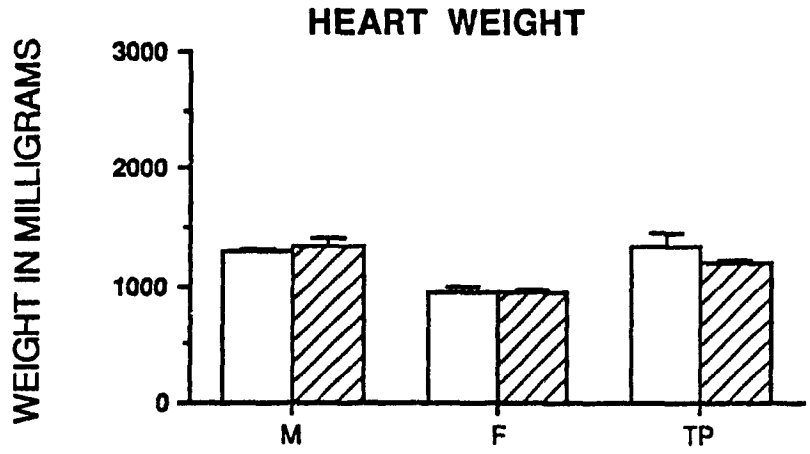

GONAD WEIGHT
MALES
SAL
ISO
WEIGHT IN MILLIGRAMS
400
300
200
100
0
I
FEMALES
WEIGHT IN MILLIGRAMS
200
100
0
F
TP FEMALES
WEIGHT IN MILLIGRAMS
200
100
0
GROUP

Figure 8. Mean (± 1 S.E.M.) brain, heart and kidney weight in milligrams for males, females and TP-females.


BRAIN WEIGHT
WEIGHT IN MILLIGRAMS
3000
2000
1000
0
SAL
ISO
M
F
TP


HEART WEIGHT
WEIGHT IN MILLIGRAMS
3000
2000
1000
0
M
F
TP

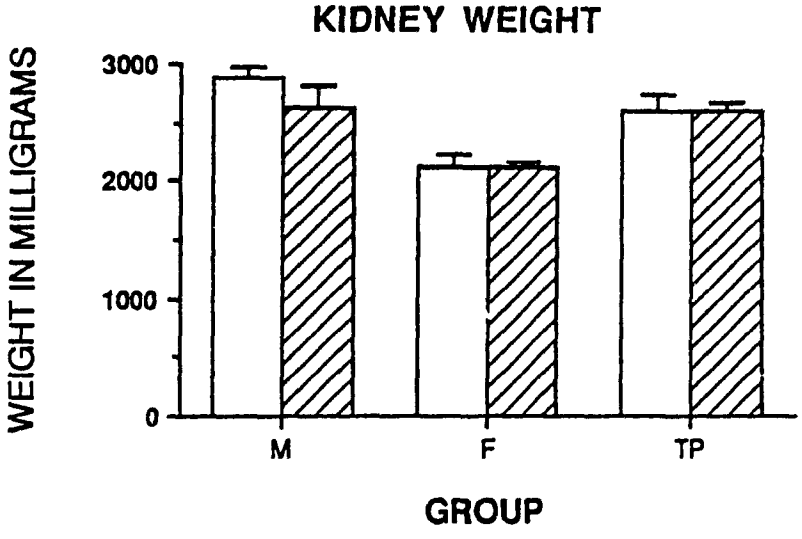
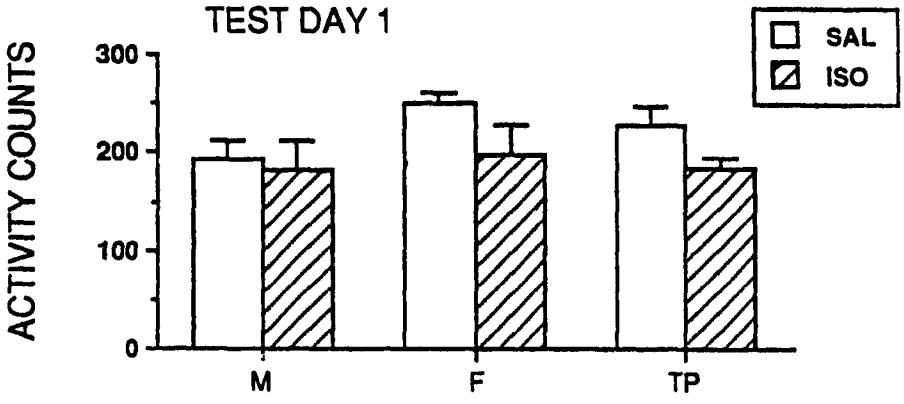

KIDNEY WEIGHT
WEIGHT IN MILLIGRAMS
3000
2000
1000
0
M
F
TP
GROUP

Figure 9. Mean (± 1 S.E.M.) activity scores after 5 min in the open field for males, females and TP-females on the two test days.

OPEN FIELD


TEST DAY 1
SAL
ISO
ACTIVITY COUNTS
300
200
100
0
M
F
TP

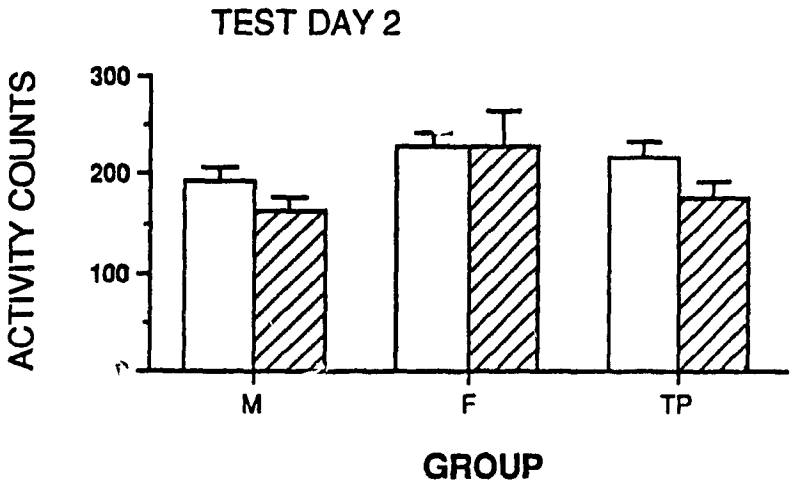

TEST DAY 2
ACTIVITY COUNTS
300
200
100
M
F
TP
GROUP

Figure 10. Mean (± 1 S.E.M.) lordosis score after 10 mounts by a stud male for males, females and TP-females on the two tests for female sexual behavior.

FEMALE SEXUAL BEHAVIOR

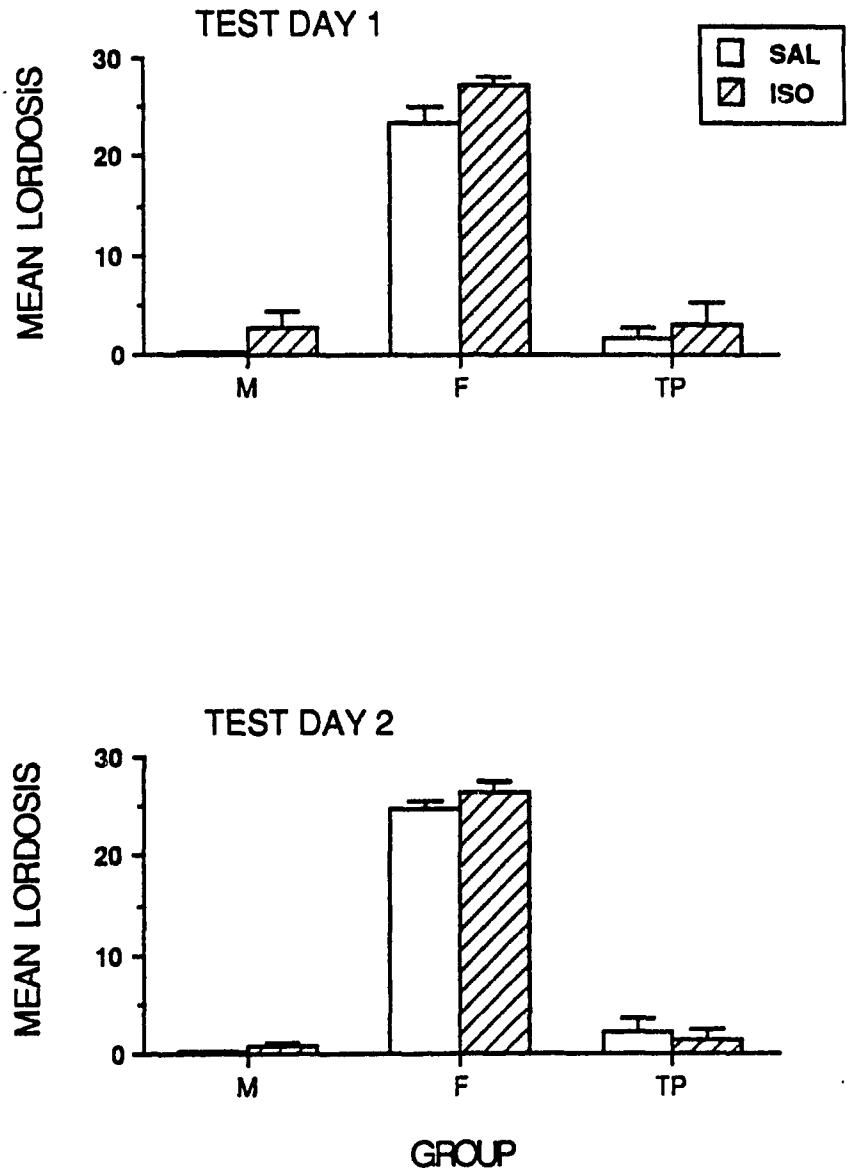


Figure 10 shows the mean total lordosis scores for SAL and ISO-treated animals in each of the three groups of animals on the two test days. A three-way split plot analyses of variance revealed a main effect of group, $F(2,38)=182.50, p<.0001$ reflecting the fact that lordosis was dramatically suppressed in males and TP-females, relative to females. There was also a small but significant effect of dose, $F(1,30)=5.31$, $p<.03$ indicative of the consistent trend for ISO to increase lordosis behavior in all groups.

Male Sexual Behavior.

All animals were tested for male sexual behavior, after TP replacement, however, sexual behavior was so infrequent that the groups could not be compared. Although these animals were younger than the animals in Experiment 1 and replacement doses were given daily, as opposed to every second day conducted in Experiment 1, this still proved to be inadequate to restore sexual behavior to testable levels.

Discussion

In this experiment, when ISO was administered intracisternally at a dose previously shown to evoke maximal stimulation of ß-adrenergic receptors in the developing rat CNS (Morris and Slotkin, 1985), it caused a small, though significant increase in female sexual behavior in all groups. This finding might be seen to lend more support to the hypothesis that stimulation of ß-adrenergic receptors produces a protective effect against the defeminizing actions of T, however, at the dose given the effect was minimal and clearly did not reverse the actions of T in

either males or TP females.

As in Experiment 1, neonatal TP clearly defeminized female sexual behavior but at the dose given, did not suppress open field activity to levels found in males. Curiously, the only group which showed an effect of ISO-treatment on activity were TP-females and contrary to the hypothesis, it acted to suppress ambulation to male-typical levels. Clearly, ISO-treatment did not act to block the action of T in males.

Isoproterenol given intercisternally had no effect on body weight or on any of the organs. This is not surprising in the case of heart and kidney, but one might have expected to see some effect in the brain (Slotkin, Grignolo, Whitmore, Lerea, Trepanier, Barnes, Weigel, Seidler and Bartolome, 1982; Slotkin, Seidler, Trepanier, Whitmore, Lerea, Barnes, Weigel and Bartolome, 1982).

General Discussion

The purpose of these experiments reported in this thesis was to test the hypothesis that noradrenergic systems are involved in the mediation of the early organizational effects that gonadal hormones have on the differentiation of the mechanisms which underlie adult anatomy and sexual behavior in the rat. More specifically, the experiment was designed to test the proposal by Raum and Swerdloff (1981) that stimulation of ß-adrenergic receptors would provide protection against the known defeminizing actions of T during the perinatal period. Only limited support for their hypothesis was found in these experiments, and discrepancies

exist between the data from the two experiments.

Female Sexual Behavior. In Experiment 1 there was no effect of ISO in female sexual behavior, overall. This lack of the predicted effect of ISO, however, could have been due to insufficient levels of the drug reaching the brain. When ISO was given directly into the brain, in Experiment 2, there was a statistically significant tendency for ISO-treated animals to show elevated lordosis scores. This effect was extremely small, however, and could not be said to be behaviorally significant in either males or TP-females. It is possible that although the dose of ISO given to animals in Experiment 2 has been shown to be capable of stimulating central ß-adrenergic receptors (Slotkin, Windh, Whitmore, and Seidler, 1988), it was still too low to protect against the defeminizing actions of T even at the levels of TP administered to the TP-females.

Open Field. In the open-field test, normal adult females are reported to be more active than normal adult males (Broadhurst, 1957, 1958), a sex difference which is known to be due to exposure to androgens during the perinatal period. Testosterone administered to females shortly after birth leads to adult ambulation scores closer to those seen in adult males (Gray, Lean and Keynes, 1969; Quadagno, Anderson, Shryne and Gorski, 1972; Stewart, Skvarenina and Pottier, 1975) and neonatal castration of males results in female-typical ambulation scores (Pfaff and Zigmond, 1971). The reported sex difference in ambulation between normal males and normal females was replicated in Experiment 2.

The doses of TP given in this experiment, however, did not produce ambulation scores in TP-females equivalent to those seen in males. Investigators who have found a defeminizing effect of TP on ambulation, generally used doses much larger than the one given in this experiment. Therefore, although the TP injections given were large enough to increase body weight, they were not sufficient to suppress ambulation to male-typical levels.

Isoproterenol did not significantly alter activity scores for males or females, but curiously, there was a significant lowering of ambulation in TP-females. This finding was not predicted from the hypothesis and it is difficult to draw any meaningful conclusions about it.

Body Weight. Rats show a sexual dimorphism in body weight and size; adult males are typically larger and heavier than adult females (Wade, 1972). When TP is administered to females during the postnatal period, it results in increases in adult body weight which tend toward weights typical of normal males (Slob and Van Der Werff Ten Bosch, 1975). This documented effect of neonatal TP-treatment on females was replicated in these experiments.

Although, the adult weights of TP females were greater than those of normal females, TP-females did not attain adult body weights equivalent to normal males (Experiment 1: females, x=268, TP females, x= 350; males,x=375; Experiment 2: females, x=245, TP females, x=303; males,x=3[illegible]). This is probably due to two factors, the low dose of TP used and the fact that they also retained their ovaries until 75 days

of age. Although TP acts to accelerate body weight, estrogen has been shown to decelerate growth, and the critical period for these effects is between birth and 21 days of age (Slob and Van Der Werff Ten Bosch, 1974).

Interestingly, TP-females that were given systemic injections of ISO, appeared to be protected from the effects of TP on body weight. This finding is in keeping with the proposed hypothesis that stimulation of ß-adrenergic receptors, blocks defeminizing actions of T. Similar protective effects of ISO against endogenous T were not seen in males, however. This difference in the effect of ISO in normal males and the TP-females in the study is probably due to the relatively small amount of TP injected postnatally (50 ug) to females on days 1, 3 and 5. Slob and Van Der Werff Ten Bosch (1975) for example, found that adult females most resembled normal adult males in weight when TP (2 mg) was given to their mother on days 16-20 of gestation, they were ovariectomized at birth, and TP (0.5 mg) was given again postnatally every other day from day 2 until day 20. The protective effect of ISO on weight gain was not seen in Experiment 2. This would suggest that the effects seen in ISO-TP females in Experiment 1 were not mediated centrally, but rather, were produced by some genomic event in peripheral cells.

Organ Weight. It has been shown that stimulation of ß-adrenergic receptors by NE in the developing CNS activates ornithine decarboxylase, an enzyme which regulates cellular replication and differentiation (Morris, Seidler and

Slotkin, 1983; Morris and Slotkin, 1985). Furthermore, there is evidence that catecholamines have trophic influences on peripheral organs. For example, Claycomb (1976) found that when cardiac muscle cells were exposed to ISO, they changed from the replication phase to differentiation, and that the effect was mediated through stimulation of cyclic AMP levels. Slotkin, Whitmore, Orband-Miller, Queen and Haim (1987) found that sympathetic innervation of the heart, lung and kidney, regulates the pattern of cell replication in the rat through stimulation of ß-adrenergic receptors, by NE, which during critical periods of neonatal development, are coupled to DNA. Direct stimulation of ß-adrenergic receptors by the in vivo administration of ISO caused acute reductions in DNA synthesis in an age dependent manner. In the heart, there was a substantial reduction in DNA synthesis by 5 days of age and the effect was maximal by 9 days of age. A reduction of DNA was seen in the lung and kidney at 2 days of age, following ISO challenge, and the effected peaked by 9 days of age. The study by Slotkin et al, (1987) supports the hypothesis that sympathetic innervation regulates cell replication in the developing peripheral organs, however, since the coupling of ß-adrenergic receptors to DNA synthesis disappears as the organism matures, ß-adrenergic receptor stimulation controls cell replication only during critical periods in neonatal development. It was possible therefore, that the postnatal administration of ISO in Experiment 1 and 2, had effects on DNA synthesis that would

be reflected in differences in weight. Indeed, this proved to be the case in Experiment 1. Kidney weights were reduced in a dose dependent manner, with the highest doses of ISO producing the largest decreases in weight. Although, it is clear that ISO did affect heart weight, the pattern of weight change was not the same as that seen in the kidneys. Not surprisingly, an effect of ISO was not seen in animals given intercisternal injections in Experiment 2.

Day of Vaginal Opening. In Experiment 2, females injected with ISO did not show any irregularity in their day of vaginal opening or in their estrous cycle compared to SAL-females. Isoproterenol-treatment did not block defeminization by TP on day of vaginal opening as was predicted by the hypothesis. It is unclear why TP females in this experiment did not experience vaginal opening even into adulthood.

These findings suggest overall, that the ß-adrenergic receptor stimulation does not block the defeminizing effect of T during neonatal development in the rat; it remains possible, however, that both of these sets of results can be explained by inadequate stimulation of central ß-adrenergic receptors by the injections of ISO.

If the hypothesis were true, it might help to explain the reported effects of prenatal stress on adult male sexual behavior. Ward (1972) discovered that the male offspring of mothers stressed during pregnancy have a reduced potential for the male ejaculatory pattern when tested for adult sexual behavior, while at the same time demonstrating

sexual behavior, while at the same time demonstrating abnormally high levels of female lordotic behavior. This collective pattern of sexual responses was termed the Prenatal Stress Syndrome (Ward, 1972, Ward, 1984). Closer investigation of this phenomenon, has revealed that while normal male fetuses experience a sharp increase in T on gestational days 18 and 19, this pattern is reversed in the male fetuses of stressed mothers. They experience a T surge day 17, a time when T levels in normal male fetuses are indistinguishable from normal female fetuses. At days 18 and 19 when T levels sharply increase in normal male fetuses, T levels remain low in stressed male fetuses.

One of the known consequences of stress is an increases in the levels of NE (Stone, 1987). Although, Ward argues that the feminization and demasculinization of prenatally stressed males is a consequence of the shift in the gestestional T surge, it is possible that the increased levels of NE produced by stress, also contributes to the Prenatal Stress Syndrome by ß-adrenergic receptor stimulation.

In conclusion, however, the findings from the experiments reported here suggest that ß-adrenergic receptor stimulation does not block the defeminizing effect of T during neonatal development in the rat. The only significant support for the hypothesis came from the results on body weight, an effect which appears not to be centrally mediated. It is still possible to argue, however, that both of these sets of results might be explained by inadequate

injections of ISO.

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