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Sex Differences in the Behavioral Response to Amphetamine in Adult Rats: Interactions Between Exposure to Gonadal Hormones in Early Development and in Adulthood

Margaret L. Forgie

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ABSTRACT

Sex Differences in the Behavioral Response to Amphetamine in Adult Rats: Interactions Between Exposure to Gonadal Hormones in Early Development and in Adulthood

Margaret L. Forgie, Ph.D.
Concordia University, 1993

Previous research has demonstrated that adult female rats exhibit greater behavioral activation following an acute injection of AMPH, and greater sensitization following repeated administrations of the drug, than adult male rats. This sex difference appears to result from a sexually dimorphic response of the central nervous system (CNS) to AMPH. Sex differences in the CNS have been shown to depend on the interaction between exposure to gonadal hormones in early development and circulating gonadal hormones in the adult animal. In the present series of experiments, the effect of exposure to testicular androgens in the perinatal period, to ovarian hormones in the peripubertal period, and to circulating ovarian and testicular hormones in adulthood on the response of adult animals to AMPH was studied.

The main findings were, first, that exposure to testosterone (T) during the perinatal period decreased the responsiveness of the adult animal to AMPH. Second, circulating estradiol (E_2) enhanced the acute response of the female animal to AMPH. Circulating E_2 also enhanced sensitization to repeated injections of AMPH, and this effect was greater in those animals not exposed to T at birth. Third, when sensitization was measured as the difference in locomotor activity between animals pre-exposed to AMPH, and animals receiving AMPH for the first time, neither perinatal exposure to T nor circulating E_2 greatly altered sensitization. It was evident, however, that animals tested in the presence of E_2 were more like intact animals than under any other condition.

Taken together, the findings indicate that exposure of the male animal to testicular

hormones during the early perinatal period, and the presence of circulating E_2 in the adult female at the time of testing, are major contributors to the sex difference seen in adult animals. These hormonal factors, however, do not provide a complete explanation for the results obtained in the present experiments.

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DEDICATION

This thesis is dedicated with love to Dic, my I.U.B.O.B.P.B.

Wagons Ho!!

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GENERAL INTRODUCTION

The psychostimulant drugs, amphetamine (AMPH) and cocaine, produce a well-characterized syndrome of behavioral hyperactivity in a variety of species including humans (Angrist & Sudilovsky, 1978; Post & Contel, 1983; Randrup & Munkvad, 1967). In rats, for example, low doses of AMPH produce an increase in locomotor activity and activity directed towards environmental stimuli (e.g., sniffing and exploratory behavior), whereas higher doses produce a more rigid and restricted behavioral syndrome consisting of behaviors such as focused sniffing, repetitive head and limb movements, and compulsive chewing, licking, and gnawing (Kuczenski & Segal, 1988; Segal & Schuckit, 1983; Sharp, Zetterström, Ljungberg, & Ungerstedt, 1987). It has been suggested that both the locomotor hyperactivity and the stereotyped behaviors seen in nonhuman animals have counterparts in the human AMPH response (Ellinwood, Sudilovsky, & Nelson, 1973; Randrup & Munkvad, 1967; Segal & Schuckit, 1983).

In both animals and humans, the repeated, intermittent administration of these drugs produces an enhancement of their behavioral activating effects. This phenomenon has been referred to as behavioral sensitization (Kalivas & Stewart, 1991; Post, 1981; Robinson & Becker, 1986; Robinson, 1988). In humans, repeated use of AMPH and cocaine can produce psychotic episodes that have been likened to the psychotic episodes seen in idiopathic, paranoid schizophrenia (Angrist & Gershon, 1970; Angrist & Sudilovsky, 1978; Ellinwood, 1967; Ellinwood et al., 1973; Griffith, Cavanaugh, & Oates, 1970; Post & Contel, 1978). It has been proposed that sensitization is the mechanism by which psychostimulant psychosis in humans is produced (Angrist & Sudilovsky, 1978; Antelman & Chiodo, 1983; Ellinwood et al., 1973; Kokkinidis & Anisman, 1980; Lieberman, Kenon, & Loebel, 1990; Robinson, 1991; Robinson & Becker, 1986; Robinson & Camp, 1987).

The enhancement of behavioral effects that is brought about by repeated exposure to these drugs is very long-lasting; the psychotogenic effects of methamphetamine, for example, can remain for years, with a single re-exposure to the drug producing a psychotic state (Sato, Chen, Akiyama, & Otsuki, 1983). The sensitization produced by psychostimulants in animals is also long-lasting, having been found to be present after several months (Paulson, Camp, & Robinson, 1991; Post & Weiss, 1988; Robinson, 1984; Robinson & Becker, 1986; Robinson, Becker, & Presty, 1982). These findings suggest that there are long-lasting changes in the neural system(s) that mediate responsiveness to the psychostimulants.

Both the acute behavioral activating effects of the psychostimulants, and the sensitization that develops upon repeated administration of these drugs, depend critically on the integrity of the midbrain dopamine (DA) systems (Clarke, Jakubovic, & Fibiger, 1988; Creese & Iversen, 1975; Kelly & Iversen, 1975; Kelly, Seviour, & Iversen, 1975). The DA neurons of these systems have their cell bodies in the substantia nigra and the ventral tegmental area of the midbrain and send their axons into forebrain terminal regions such as the striatum, nucleus accumbens septi (NAS), olfactory tubercle, and medial prefrontal cortex (mPFC; Björklund & Lindvall, 1984). The ability of low to moderate doses of AMPH to elicit locomotor activation in the rat, for example, is dependent upon the integrity of the mesoaccumbens system (Clarke et al., 1988; Kelley & Iversen, 1975).

The long-term changes brought about during sensitization of psychostimulant-induced behaviors appear to result from long-term changes to the DA neurons. Initiation of sensitization has been found to depend on changes produced in the cell body region (e.g., changes in somatodendritic transmitter release; Kalivas & Duffy, 1993b) and appears to involve the D_1 dopamine receptor (Drew & Glick, 1990; Stewart & Vezina, 1989). The expression of sensitized behavior, on the other hand, appears to involve an increased release of transmitter from the terminal region of the neuron (Akimoto, Hamamura, Kazahaya, Akiyama, & Otsuki, 1990; Kalivas & Duffy, 1990; Kalivas & Duffy, 1993a;

Robinson, Jurson, Bennett, & Bentgen, 1988; Vezina, 1993; see Kalivas & Stewart, 1991 and Robinson & Becker, 1986 for reviews). Given that psychostimulant-induced behaviors are critically dependent on the midbrain DA systems, it might be supposed that factors that influence the functioning of these systems in individual animals would result in individual differences in the behavioral expression of responsiveness to the psychostimulant drugs.

Individual rats, like individual humans, vary in their sensitivity to the acute behavioral activating effects of the psychostimulants and to the sensitization of these effects produced after repeated intermittent exposure to these drugs (Angrist & Sudilovsky, 1978; Segal & Kuczenski, 1987; Segal & Schuckit, 1983). Many factors have been proposed to influence this sensitivity, including sex, strain, prior drug history, and prior exposure to stressful stimuli (see Robinson, 1988 for review). One of the most robust individual differences is that due to sex; it has been found repeatedly that female rats are more responsive to these drugs than are males (Beatty & Holzer, 1977; Beatty, Dodge, & Traylor, 1982; Becker, Robinson, & Lorenz, 1982; Camp, Becker, & Robinson, 1986; Camp & Robinson, 1988a, 1988b; Glick & Hinds, 1984; Post & Contel, 1978; Robinson, 1984; Robinson et al., 1982; Savageau & Beatty, 1981; Van Haaren & Meyer, 1991). This sex difference has been attributed, for the most part, to sex differences in the amount and kind of circulating gonadal hormones at the time of testing in the adult animal (e.g., Becker et al., 1982; Camp & Robinson, 1988a, 1988b; Robinson, 1984), and it is clear that both circulating ovarian and testicular steroids play a role in the expression of these sex differences.

Previous studies have not taken into account, however, the fact that male and female animals receive differential hormonal exposure at various times during development that could influence the development of the DA systems and, thus, the behaviors that depend on them. For example, male animals are exposed to testosterone (T) during the early critical period for sexual differentiation of the brain. This exposure is known to affect

the development of brain regions involved in both reproductive and non-reproductive behaviors and has been shown to alter responsiveness to circulating gonadal hormones in the adult animal (see Arnold & Breedlove, 1985; Beatty, 1992; Toran-Allerand, 1986; and Yahr, 1988 for reviews).

In addition, the DA neurons are steroid sensitive, and sex differences in these cells appear very early during the fetal development of the rat (Reisert & Pilgrim, 1991). Given that DA neurons have been shown to influence the development of at least one of their target regions, the mPFC (Kalsbeek et al., 1987; Kalsbeek, Matthijssen, & Uylings, 1989), it is possible that sex differences in the DA neurons could also translate into the sex-specific development of the target region. These sex differences could be reflected in later sex differences in DA-dependent behaviors. Thus, differential exposure of male and female animals to gonadal hormones in early development might influence the expression of psychostimulant-induced behaviors in a sex-specific manner and, further, may alter the influence of circulating gonadal hormones on these behaviors in the adult.

The experiments that comprise the present thesis were conducted to systematically investigate these possibilities. Different hormonal manipulations were made in male and female animals early in development and then locomotor activity in response to repeated, intermittent treatments with a moderate dose of AMPH was measured in adulthood. In addition, circulating testicular and ovarian hormones were manipulated in the adult animal at the time of behavioral testing with AMPH. The background literature and rationale for these experiments is addressed in greater detail in the following sections.

Sex Differences in Responsiveness to AMPH in the Adult Rat

Sex differences in response to both acute and repeated injections of AMPH have been demonstrated for a variety of different AMPH-induced behaviors including stereotypy (Beatty & Holzer, 1977; Beatty et al., 1982; Camp & Robinson, 1988a, 1988b), rotational behavior (Becker et al., 1982; Camp et al., 1986; Robinson, 1984; Robinson et al., 1982),

and locomotor activity (Camp & Robinson, 1988a, 1988b; Savageau & Beatty, 1981). These sex differences may be due, in part, to the known sex difference in peripheral metabolism of AMPH (see Becker et al., 1982). It has been shown that for a given dose of AMPH, higher brain levels are attained in females than in males (Becker et al., 1982; Meyer & Lytle, 1978), reflecting the increased activity of hepatic metabolic enzymes in males (Conney, 1967; Meyer & Lytle, 1978). Circulating T is thought to be responsible for the more rapid metabolism in males. Changes in circulating ovarian hormones do not affect brain levels of AMPH in females (Becker et al., 1982), whereas in adult males, castration increases brain levels of AMPH following systemic injections to levels similar to those found in intact and ovariectomized females (J. Becker, personal communication, and see Camp & Robinson, 1988b; Robinson, 1984). When, however, doses of AMPH have been adjusted to take into account this sex difference (e.g., Becker et al., 1982; Camp et al., 1986; Camp & Robinson, 1988a, 1988b), and females have been given a lower dose of the drug than males, the sex difference in AMPH-induced behavior persists. Thus, the sex difference in behavior appears to result from a sexually dimorphic response of the brain to AMPH. A possible contributing factor to this sexually dimorphic response is the known influence of circulating gonadal hormones on the DA systems.

In the adult female rat, AMPH-induced behaviors have been found to fluctuate with the estrous cycle (Becker & Cha, 1989; Becker et al., 1982; Joyce & Van Hartesveldt, 1984). An increase in AMPH-induced behavior is observed at estrus and is accompanied by increases in extracellular levels of DA in the striatum (Becker & Cha, 1989). In addition, AMPH-stimulated release of DA, *in vitro*, is enhanced in striatal slices taken from estrous females (Becker & Cha, 1989; Becker & Ramirez, 1981b). Ovariectomy of the adult female rat has been found to attenuate the behavioral response to AMPH (Becker & Beer, 1986; Camp et al., 1986; Joyce, Montero, & Van Hartesveldt, 1984; but see Beatty et al., 1982 and Camp & Robinson, 1988b), and to attenuate AMPH-stimulated release of DA from striatal slices (Becker & Ramirez, 1981b). It has been suggested that these effects

are due to some effect of estradiol on the functioning of the DA systems (Becker 1990a, 1990b; Becker & Beer, 1986; Becker, Beer, & Robinson, 1984).

The literature concerning the effects of estradiol on these systems, and on DA-dependent behaviors, is both complex and controversial. Depending on the dose of the hormone used, and the time after administration that the effect is measured, the direction of the effect produced has been shown to vary (see Van Hartesveldt & Joyce, 1986 for review). Recent experiments have shown, however, that the acute administration of low doses of estradiol to ovariectomized female rats enhances turnover of DA in the striatum (Di Paolo, Rouillard, & Bédard, 1985), enhances AMPH-stimulated release of DA from striatal slices (Becker, 1990a), increases AMPH-induced extracellular DA in the striatum as measured by *in vivo* microdialysis (Becker, 1990b; Castner, Xiao, & Becker, 1993), and facilitates AMPH-induced behaviors (Becker, 1990b; Castner et al., 1993). In addition, other ovarian and pituitary hormones, such as progesterone and prolactin, that fluctuate with the estrous cycle, can also have a facilitatory effect on the DA systems and the effects of psychostimulants (Becker et al., 1984; Becker & Ramirez, 1981a; Dluzen & Ramirez, 1990; Gonzalez-Mora, Guadalupe, & Mas, 1990; Morissette, Lévesque, Bélanger, & Di Paolo, 1990). Thus, circulating ovarian and pituitary hormones appear to have facilitatory effects on AMPH-induced behaviors in the female animal.

There is also evidence to suggest that circulating androgens can modulate the effects of AMPH. Studies done in the 1980's reported that castration of adult male rats tended to increase the acute behavioral response to AMPH (Beatty et al., 1982; Dluzen, Green, & Ramirez, 1986; Menniti & Baum, 1981), whereas the administration of T to either males or females tended to decrease it (Beatty et al., 1982; Menniti & Baum, 1981). These studies failed, however, to adjust drug doses to take into account the effect of circulating T on drug metabolism. In studies in which the doses of AMPH have been adjusted, castration of males has been found to either increase the behavioral response to an acute injection of AMPH (Camp & Robinson, 1988b) or to have no effect (Camp et al., 1986).

Furthermore, neither castration nor T-replacement has been found to affect AMPH-induced DA release from striatal slices (Becker & Ramirez, 1981b). There is some evidence, however, that castration of adult male rats does produce a decline in baseline levels of DA turnover in the NAS (assessed by post-mortem assay) that can be restored by administration of T (Alderson & Baum, 1981; Mitchell & Stewart, 1989; but see Baum, Melamed, & Globus, 1986).

In a series of experiments done to examine the contribution of circulating gonadal hormones to sex differences in sensitization to repeated injections of AMPH, it was found that ovariectomy did not attenuate the development of sensitization of stereotypy (Camp & Robinson, 1988b), locomotor activity (to a high dose of AMPH; Camp & Robinson, 1988b) or rotational behavior (Robinson, 1984; Robinson et al., 1982) in the female rat. In contrast, castration of the male rat enhanced the development of sensitization over that seen in intact male animals, and further, the behavior of castrated males was similar to that shown by intact and ovariectomized females (Camp & Robinson, 1988b; Robinson, 1984; Robinson et al., 1982). In an attempt to reconcile these findings with those demonstrating that ovarian hormones have a facilitatory effect on the response of the female animal to an acute injection of the drug (e.g., Becker & Beer, 1986; Camp et al., 1986), Camp and Robinson (1988b) proposed that circulating testicular hormones are responsible for the sex difference in the development of sensitization to repeated injections of AMPH (see also Robinson, 1988, 1991), whereas circulating ovarian hormones are responsible for the sex difference in the acute effects of the drug. This broad conclusion may be premature as there are several findings in the literature that are not consistent with this proposal. For example, it is curious that ovariectomy did not attenuate the response of the female animal to the first injection of the drug (i.e., intact females were not found to be different from ovariectomized females; Camp & Robinson, 1988b) as had been found previously (e.g., Camp et al., 1986), and as would be expected if ovarian hormones are responsible for the greater response of the intact female animal to an acute injection of AMPH in comparison to

the intact male (see also Beatty et al., 1982). Thus, although circulating gonadal hormones appear to participate in the sex difference in intact animals, in light of the inconsistencies observed, further study is required to determine the exact nature of the contribution of circulating ovarian and testicular hormones to the behavior of the adult male and female rat.

Few studies have taken into account either the perinatal organizational actions of testicular hormones or the differential history of hormonal exposure over the life-span of the two sexes. Both of these factors could be expected to have considerable impact on the behaviors exhibited by intact, adult animals either by affecting directly the response to AMPH, or by affecting the response of adult animals to circulating gonadal hormones. Moreover, there is ample evidence to suggest that the DA systems develop in a sexually dimorphic manner, and that these developmental differences between male and female animals could contribute to sex differences in DA-dependent behaviors observed in adulthood.

Development of Sex Differences

In the classic view of sexual differentiation, exposure to testicular androgens in the immediate pre and postnatal period is considered sufficient for the development of the adult male nervous system, whereas the absence of these androgens is considered sufficient for the development of the female nervous system (e.g., Jost, Vigier, Prépin, & Perchellet, 1973; MacLusky & Naftolin, 1981; Young, Goy, & Phoenix, 1964). Although it is clear that exposure of the male animal to testicular androgens in the perinatal period has profound effects on the development of brain and behavior, a number of researchers have suggested that other factors may be involved in sexual differentiation (Gerall, Dunlap, & Hendricks, 1973; Stewart & Cygan, 1980; Stewart, 1988; Toran-Allerand, 1984; MacLusky & Naftolin, 1981). In particular, it has been suggested that exposure of the developing female animal to ovarian hormones is necessary for the full development of adult, female-typical patterns of brain and behavior (e.g., Döhler et al., 1984; Fitch, Cowell, Schrott, &

Denenberg, 1991; Gerall et al., 1973; Stewart & Cygan, 1980; Toran-Allerand, 1984).

A second, related issue is that many of the effects of ovarian hormones on the development of the female brain and behavior have been found to occur after the traditionally defined perinatal critical period for the actions of testicular androgens (e.g., Fitch et al., 1991; Gerall et al., 1973; Hendricks & Weltin, 1976; Matsumoto & Arai, 1976; Stewart & Cygan, 1980), that is, during the late postnatal (approximately days 10 to 20) or peripubertal (approximately days 20 to 40) periods. Because of these findings, and the finding that testicular androgens may have effects at these later time-points (e.g., Stevens & Goldstein, 1983) it seems probable that differential exposure of the two sexes to ovarian and testicular hormones throughout postnatal development contributes to the sex differences observed in adult animals.

Perinatal Exposure to Testicular Hormones. During the late fetal and immediate postnatal periods in the rat, male and female animals are exposed to differential levels of testicular androgens. Plasma levels of T are higher in males than in females, but the actual sex differences during the postnatal period, although significant, are relatively small (Weisz & Ward, 1980). Prior to birth, however, the level of plasma T in male animals rises dramatically on embryonic day 18 (E18), and it is thought that this surge in plasma T sensitizes the male rat to the relatively lower levels of the hormone that are present after this day (Hoepfner & Ward, 1988; Weisz & Ward, 1980; Ward & Ward, 1985).

This differential exposure of the two sexes to T is known to produce sex-specific changes in the development of the hypothalamic-pituitary-gonadal axis and to produce sexual dimorphisms in many brain and spinal cord nuclei (e.g., the preoptic area and spinal nucleus of the bulbocavernosus muscle) that are associated with reproductive behaviors (see Arnold & Breedlove, 1985; Arnold & Gorski, 1984; Rand & Breedlove, 1988; and Toran-Allerand, 1986 for reviews). These effects on the central nervous system involve basic alterations in the morphology of the brain regions, including changes in the gross size of the structure, cell number, dendritic arborization, and synaptic connectivity (Ayoub,

Greenough, & Juraska, 1983; Gorski, Harlan, Jacobson, Shryne, & Southam, 1980; Greenough, Carter, Steerman, & De Voogd, 1977; Hammer & Jacobson, 1984; Jacobson, Shryne, Shapiro, & Gorski, 1980; Raisman & Field, 1973; Tobet & Fox, 1992).

Although the testicular steroid implicated in these effects is T, this hormone is thought to act primarily via one of its metabolites, estradiol, which is produced locally in brain tissue via the enzyme aromatase (MacLusky, Phillip, Hurlbert, & Naftolin, 1985). Estradiol has been shown to enhance neurite outgrowth in a number of model systems (Honjo et al., 1992; Lorenzo, Díaz, Carrer, & Cáceres, 1992; Reisert et al., 1987; Toran-Allerand, 1976, 1978, 1980, 1991). For example, in cultures of explants of mouse hypothalamic tissue, estradiol has been shown to produce dramatic effects on the extent of neurite outgrowth (e.g., Toran-Allerand, 1976, 1980) and dendritic differentiation within the explant (Toran-Allerand, Hashimoto, Greenough, & Saltarelli, 1983). T may also influence sexual differentiation of the central nervous system via its androgenic properties (e.g., Breedlove & Arnold, 1983a, 1983b; Goldstein & Sengelaub, 1992; Grisham, Casto, Kashon, Ward, & Ward, 1992; Rand & Breedlove, 1988).

In recent years it has become apparent that brain regions not specifically involved in reproductive behaviors are also sexually dimorphic, for example, the cerebral cortex (Juraska, 1990b, 1991; Kolb & Stewart, 1991; Muñoz-Cueto, García-Segura, & Ruiz-Marcos, 1990, 1991; Reid & Juraska, 1992; Stewart & Kolb, 1988; Van Eden, Uylings, & Van Pelt, 1984) and the hippocampus (Juraska, 1990a, 1991; Juraska, Fitch, Henderson, & Rivers, 1985; Juraska, Fitch, & Washburne, 1989; Roof & Havens, 1992). Again, it has been shown that measures of gross size, cell number, and cell growth and connectivity in these brain regions vary as a function of exposure to T in the perinatal critical period (Juraska, 1991; Juraska, Kopcik, Washburne, & Perry, 1988; Kolb & Stewart, 1991; Milner & Loy, 1982; Stewart & Kolb, 1988;).

The brain regions that show these dimorphisms express estrogen receptors during development (Gerlach, McEwen, Toran-Allerand, & Freidman, 1983; MacLusky, Brown,

& Hochberg, 1992; O'Keefe & Handa, 1990; Sheridan, 1979; Shughrue, Stumpf, MacLusky, Zielinski, & Hochberg, 1990; Miranda & Toran-Allerand, 1992; Toran-Allerand, Miranda, Hochberg, & MacLusky, 1992), and the enzyme for conversion of T to estradiol has been found in these tissues (MacLusky, Clark, Naftolin, & Goldman-Rakic, 1987; Shinoda, Yagi, Fujita, Osawa, & Shiotani, 1989). Furthermore, cultures of neurons from these regions have been shown to be sensitive to estradiol (García-Segura, Olmos, Robbins, Hernandez, Meyer, & Naftolin, 1989; Toran-Allerand, 1991; Uchibori & Kawashima, 1985).

The developing monoaminergic neurotransmitter systems also appear to be sensitive to the presence of gonadal hormones (e.g., Guillamón, De Blas, & Segovia, 1988; Küppers, Pilgrim, & Reisert, 1991; Reisert et al., 1987; Wilson & Agrawal, 1979) and, thus, may develop in a sex-specific manner. Of primary importance to the present thesis is the work of Reisert and her colleagues demonstrating that DA neurons, in culture, respond to both T and estradiol with an enhancement of neurite outgrowth (Reisert et al., 1987). This finding suggests that the midbrain DA neurons, upon which AMPH-induced behaviors are dependent, may be influenced in a sex-specific manner by the perinatal hormonal environment. In support of this hypothesis is the evidence that although neither the mature striatum nor midbrain have been shown to express receptors for estradiol, the mRNA for these receptors has been shown to be expressed in the developing striatum (Toran Allerand et al., 1992).

In addition to steroid sensitivity, evidence has been presented to demonstrate that the DA neurons are already sexually dimorphic at the time of onset of the perinatal critical period for sexual differentiation. In culture, DA neurons taken from each sex, early in development (i.e., on day E14), show differences in neuron number, somal size and functional competence (e.g., transmitter uptake and synthesis; Beyer, Eusterschulte, Pilgrim, & Reisert, 1992; Beyer, Pilgrim, & Reisert, 1991; Engele, Pilgrim, & Reisert, 1989; Kolbinger, Trepel, Beyer, Pilgrim, & Reisert, 1991; Reisert & Pilgrim, 1991;

Reisert, Schuster, Zienecker, & Pilgrim, 1990). Because these cells are presumed to be taken at a time before the rise in testicular androgens, and because differences are observed in culture in the absence of steroids, it has been argued that there is a sex-specific genetic program in DA cells independent from responsivity to gonadal hormones. As has been pointed out, however (e.g., Beyer et al., 1991), the very fact that the sexes can be identified at this early stage of development (based on differences in gonad morphology) may point to the presence of unidentified hormonal factors that influence the sexes differently, even at this early stage. If such a factor exists, however, it may not be an early influence of T or estradiol (Beyer et al., 1992). Thus, although the basis of the effect is yet to be specified, it does appear that DA neurons express a sex-specific developmental pattern that can be further modified by the presence of gonadal hormones.

In addition to the implications that these early sex differences have for sex differences in development of the DA neurons, themselves, these differences, whether steroid dependent or independent, could have profound effects on the target regions that this cell group comes to innervate (e.g., NAS, striatum, and mPFC). A number of researchers have suggested that ingrowing cell groups, and the neurotransmitters that they express, influence the structure of their developing target region (Coyle & Molliver, 1977; Kalsbeek et al., 1987, 1989; Stewart, Kühnemann, & Rajabi, 1991; Mattson, 1988; Voorn, Kalsbeek, Jorritsma-Byham, & Groenewegen, 1988).

DA cells arise very early in development (Kalsbeek, Voorn, Buijs, Pool, & Uylings, 1988; Lauder & Bloom, 1977; Reisert et al., 1990; Voorn et al., 1988), and the first axonal outgrowths reach their target regions during a time when these regions are undergoing massive neurogenesis (Kalsbeek et al., 1988; Voorn et al., 1988). Kalsbeek and his colleagues (Kalsbeek et al., 1987, 1989) have shown that destruction of the mesocortical DA projection in early development radically alters the cytoarchitecture of the mPFC. Furthermore, Stewart et al. (1991) have pointed out that the pattern of estrogen receptors in the mPFC during development overlaps strikingly with the pattern of invading

DA terminals. Thus, sex differences could be expressed directly in the DA neurons themselves and through sex-specific ingrowth of these afferent neurons into their target areas. In fact, sex differences in the number of tyrosine hydroxylase-immunoreactive axon profiles in the striatum that are presumed to reflect sex differences in DA ingrowth (viewed on E16, just prior to the onset of the perinatal critical period) have been demonstrated (Ovtscharoff, Eusterschulte, Zienecker, Reisert, & Pilgrim, 1992). Although the mRNA for the estrogen receptor has been shown to be expressed in the developing striatum (Toran-Allerand et al., 1992), it is not known if the pattern of these receptors coincides with the ingrowing DA neurons.

Although there have been few studies investigating the effects of differential hormonal exposure in early development on sex differences in the adult DA system, Stewart et al. (1991) have demonstrated that there are sex-specific alterations in DA levels in the mPFC during development that are dependent on the presence of T at birth, and Siddiqui and Gilmore (1988) have shown that early exposure to gonadal hormones alters levels of striatal DA in adult animals (see also Leret, Gonzalez, Tranque, & Fraile, 1987; Leret & Fraile, 1985). On the other hand, although Crowley, O'Donohue, and Jacobowitz (1978) found sex differences in the levels of DA in the striata of adult male and female rats (males greater than females; but see Siddiqui & Gilmore, 1988), they found no effect of early exposure to T on these sex differences.

In addition to these attempts to assess the effects of early hormone exposure on the overall levels of transmitter in particular brain regions, Becker and Ramirez (1981a) have examined the effects of early exposure to T on sex differences in AMPH-stimulated release of DA from striatal slices, *in vitro*. In adult animals, ovariectomy of the female, but not castration of the male, attenuated AMPH-stimulated release of DA from striatal slices (Becker & Ramirez, 1981b). Exposure to T in the neonatal period did not influence this sex difference (i.e., males castrated on postnatal day 2 and females treated with T on postnatal day 2 showed the male pattern of response in adulthood; Becker & Ramirez,

1981a).

Prepubertal Exposure to Ovarian Hormones. Plasma levels of ovarian hormones, in particular estradiol, increase during postnatal days 10 to 20 in the rat (Döhler & Wuttke, 1975; Meijs-Roelofs, Uilenbroek, de Jong, & Welschen, 1973). Their potential to influence the development of female-typical patterns of brain and behavior has been considered by several groups of researchers (e.g., Becker & Ramirez, 1981a; Denti & Negroni, 1975; Döhler et al., 1984; Fitch et al., 1991, 1992; Gerall et al., 1973; Hendricks & Duffy, 1974; Hendricks & Weltin, 1976; Hines & Goy, 1985; Mack, Fitch, Cowell, Schrott, & Denenberg, 1993; Matsumoto & Arai, 1976; Stewart & Cygan, 1980; Toran-Allerand, 1984, 1991; Watanobe & Takebe, 1987). For example, Gerall et al. (1973) have shown that ovariectomy during the neonatal period defeminizes several aspects of adult female sexual behavior and, further, that male animals castrated at birth and implanted with ovaries show greater levels of female sexual behavior in adulthood than do similarly castrated males without ovarian transplants (see also Hendricks & Duffy, 1974). Others have demonstrated that ovariectomy during the perinatal or late postnatal period defeminizes adult open-field behavior (Blizard & Denef, 1973; Denti & Negroni, 1975; Stewart & Cygan, 1980). Thus, there is evidence to suggest that adult, female-typical patterns of behavior can be influenced by exposure to ovarian steroids in early development.

There is also evidence that exposure to ovarian hormones during early development can produce structural changes to the brain (Fitch et al., 1991; Kolb & Stewart, 1991; Mack et al., 1993; Matsumoto & Arai, 1976; Matsumoto, 1991; Muñoz-Cueto et al., 1991). For example, Matsumoto and Arai (1976) have shown that treatment of female animals with exogenous estradiol from birth to 30 days of age increases the number of axodendritic synapses in the hypothalamic arcuate nucleus to levels similar to those seen in the normal, postpubertal female. Similarly, Denenberg and colleagues have demonstrated that the normal sexual dimorphism of the corpus callosum (male larger than female) is greatly influenced by exposure of the female animal to ovarian hormones in the late

postnatal period (Fitch et al., 1991; Mack et al., 1993).

These data demonstrate two important points. First, that ovarian hormones can influence the development of the female animal, and second, that gonadal hormones can have effects well outside of the traditionally defined perinatal critical period. In fact, recent studies have shown that structural changes to the brain of female animals may occur well into adulthood in response to the fluctuations of ovarian hormones that occur over the estrous cycle (Gould, Woolley, Frankfurt, & McEwen, 1990; Woolley, Gould, Frankfurt, & McEwen, 1990; Woolley & McEwen, 1992).

One factor that complicates the interpretation of the effects of ovarian hormones during early development is the finding that in adult animals sensitivity to estradiol is decreased following long-term absence of circulating hormones (Beach & Orndoff, 1974; Czaja & Butera, 1985; Damassa & Davidson, 1973; Gerall & Dunlap, 1973). For example, it is known that female rats that have been ovariectomized for some time require several priming injections of estradiol to achieve full sensitivity to the hormone (i.e., in the context of female sexual behavior; Beach & Orndoff, 1974; Czaja & Butera, 1985; Damassa & Davidson, 1973; Gerall & Dunlap, 1973). Thus, it has been argued that the apparent organizational actions of estradiol in the female may reflect only these changes in sensitivity to later estradiol (see Hendricks, 1992).

It is not clear, however, that this explanation is sufficient to account for all the findings concerning the effects of ovarian hormones on the development of the female animal. For example, it cannot account for the effects of prepubertal ovariectomy on open-field behavior (Stewart & Cygan, 1980) because sex differences in this behavior have been shown not to depend upon circulating estradiol in the adult animal (Blizard, Lippman, & Chen, 1975; Slob, Bogers, & Van Stolk, 1981; Stewart, Skvarenina, & Pottier, 1975). Furthermore, Becker and Ramirez (1981a) report that prepubertal ovariectomy of the female rat results in a male-like pattern of AMPH-stimulated DA release from striatal slices, *in vitro*. This effect is different from the effect of adult ovariectomy even when the time

after ovariectomy is held constant. Finally, the structural differences in certain brain regions that are thought to depend on early exposure to ovarian hormones cannot be accounted for by this explanation. These considerations make it probable that exposure to ovarian hormones could modify the developing DA neurons or the targets upon which they act.

Many aspects of dopaminergic function continue to develop well into the late postnatal period, reaching adult levels of functioning by the fourth or fifth postnatal week (Ferretti, Blengio, Vigna, Ghi, & Genazzani, 1992; Gazzara, Fisher, & Howard, 1986; Hedner & Lundborg, 1985; Murrin & Zeng, 1990; Pitts, Freeman, & Chiodo, 1990; Rao, Molinoff & Joyce, 1991; Sales, Martres, Bouthenet, & Schwartz, 1989; Shalaby & Spear, 1981; Shalaby, Dendel, & Spear, 1981; Tepper, Trent, & Nakamura, 1990; Trent, Nakamura, & Tepper, 1991). Thus, it remains possible that gonadal hormones present in later postnatal periods could produce further modification of the sex-specific development of the DA system.

One finding of particular relevance to the present thesis is that sensitization to the behavioral effects of AMPH does not occur prior to the fourth or fifth postnatal week in the rat, even though the drug is capable of eliciting behavioral activation in very young animals (Fujiwara, Kazahaya, Nakashima, Sato, & Otsuki, 1987; Kolta, Scalzo, Ali, & Holson, 1990). Sensitization did not develop in animals pre-exposed to either d-amphetamine (Kolta et al., 1990) or methamphetamine (Fujiwara et al., 1987) prior to postnatal day 21. Fujiwara et al. (1987) report weak sensitization when animals were pre-exposed to the drug between 22 and 31 days of age and challenged with the drug at 35 days of age, whereas Kolta et al. (1990) did not find sensitization to d-amphetamine developed until after 40 days of age. Moreover, it has been reported that no sex difference in responsiveness to the drug appears prior to puberty (Kolta et al., 1990), although in this study only a weak sex difference was observed in adult animals. Nevertheless, it seems possible that the development of sensitization to AMPH requires a mature nervous system, and that the sex

difference in sensitization depends on gonadal hormones at puberty in order to be expressed. Although few differences in the functioning of the DA system between pre and postpubertal animals have been reported, one group of researchers has suggested that the negative, long-loop feedback systems (e.g., striatonigral efferents) are not fully developed until after puberty (Andén, Grenhoff, & Svensson, 1988).

The Present Thesis

The present thesis was conducted to answer the question of whether the adult sex difference in behavioral responsiveness to acute and repeated injections of AMPH is due solely to differences between the sexes in the amount and kind of circulating gonadal hormones at the time of testing in adulthood, or whether exposure to gonadal hormones in early development contributes as well.

The development of sensitization can be measured by an increase in behavior over repeated administrations of the drug, increased responsiveness to a challenge injection of the drug following repeated administration, or by comparing the behavior of animals previously exposed to the drug with the behavior of animals receiving the drug for the first time. Previous studies investigating sex differences in response to AMPH have generally examined the change in behavior over days of testing and the response of the animals to a challenge injection of the drug following repeated exposure (e.g., Camp & Robinson, 1988a, 1988b).

In the present experiments, locomotor activity was measured in response to repeated treatments with either a moderate dose of AMPH or the saline vehicle. Following these repeated treatments, all animals were challenged with a low dose of AMPH in a test for sensitization. This methodology allows for a more complete analysis of the effects of gonadal hormones on sex differences in AMPH-induced activity, while still allowing data from the present thesis to be compared to the results obtained by previous researchers. Moreover, because previous investigations of sex differences in the locomotor-activating

effects of AMPH have either used high doses of the drug (Camp & Robinson, 1988b), or have not adjusted doses of the drug to take into account sex differences in peripheral metabolism of AMPH (Savageau & Beatty, 1981), an additional contribution to the literature is made by the present thesis.

Seven experiments were conducted to explore the interaction between early exposure to gonadal hormones in male and female animals and the effects of circulating gonadal hormones in the adult animal on the sex difference in responsiveness to AMPH. In Experiment 1, intact, adult animals were tested in order to determine the extent and the pattern of the sex differences under the testing conditions to be used in all subsequent experiments. In Experiment 2, the contribution of circulating T in the adult animal to the sex difference in responsiveness to AMPH was assessed. Experiments 3 and 4 were conducted to determine whether exposure to T during the early neonatal period contributed to the sex difference in responsiveness to AMPH in adulthood, and whether early hormone exposure changed the response of the animal to circulating estradiol in adulthood. The effect of prenatal androgen exposure on subsequent responsiveness to AMPH in male animals was tested in Experiment 5. Finally, the hypothesis that exposure of the female animal to ovarian hormones over the life-span enhances adult responsiveness to AMPH was tested in Experiments 6 and 7.

EXPERIMENT 1

Sex differences in response to AMPH have been reported for a variety of behaviors including rotation, stereotypy, and locomotor activity (Beatty & Holzer, 1977; Beatty et al., 1982; Becker et al., 1982; Camp et al., 1986; Camp & Robinson, 1988a, 1988b; Robinson, 1984; Robinson et al., 1982; Savageau & Beatty, 1981). Previous studies done to examine sex differences in AMPH-induced locomotor activity have either used high doses of the drug (Camp & Robinson, 1988a, 1988b) or have not corrected for sex differences in peripheral metabolism of AMPH (Savageau & Beatty, 1981). The present experiment was undertaken to determine the sex difference in locomotor activity in response to AMPH, taking these issues into consideration.

Locomotor activity of intact, adult male and female animals was measured in response to repeated treatments with either a moderate dose of AMPH (corrected for sex) or the saline vehicle. Following this pre-exposure period, all animals were challenged with a low-dose of AMPH in a test for sensitization.

Method

Subjects

Subjects were 36 male and 36 female New Colony Wistar rats obtained from eight litters born in the laboratory at Concordia University. Throughout their lives, animals were housed in a temperature and humidity controlled room, under a 12-hour light/dark cycle, with lights on between 8:00 h and 20:00 h. All testing took place during the light cycle. Food and water were available ad libitum throughout the course of the experiment.

Breeding. Adult male and female rats obtained from Charles River Breeding Farms (St. Constant, Québec) were mated in groups of three or four females to one male.

Successful mating was assessed by noting the presence of sperm in daily vaginal smears and this day was defined as embryonic day zero (E0). Females with sperm-positive vaginal smears were removed from the mating cage and individually housed in standard, wire-mesh hanging cages. On E17 to E19, pregnant females were moved to wire-topped, polypropylene shoebox cages with hardwood chip bedding.

Neonatal Manipulations. Beginning on E21, the breeding cages were checked every few hours for the presence of pups. When pups were found (designated postnatal day zero; PN0), the mother was removed from the breeding cage and the litter was sexed and culled to 10 pups, including five male and five female animals. Following these manipulations the pups were returned to the nest, and after a few minutes, the mother was re-introduced. These animals were part of an experiment done to examine the effects of handling on responsiveness to AMPH in the adult animal. Four of the litters were removed from the nest for 15 minutes each day from PN1 to PN21 (weaning), whereas the remaining litters were left undisturbed during this time. On PN25, all animals were housed in same-sex groups (two to three individuals per group) in standard, wire-mesh hanging cages. Animals remained in these groups until they were between 60 and 63 days of age at which time they were individually housed. Animals remained individually housed throughout the experiment.

Apparatus

Locomotor activity was measured in a bank of 12 activity boxes, each box measuring 20 cm (width) x 40 cm (length) x 25 cm (height). Each box was constructed of pressed wood on the sides and back, with a hinged Plexiglas wall on the front. The floor of the cage consisted of stainless steel rods set 1 cm apart and the top was of wire-mesh screen. Four photocells were located around the perimeter of the box. Two were located at a height of 3.5 cm above the floor along the front and rear walls and spaced 20 cm apart to measure horizontal activity, and two were located in the side walls, 20 cm above the floor

and spaced evenly apart to measure vertical movements. Each time the animal entered or exited a photocell beam an activity count was recorded. The room in which the boxes were located was illuminated by the red photocell lights. A 75 dB white noise generator was used to mask extraneous sounds.

Procedure

Subject Assignment. Two pairs of male and two pairs of female animals were chosen at random from each litter (64 subjects), and an additional eight subjects (four male and four female) were chosen at random from the remaining 16 animals. One member of each littermate pair was assigned to receive either D-amphetamine sulphate (AMPH; Smith, Kline and French) or the 0.9% sterile saline vehicle (SAL), whereas the additional eight animals were randomly assigned to either the AMPH or SAL condition. Thus, a 2 (MALE vs. FEMALE) x 2 (AMPH vs. SAL) design was created, with 18 subjects in each group. Animals were assigned to activity boxes such that no two animals of the same group were tested in the same box more than once, and the order of the groups in the 12 boxes was counterbalanced across testing squads.

Pre-exposure Period. Activity testing began when the animals were between 90 and 93 days of age. Animals received an intraperitoneal (ip) injection of either AMPH (1.5 mg/kg or 1.3 mg/kg) or SAL (1.0 ml/kg) just prior to being placed into the activity boxes. The two different doses of AMPH were used to attempt to correct for sex differences in peripheral metabolism of the drug, thus equating brain levels of AMPH. Males received 1.5 mg/kg of AMPH and females received 1.3 mg/kg of AMPH. These doses were estimated from the data provided by Becker et al. (1982) and the ratio of higher doses used by other researchers (i.e., 3.0 mg/kg to males and 2.6 mg/kg to females; Camp et al., 1986; Camp & Robinson, 1988b; Robinson, 1984). Collection of activity data commenced a few minutes after injection of the last rat and continued for two hours. At the end of the activity session, animals were returned to their home cage. Animals were tested every third

day for a total of five pre-exposure days.

Test for sensitization. Three days following the last pre-exposure day, all animals received an injection of AMPH prior to being placed into the activity box in a test for sensitization. Because sensitization to AMPH can be manifested by an increase in stereotyped behaviors that reduce overall locomotor activity (Kuczenski & Segal, 1988; Segal & Kuczenski, 1987; Segal & Schuckit, 1983; Stewart & Vezina, 1987), lower doses were used for the test day. Females received 0.65 mg/kg and males received 0.75 mg/kg, representing one-half of the dose used in the pre-exposure period for each group, respectively. These doses would not be expected to produce stereotypy even in sensitized animals (c.f., Segal & Schuckit, 1983).

Data Analyses. Total photocell counts recorded for each animal on each day of the pre-exposure period were analyzed by a three-way repeated measures analysis of variance (ANOVA) with sex (MALE vs. FEMALE) and drug (AMPH vs. SAL) as the between-subjects factors and pre-exposure day as the within-subjects factor. Total photocell counts recorded for each animal during each 30-minute time-block across the two-hour session on the test day for sensitization were analyzed by means of a three-way repeated measures ANOVA with sex and pre-exposure drug as the between-subjects factors and time-block as the within-subjects factor. (Significance level for all ANOVA effects, $\alpha = .05$). Post-hoc analyses of significant interaction effects were made using simple main effects and Tukey tests where appropriate (Kirk, 1982). Significance levels for post-hoc comparisons were set individually for each interaction investigated using a Bonferroni correction (α/number of comparisons) where the level of α was based on the familywise error rate for the interaction (see Kirk, 1982, pp. 367-371).

Results

Pre-exposure Period

Mean total locomotor activity scores for each group on each of the five days of the pre-exposure period are shown in Figure 1. AMPH increased activity for both sexes (drug: $F(1,68) = 265.9$, $p < .0005$). Female animals showed greater baseline and AMPH-induced locomotion than male animals (sex: $F(1,68) = 140.7$, $p < .0005$). Although female animals showed significantly greater SAL-induced locomotor activity than males ($p < .01$), as can be seen in Figure 1, the sex difference was much larger in those animals treated with AMPH (sex x drug: $F(1,68) = 62.45$, $p < .0005$).

Both sexes showed sensitization of AMPH-induced locomotor activity across days of testing (day x drug: $F(4,272) = 40.22$, $p < .0005$), and this effect was greater in females than in males (day x sex x drug: $F(4,272) = 7.6$, $p < .0005$). Female animals showed a significant increase in activity from days 1 to 3 of the pre-exposure period ($p < .01$). Although male animals also showed an increase from days 1 to 3 of the pre-exposure period ($p < .05$), slightly higher levels of activity were observed on the last day of the pre-exposure period (Day 1 vs. Day 5, $p < .01$). As can be seen in Figure 1, however, the increase in AMPH-induced activity shown by female animals across days of the pre-exposure period was about twice that shown by the male animals.

Test for Sensitization

Mean total locomotor activity scores for each group, at each 30-minute time-block across the two-hour test for sensitization, are shown in Figure 2. Animals pre-exposed to AMPH showed greater activity than animals that received the drug for the first time (pre-exposure drug: $F(1,68) = 11.12$, $p < .005$). Females again showed greater activity than males (sex: $F(1,68) = 138.6$, $p < .0005$), and this effect did not vary significantly as a function of pre-exposure drug (sex x pre-exposure drug: $F(1,68) = 2.2$, $p = .14$).

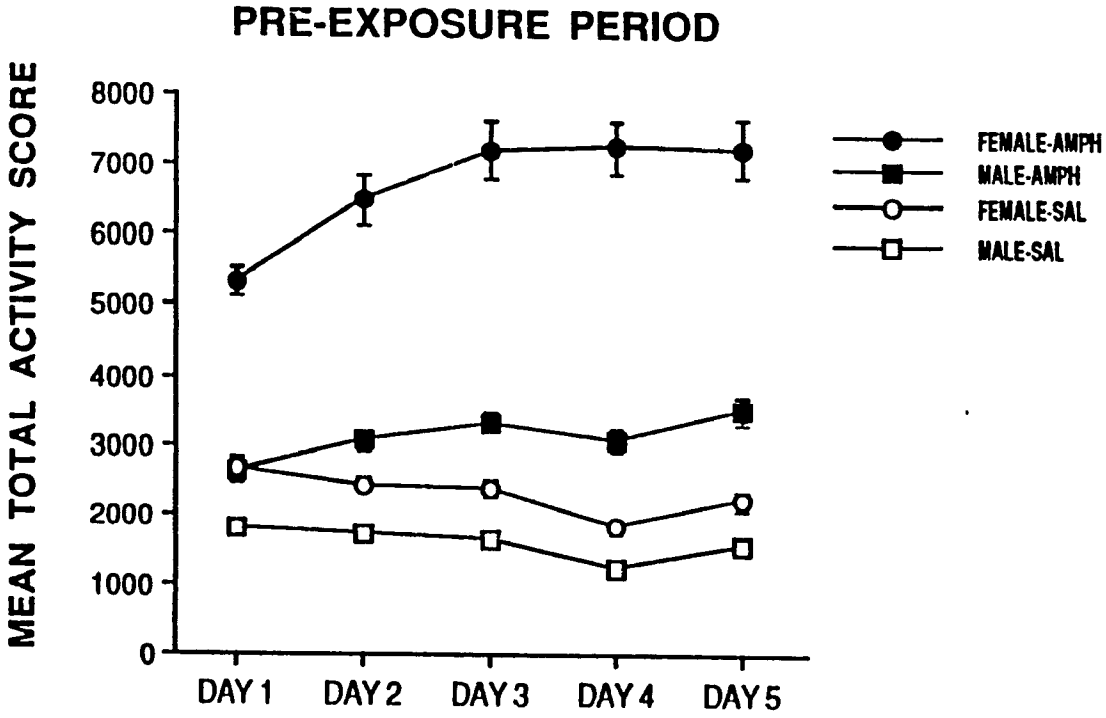


Figure 1. Mean total locomotor activity scores (± SEM) in response to either AMPH (males: 1.5 mg/kg; females: 1.3 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period.

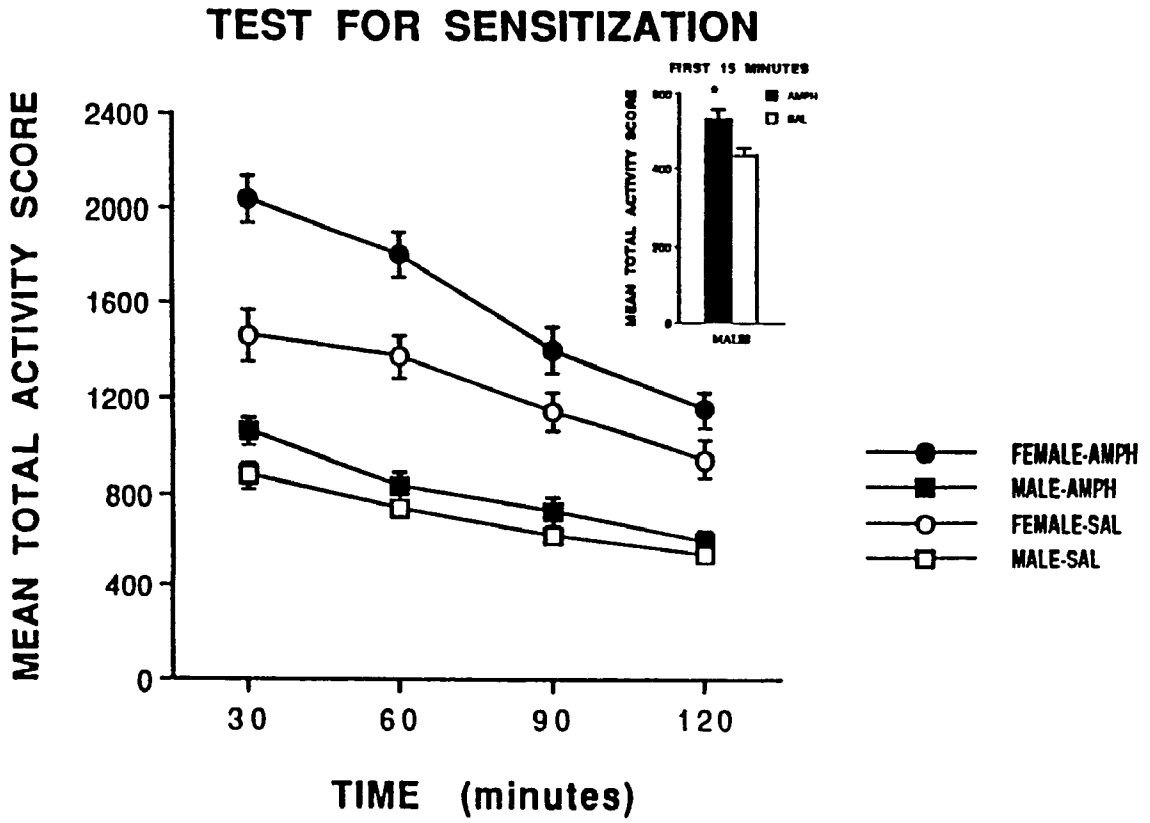


Figure 2. Mean total locomotor activity scores (± SEM) during each 30-minute time-block on the test day for sensitization when all animals received AMPH (males: 0.75 mg/kg; females: 0.65 mg/kg). Inset: Mean total locomotor activity scores (± SEM) during the first 15 minutes of the test session for male animals. (Asterisk indicates significant difference between groups: $t(34) = 2.48$, $p < .01$; one tailed).

Thus, in response to the low-dose challenge injection of AMPH, there was a sex difference in acute responsiveness to the drug (as measured by the difference between male and female animals pre-exposed to SAL) and a sex difference in the sensitized response to AMPH (as measured by the difference between male and female animals pre-exposed to AMPH).

Although the overall sex x pre-exposure drug interaction did not reach significance, females did show greater sensitization than males when sensitization is measured as the difference between animals pre-exposed to AMPH and animals pre-exposed to SAL, as revealed by the significant time-block x sex x pre-exposure drug interaction ($F(3,204) = 3.24$, $p < .05$). Females pre-exposed to AMPH showed significantly greater activity in response to the low-dose AMPH challenge than females pre-exposed to SAL during the first 60 minutes of the test session ($ps < .01$). Males pre-exposed to AMPH, on the other hand, did not show significantly greater activity than their SAL pre-exposed counterparts at any time-block in the present analysis. Thus, although males show a small sensitization effect during the first 30 minutes of the test session (see Figure 2), this effect did not reach statistical significance ($p < .10$) by the simple main effects analysis. As can be seen in the inset of Figure 2, male animals pre-exposed to AMPH did show significantly greater activity than male animals pre-exposed to SAL during the first 15 minutes of the test session.

Discussion

The results of the present experiment confirm the findings of previous researchers for other AMPH-induced behaviors (e.g., Becker et al., 1982; Camp et al., 1986; Camp & Robinson, 1988a, 1988b; Robinson, 1984; Robinson et al., 1982). First, there was a significant sex difference in the locomotor activity produced by an acute injection of AMPH. Female animals showed greater AMPH-induced activity on the first day of the

pre-exposure period than male animals, and in addition, females pre-exposed to SAL showed greater responsiveness to AMPH than males pre-exposed to SAL on the test day for sensitization.

Second, females that received repeated injections of AMPH showed greater sensitization than males. This was evident regardless of whether sensitization was measured as a change in behavior with repeated injections of the drug or as a difference in the responsiveness of drug pre-exposed animals to the subsequent challenge injection of AMPH. Female animals showed a larger increase in AMPH-induced activity over days of the pre-exposure period than did males, and on the test for sensitization when all animals were treated with AMPH, females pre-exposed to the drug showed greater activity than males pre-exposed to the drug.

Finally, when sensitization was measured as the difference in locomotor activity in response to the AMPH-challenge between animals pre-exposed to AMPH and animals pre-exposed to SAL, females showed a greater difference than males. It should be pointed out, however, that this latter sensitization effect was small in the intact male animals tested in this study. Previous research has shown a somewhat larger effect of pre-exposure drug in intact male animals (relative difference between AMPH and SAL pre-exposed animals; c.f., Vezina & Stewart, 1989).

In addition to the significant sex differences in AMPH-induced activity, there was also a significant sex difference in baseline locomotor activity. Previous researchers have reported females to be more active than males under a variety of different conditions (e.g., in the open-field, Blizard et al., 1975; Slob et al., 1981; Stewart et al., 1975). Thus, part of the sex difference in the locomotor response to AMPH can be attributed to this sex difference in baseline activity. It is quite clear, however, that the sex difference in AMPH-induced activity observed in the present experiment was greater than that accounted for by baseline activity alone.

EXPERIMENT 2

As demonstrated by the results of Experiment 1, a significant sex difference exists in AMPH-induced locomotor activity in intact, adult animals. Previous researchers have reported that castration of the adult male enhances their response to both acute and repeated injections of AMPH (e.g., Camp & Robinson, 1988b; Robinson, 1984; but see also Camp et al., 1986). This, coupled with findings from the same experiments showing little effect of ovariectomy in females on responsiveness to repeated AMPH, has led to the suggestion that circulating testicular hormones in the male are responsible for the sex difference in sensitization observed in intact animals (e.g., Robinson, 1988).

The present experiment was undertaken to determine whether the sex difference observed in Experiment 1 could be decreased, or eliminated, when both male and female animals were gonadectomized in adulthood and tested in the presence of circulating T. In addition, in order to determine whether the sex difference in AMPH-induced locomotor activity would persist in the absence of circulating gonadal hormones, a second group of animals was tested without hormone replacement.

Method

Subjects

Subjects were 32 male and 32 female New Colony Wistar rats obtained from Charles River Breeding Farms (St. Constant, Québec). Animals were between 80 and 85 days of age upon arrival in our facility and were housed individually in standard wire-mesh hanging cages. Colony conditions were as described previously. All testing took place during the light cycle.

Surgery

Five to six days after arrival, all animals were gonadectomized under methoxyflurane (Metofane; Pitman-Moore) anaesthesia. At this time, half the animals of each sex were randomly assigned to receive a silastic implant containing either crystalline T (Steraloids) or cholesterol (CHOL; Sigma). The implants were constructed from 50 mm lengths of silastic tubing (ID = 1.57 mm; OD = 3.18 mm; Dow Corning) and were sealed at both ends with silastic adhesive (Dow Corning) such that the exposed length of the implant measured 45 mm. Similar implants have been shown to produce circulating levels of T comparable to those of intact males (Damassa, Smith, Tennent, & Davidson, 1977) and will maintain sexual behavior in castrated male rats (Baum et al., 1986; Mitchell & Stewart, 1989).

Implants were incubated in 0.9% sterile physiological saline for 24 hours and then transferred to 70% ethanol, one to three hours immediately prior to implantation. Implants were placed subcutaneously along the spine via a small incision in the dorsal surface of the neck. Animals received an intramuscular (im) injection of 0.1 ml of penicillin G (Ayerst) after the surgery.

Apparatus

Locomotor activity was measured in the same activity boxes described in Experiment 1.

Procedure

Subject Assignment. Half the animals of each sex and hormone condition were randomly assigned to receive either AMPH or SAL, creating a 2 (MALE vs. FEMALE) x 2 (T vs. CHOL) x 2 (AMPH vs. SAL) design with eight subjects in each group. Assignment of animals to the activity boxes was counterbalanced both within and between testing squads.

Activity Testing. Activity testing began 15 days following the adult surgeries. The procedures used for the pre-exposure period and the test for sensitization were identical to those described for Experiment 1. As in Experiment 1, doses of AMPH were adjusted in an attempt to correct for differences in peripheral metabolism of the drug between the T and CHOL groups, thus equating brain levels of AMPH. Animals with T implants received 1.5 mg/kg of AMPH in the pre-exposure period and 0.75 mg/kg of AMPH on the test for sensitization, whereas animals with CHOL implants received 1.3 mg/kg and 0.65 mg/kg of AMPH, respectively.

Data Analyses. Total photocell counts for each animal on each day of the pre-exposure period were analyzed by a four-way repeated measures ANOVA with sex (MALE vs. FEMALE), hormone (T vs. CHOL), and drug (AMPH vs. SAL) as the between-subjects factors and pre-exposure day as the within-subjects factor. Total photocell counts for each animal during each 30-minute time-block across the two-hour session on the test day for sensitization were analyzed by a four-way repeated measures ANOVA with sex, hormone, and pre-exposure drug as the between-subjects factors and time-block as the within-subjects factor. Post-hoc analyses of significant interaction effects were made using the method described in Experiment 1.

Results

Pre-exposure Period

Figure 3 shows the mean total locomotor scores for each of the eight experimental groups on each day of the pre-exposure period. The activity scores of one MALE-T-AMPH animal ranged from three to five standard deviations above the MALE-T-AMPH group mean on each day of the pre-exposure period and data for this animal were dropped from the analyses.

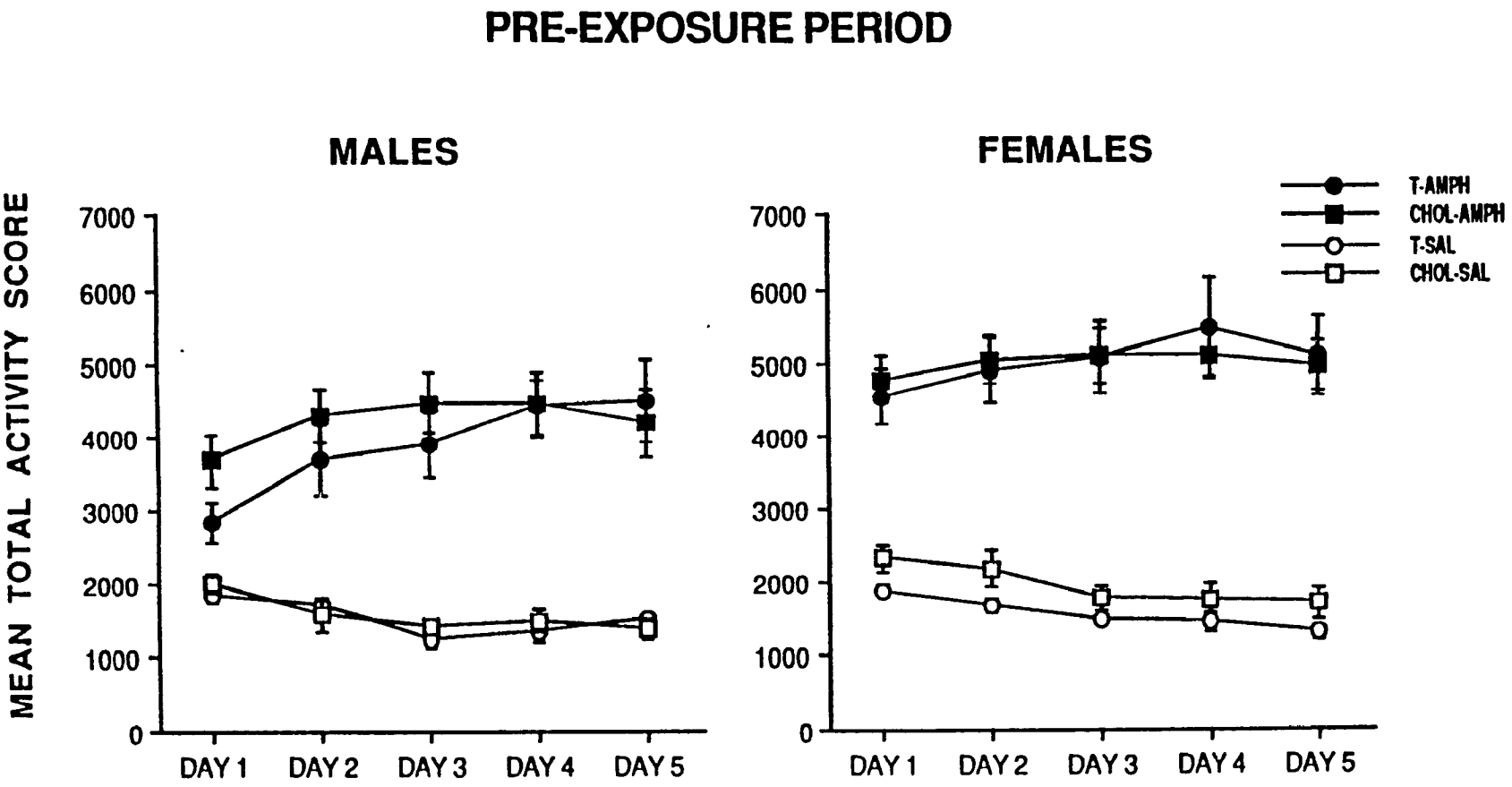


Figure 3. Mean total locomotor activity scores (± SEM) for males (left panel) and females (right panel) in response to either AMPH (T-treated: 1.5 mg/kg; CHOL-treated: 1.3 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period.

AMPH enhanced activity for all groups (drug: $F(1,55) = 235.27$, $p < .0005$), and this effect increased slightly with days of testing (day x drug: $F(4,220) = 17.71$, $p < .0005$). Female animals showed greater activity than male animals (sex: $F(1,55) = 9.59$, $p < .005$), and this sex difference depended on drug group (sex x drug: $F(1,55) = 4.14$, $p = .05$). Post-hoc analyses revealed that the females showed significantly greater AMPH-induced locomotor activity than males ($p < .01$), but there was no sex difference in SAL-treated rats. This effect did not vary over days of testing (day x sex x drug: $F(4,220) < 1$).

There were no differences as a function of hormone. In particular, the three way interaction of sex x hormone x drug was not significant ($F(1,55) < 1$), and therefore, females showed greater AMPH-induced activity than males, both in the absence of circulating gonadal hormones and in the presence of T. Although the overall ANOVA did not reveal significant effects of hormone, T appeared to decrease the AMPH-induced activity of male animals on the first day of the pre-exposure period (see Figure 3). A comparison of these two groups using a t-test revealed that group MALE-T-AMPH showed significantly lower levels of activity than group MALE-CHOL-AMPH ($t(13) = 1.85$, $p < .05$; one-tailed).

Because the overall analysis revealed a sex difference only in AMPH-treated animals, a second, four-way ANOVA with sex and hormone as the between-subjects factors and pre-exposure day and test hour as the within-subjects factors was conducted on the locomotor scores for the AMPH-treated animals. As expected, the main effects for sex and day were significant (sex: $F(1,27) = 7.13$, $p < .02$; day: $F(4,108) = 6.7$, $p < .005$). In addition, this analysis revealed a complex three-way interaction for sex x hormone x hour ($F(1,27) = 7.32$, $p < .02$). Inspection of Figure 4 reveals that it was the high levels of activity for group FEMALE-T during the first hour of testing that contributed most to the sex difference observed in the overall ANOVA.

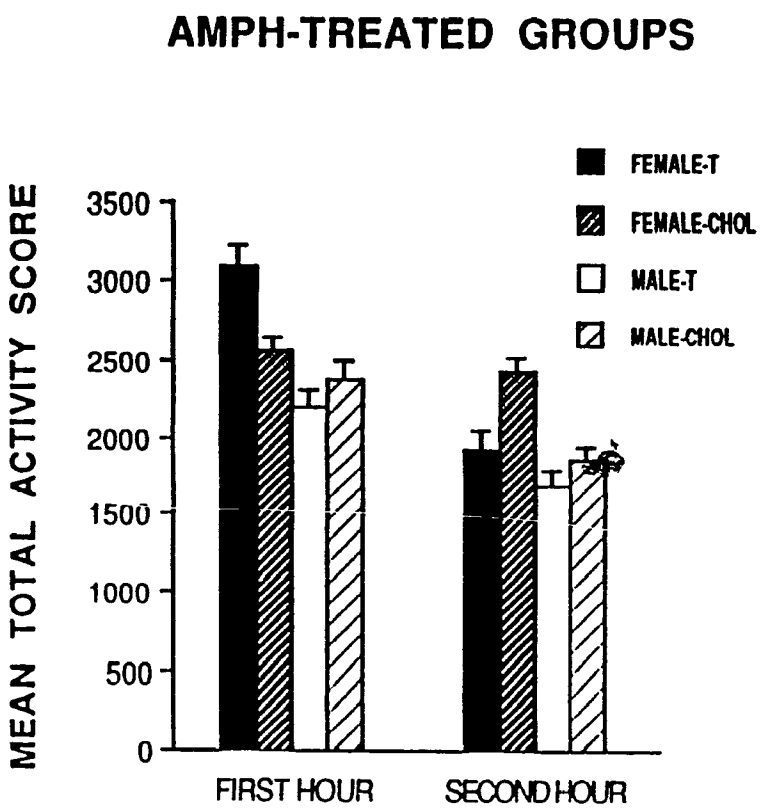


Figure 4. Mean total locomotor activity scores (± SEM) for the first and second hours of each test session, collapsed across the five days of the pre-exposure period for animals treated with AMPH (T-treated: 1.5 mg/kg; CHOL-treated: 1.3 mg/kg).

Test for Sensitization

Figure 5 shows the mean total locomotor scores, at each 30-minute time-block on the test day for sensitization, when all animals received a challenge injection of AMPH. The ANOVA revealed that all groups showed sensitization; animals that had been pre-exposed to AMPH showed higher levels of activity than those animals receiving the drug for the first time (pre-exposure drug: $F(1,55) = 18.08$, $p < .0005$) during the first 90 minutes of the test session (time-block x pre-exposure drug: $F(3,165) = 12.7$, $p < .0005$). Female animals tended to show higher levels of activity than male animals across drug and hormone groups (sex: $F(1,55) = 3.89$, $p = .054$); this effect was significant during the first 60 minutes of the session (time-block x sex: $F(3,165) = 4.27$; $p < .05$). Although there was no main effect for hormone ($F(1,55) < 1$), animals with T implants showed significantly greater activity than animals with CHOL implants during the first 30 minutes of the session (hormone x time-block: $F(3,165) = 6.33$, $p < .005$). As can be seen in Figure 5, this effect appears to be due to the high levels of activity shown by group FEMALE-T-AMPH.

The magnitude of sensitization (as defined by the difference between animals previously exposed to AMPH and those previously exposed to SAL) did not differ as a function of either sex or hormonal treatment (sex x pre-exposure drug: $F(1,55) < 1$; hormone x pre-exposure drug: $F(1,55) = 1.19$, $p > .05$).

Discussion

In this study, a sex difference in overall levels of AMPH-induced locomotor activity was found regardless of the presence of circulating T at the time of testing (see also Beatty et al., 1982). This is in contrast to reports for other AMPH-induced behaviors (Camp et al., 1986; Camp & Robinson, 1988b; Robinson, 1984) in which male and female animals tested after gonadectomy in adulthood were not found to differ in either AMPH-induced

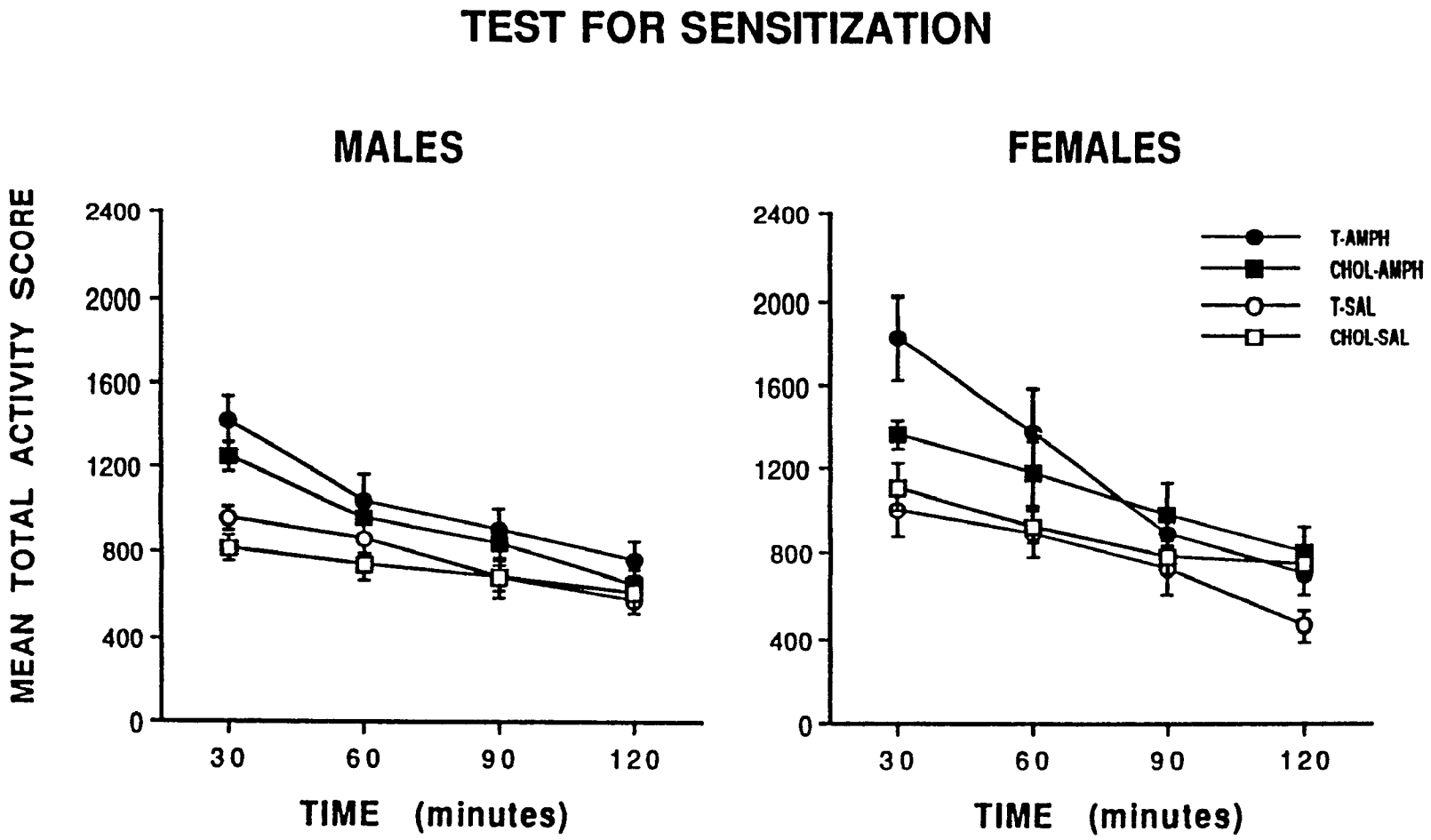


Figure 5. Mean total locomotor activity scores (± SEM) for males (left panel) and females (right panel) during each 30-minute time-block on the test day for sensitization when all animals received AMPH (T-treated: 0.75 mg/kg; CHOL-treated: 0.65 mg/kg).

rotational behavior or stereotypy. These data demonstrate that the sex difference observed in Experiment 1 does not depend solely on sex differences in levels of circulating testicular hormones at the time of testing. On the other hand, it is clear that the sex difference in overall levels of AMPH-induced activity in the present study was small in comparison to that observed for intact animals.

A second finding was that T decreased the acute locomotor response to AMPH in males, but not in females. Consistent with early reports on AMPH-induced locomotor activity (Menniti & Baum, 1981) and stereotypy (Beatty et al., 1982), T decreased the AMPH-induced activity of male animals on the first day of the pre-exposure period even when doses of AMPH were corrected for the effects of T on peripheral metabolism. This finding is also consistent with the data for intact versus castrated males reported by Camp and Robinson (1988b) for stereotypy, but not with that reported by Camp et al. (1986) for rotational behavior. This effect was not observed in SAL-treated males on the test day for sensitization in response to the low dose of AMPH.

The suppressive effect of T on the behavior of AMPH-treated male animals was lost through repeated testing; it appeared that the activity of MALE-T animals increased relative to that of MALE-CHOL animals across days of the pre-exposure period. From inspection of Figure 3, it is clear that T-treated males tended to show the greatest increase in AMPH-induced activity over days of testing observed. It is possible that the lack of change in locomotor activity reflects the development of competing stereotyped behaviors in the other groups of rats (see Experiment 3). Unfortunately, direct, systematic observations of the animals were not available.

On the test day for sensitization, when all animals were challenged with a low dose of AMPH, the magnitude of sensitization did not differ as a function of hormonal condition. That is, the difference in activity in response to the low challenge dose of AMPH between animals pre-exposed to AMPH and animals pre-exposed to SAL was not altered as a function of hormonal treatment. Thus, in contrast to the conclusion of Camp

and Robinson (1988b) that testicular hormones suppress the development of sensitization in male rats, circulating T did not retard sensitization of locomotor activity to a moderate dose of AMPH in the present experiment.

During the first hour of testing throughout the pre-exposure period, and during the initial 30 minutes of the test for sensitization, female animals with T implants tended to have the highest locomotor activity scores. Furthermore, this group tended to show the largest sensitization effect during the early part of the test for sensitization. This is interesting given the previous data of Beatty et al. (1982), showing that females given T had higher levels of stereotypy in response to an acute injection of AMPH than intact males. There are several possible explanations for the sex difference in response to AMPH in the presence of circulating T.

First, exposure of male animals to T during the neonatal period may alter the responsiveness of the adult male animal to T. For example, Beatty et al. (1982) have found male animals castrated prior to postnatal day 10 more responsive to AMPH in the presence of T than males castrated in adulthood. Thus, the fact that in the present study T given to adult females did not reduce their AMPH-induced activity to the level of males may be due to the fact that they were not exposed to T earlier in development. These organizational actions of T could be mediated via either the central nervous system or the peripheral enzymatic response to AMPH. It is thought that the male pattern of hepatic enzyme activity for many compounds is influenced by exposure to androgens prior to puberty (Denef & De Moor, 1972; Gustafsson et al., 1980). Thus, even though androgens are thought to regulate the male pattern of AMPH metabolism (Beatty et al., 1982; Becker et al., 1982), it is possible that exposure to T early in development alters the ability of T to modulate enzyme activity later in life. If this were the case, T given in adulthood would not have the same effect on peripheral metabolism of the drug in female animals.

Another possibility is that the actions of T in the adult animal are mediated by its metabolite, estradiol. Estrogens are produced from androgens via aromatization in a

number of peripheral sites including adipose tissue (e.g., Deslypere, Verdonck, & Vermeulen, 1985; Killinger, Perel, Danilescu, Kharlip, & Lindsay, 1990; Nimrod & Ryan, 1975). Estradiol has been shown to enhance both AMPH-induced behaviors (Becker, 1990b; Becker & Beer, 1986; Menniti & Baum, 1981; and see Experiments 4 and 6 of the present thesis) and AMPH-induced increases in extracellular DA in the striatum of female animals (Becker, 1990b). Thus, if significant amounts of T are converted to estradiol in peripheral sites in the female animal, this could contribute to their greater responsiveness to AMPH in the presence of T.

In summary, it is clear that the sex difference in AMPH-induced locomotor activity cannot be attributed simply to circulating T at the time of testing in the adult male animal. It is also clear, however, that in the absence of circulating hormones the sex difference in AMPH-induced activity is small in magnitude and does not have the same features as that observed for intact animals.

EXPERIMENT 3

The results of Experiment 2 reveal that regardless of the presence of circulating T at the time of testing, females remain more responsive to AMPH than males. This suggests that other variables contribute to the sex difference observed in Experiment 1. In addition to sex differences in circulating gonadal hormones in adulthood, male and female animals receive differential exposure to gonadal hormones at other time-points in development; in particular, male animals are exposed to T during the early critical period for sexual differentiation of the brain. Experiment 3 was undertaken to determine the contribution of differential exposure to T during the early neonatal period to the adult sex difference in responsiveness to AMPH. In order to isolate the contribution of this early T-exposure, all animals were tested in the absence of circulating gonadal hormones.

Method

Subjects

Subjects were 36 male and 36 female New Colony Wistar rats obtained from 10 litters born in the laboratory at Concordia University. Colony conditions were as described in Experiment 1. All testing took place during the light cycle.

Breeding. Procedures for breeding and housing of pregnant females were as described for Experiment 1.

Neonatal Hormonal Manipulations. Beginning on E21, the breeding cages were checked every few hours for the presence of pups. When pups were found (designated postnatal day zero; PN0), they were removed from the cage and transported to another room. There the litter was sexed and culled to a maximum of 12 pups and the following manipulations were made. Male animals were either gonadectomized under hypothermic

anaesthesia (GDXMALE) or were subjected to the anaesthetic procedure alone (MALE). Female animals received a subcutaneous (sc) injection of either 200 µg testosterone propionate (Sigma; TPFEMALE) or the peanut oil vehicle (0.1 ml; FEMALE) on both this and the following day. The site of the injection was sealed with Collodion (Fisher). Approximately half of the litters contained MALE, GDXMALE, and FEMALE animals, whereas the remainder contained only MALE and TPFEMALE animals. This was done to avoid the possibility of TP exposure to the FEMALE and GDXMALE groups. Each litter contained at least two individuals of each sex condition. Following these manipulations the pups were returned to the nest, and after a few minutes, the mother was re-introduced. Pups were removed briefly from the nest approximately 24 hours later on PN1 in order to administer a second treatment to the female animals.

The litters remained undisturbed except for weekly cage cleaning procedures until PN21 when weaning took place. On PN25 to 26, the animals were housed in same-condition pairs in standard, wire-mesh hanging cages. Animals remained in these pairs until they were 63 to 66 days of age at which time they were individually housed.

Adult Surgical Procedures. When animals were 84 to 87 days of age they were either gonadectomized (MALE, FEMALE, TPFEMALE) or sham-operated (GDXMALE) under Metofane anaesthesia. Animals received 0.1 ml penicillin G (im) after this surgery.

Apparatus

Locomotor activity was measured in the same activity boxes described in Experiment 1.

Procedure

Subject Assignment. Within each litter, each member of a pair of animals of each T-exposure and sex condition was randomly assigned to receive either AMPH or SAL, creating a 2 (T vs. NO-T) x 2 (male vs. female) x 2 (AMPH vs. SAL) design with nine

subjects in each group. Assignment of animals to the activity boxes was counterbalanced both within and between testing squads.

Activity Testing. Activity testing began 15 to 16 days following the adult surgeries. The procedures used for the pre-exposure period and the test for sensitization were identical to those described for Experiment 1. Because the animals were tested in the absence of circulating gonadal hormones, the same doses of AMPH were used for all groups. A dose of 1.5 mg/kg was used for the pre-exposure period and a dose of 0.75 mg/kg was used for the test for sensitization.

Scoring of Stereotypy. In a pilot study (Forgie & Stewart, 1991), it was noted that several of the animals receiving the 1.5 mg/kg dose of AMPH showed stereotyped behaviors, consisting of sniffing and rearing in one place, often for extended periods of time. In order to quantify these behaviors, the activity sessions were videotaped on the last day of the pre-exposure period. The behavior of the animals in the AMPH groups was then observed for 2 minutes, every 15 minutes, beginning 13 minutes after the start of the two-hour session, by a rater who was blind to group membership (i.e., sex and T-exposure condition). Each animal was assigned a single stereotypy score, ranging from 0 to 6 during two, 10-second scoring periods (spaced 50 seconds apart), within each of the 2-minute observation periods. The scale used was modified from two sources (Creese & Iversen, 1975; Eichler, Antelman, & Black, 1980): 0 = asleep; 1 = stationary, normal in place activity such as grooming; 2 = increased locomotor activity; 3 = predominately active with bursts of stereotyped sniffing or rearing; 4 = predominately stereotyped sniffing or rearing with bursts of locomotor activity; 5 = continuous stereotyped behavior such as sniffing over a wide area; 6 = continuous focused stereotyped sniffing or rearing in one location without locomotion.

Data Analyses. Total photocell counts recorded for each animal on each day of the pre-exposure period were analyzed by a four-way repeated measures ANOVA with T-exposure (T vs. NO-T), sex (male vs. female), and drug (AMPH vs. SAL) as the between-

subjects factors and pre-exposure day as the within-subjects factor. Total photocell counts recorded for each animal during each 30-minute time-block across the two-hour session on the test day for sensitization were analyzed by means of a four-way repeated measures ANOVA with T-exposure, sex, and pre-exposure drug as the between-subjects factors and time-block as the within-subjects factor. Post-hoc analyses of significant interaction effects were made using the method described in Experiment 1.

To obtain a single stereotypy score for each animal, the median score was calculated from the 16 individual ratings made across the two-hour test session. These median scores were then subjected to a Kruskal-Wallis ANOVA to detect overall, between-group differences. Subsequent pairwise comparisons were then made using the Mann-Whitney U test (Siegel, 1956; significance level for these tests, $\alpha = .05$).

Results

Pre-exposure Period

The mean total locomotor scores for each group on each day of the pre-exposure period are shown in Figure 6. The data for one GDXMALE-SAL animal were discarded from the experiment when the animal received the incorrect injection on Day 3. AMPH significantly increased activity for all groups (drug: $F(1,63) = 107.2$, $p < .0005$). Animals that had been exposed to T at birth were significantly less active than animals that did not receive such exposure (T-exposure: $F(1,63) = 5.40$, $p < .05$). This effect did not vary as a function of drug group, thus T-exposure decreased both baseline and AMPH-induced locomotor activity (T-exposure x drug: $F(1,63) < 1$).

There was also a significant sex difference; female animals (FEMALE + TPFEMALE) showed greater activity than male animals (MALE + GDXMALE; sex: $F(1,63) = 13.14$, $p < .001$). This sex difference tended to depend on drug condition (sex x drug: $F(1,63) = 3.95$, $p = .051$). Post-hoc analyses revealed that there was a significant

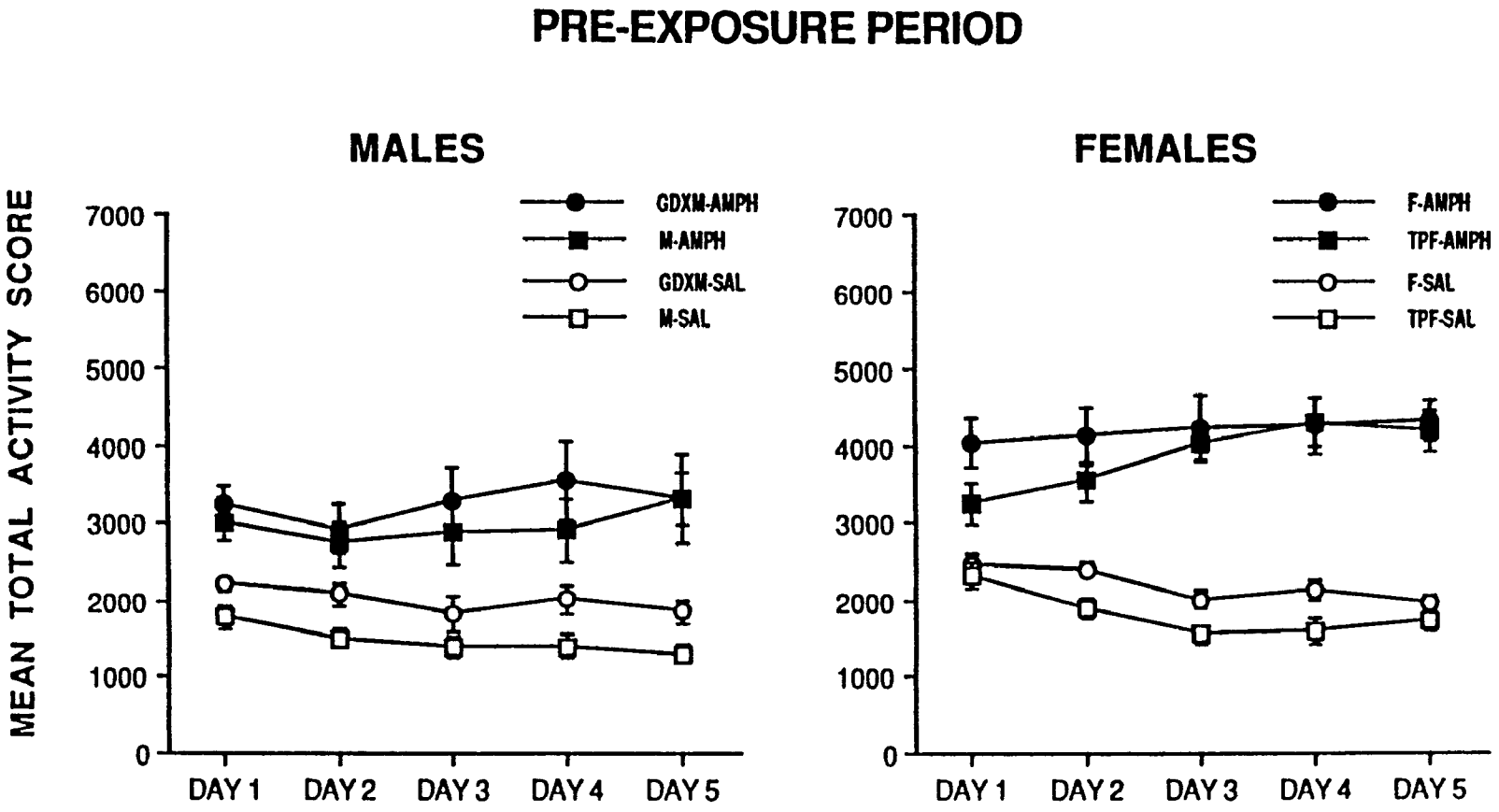


Figure 6. Mean total locomotor activity scores (± SEM) in response to either AMPH (1.5 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period. Data for males (MALE and GDXMALE) and females (FEMALE and TPFEMALE) are shown in the left and right panels of the figure, respectively.

sex difference only for AMPH-treated animals ($p < .01$). The three-way interaction of T-exposure x sex x drug was not significant ($F(1,63) < 1$), and therefore, this effect did not depend on neonatal exposure to T (thus, TPFEMALE > MALE and FEMALE > GDXMALE; see Figure 6).

There was a small increase in AMPH-induced locomotor activity across the five days of the pre-exposure period (day x drug: $F(4,252) = 11.87$, $p < .0005$). Visual inspection of the locomotor scores for individual animals made it clear that whereas some animals showed an increase in locomotor activity in response to AMPH with successive injections, other animals did not, and several showed decreasing activity scores over days of testing. Thus, although there is a tendency for some groups to show an enhancement of AMPH-induced locomotor activity with days of testing (e.g., group TPFEMALE), the effects of day in the ANOVA did not vary as a function of either sex or T-exposure group. This lack of change in behavior might be due to the development of stereotypy in some animals that would in turn reduce the levels of locomotor activity (Kuczenski & Segal, 1988; Segal & Kuczenski, 1987; Segal & Schuckit, 1983; Stewart & Vezina, 1987).

Figure 7 shows the frequency distribution of stereotypy ratings for the four groups of AMPH-treated animals on day 5 of the pre-exposure period. A Kruskal-Wallis ANOVA of the median stereotypy scores revealed a significant effect of group ($H(3) = 8.59$, $p < .05$), with group GDXMALE showing the highest median stereotypy scores and group TPFEMALE the lowest median stereotypy scores. Subsequent analyses revealed a significant effect of T-exposure group ($U = 90.5$, $p < .05$); NO-T animals showed higher median stereotypy scores than T animals. There was no difference as a function of sex.

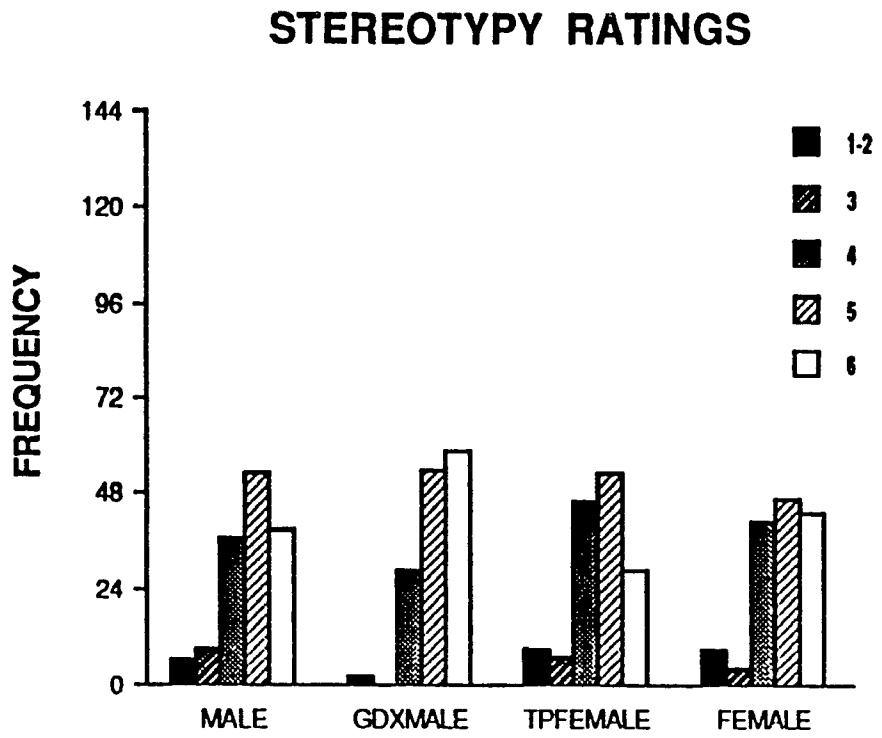


Figure 7. Frequency distribution of stereotypy ratings on pre-exposure day 5 for AMPH-treated animals.

Test for Sensitization

In response to a low-dose AMPH challenge, those animals previously exposed to AMPH showed significantly higher levels of activity than those receiving the drug for the first time (pre-exposure drug: $F(1,63) = 13.99$, $p < .001$; see Figure 8). Thus, sensitization was observed for all groups previously exposed to AMPH. Animals that had been exposed to T, neonatally, showed lower activity scores in comparison to those animals that had not (T-exposure: $F(1,63) = 14.19$, $p < .001$), but this effect did not differ as a function of pre-exposure drug (T-exposure x pre-exposure drug: $F(1,63) < 1$). Therefore, although neonatal T-exposure decreased both the acute (i.e., amongst the SAL pre-exposed animals) and the sensitized (i.e., amongst AMPH pre-exposed animals) response to the challenge injection of AMPH, the magnitude of sensitization (as defined by the difference in activity scores between those animals pre-exposed to AMPH and those pre-exposed to SAL) did not differ as a function of neonatal exposure to T. The finding that NO-T animals (FEMALE + GDXMALE) showed enhanced activity in comparison to T animals (TPFEMALE + MALE) on the test day in response to a low dose of AMPH supports the hypothesis that these animals were responding with greater stereotypy to the 1.5 mg/kg dose of AMPH used in the pre-exposure period.

Activity scores declined over the two-hour session for all groups (time-block: $F(3,189) = 76.39$, $p < .0002$; see Figure 8). This effect did not vary as a function of T-exposure group, but there was a significant interaction of sex x pre-exposure drug x time-block ($F(3,189) = 4.91$, $p < .01$). Activity scores for females (FEMALE + TPFEMALE) pre-exposed to AMPH showed a greater decline across the test session than did the activity scores of males (MALE + GDXMALE; $ps < .01$). There were no other significant effects of sex.

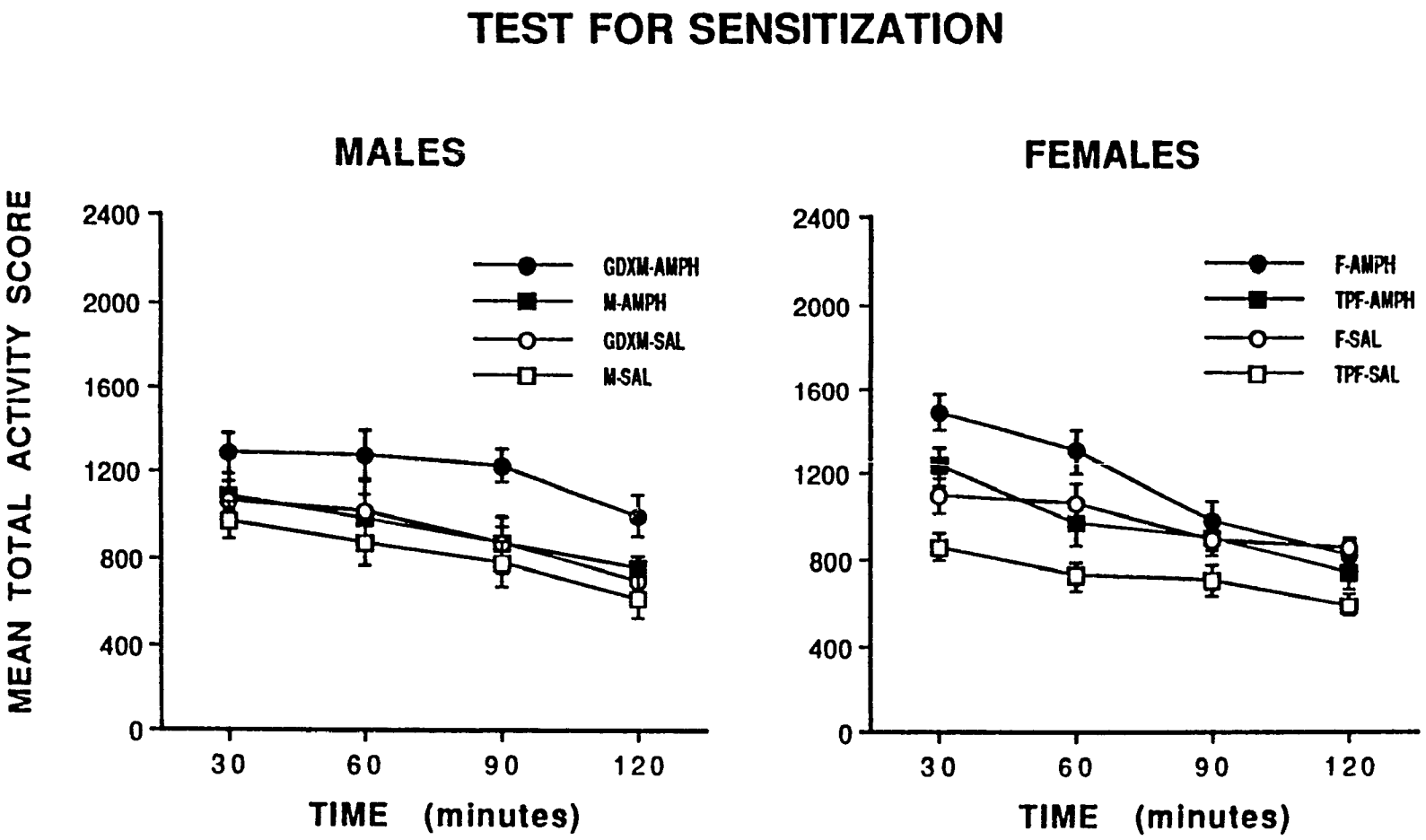


Figure 8. Mean total locomotor activity scores (± SEM) during each 30-minute time-block on the test day for sensitization, when all animals received 0.75 mg/kg AMPH. Data for males (MALE and GDXMALE) and females (FEMALE and TPFEMALE) are shown in the left and right panels of the figure, respectively.

Discussion

In the present experiment, animals exposed to T in the neonatal period (MALE + TPFEMALE) were less responsive to the effects of AMPH on locomotor activity than were those not exposed to T (FEMALE + GDXMALE). It is apparent from the data collected in the present experiment, however, that the manipulation of T in the neonatal period in both males and females was not sufficient to eliminate the sex difference in responsiveness to AMPH, at least during the pre-exposure period. Under these conditions, GDXMALE animals were not as active after AMPH as FEMALE animals, nor were TPFEMALE animals as inactive as MALE animals.

One explanation for this finding is that the timing and/or length of exposure to T during the critical period is an important contributing factor to adult responsiveness to AMPH. Normal male rats receive exposure to T both pre and postnatally (see Ward & Ward, 1985). In the present experiment, the two groups of animals for which T-exposure was experimentally manipulated (groups TPFEMALE and GDXMALE) received exposure to T during only one of these periods. For example, TPFEMALE animals were not exposed to the prenatal surge of T on E18, but by administering large doses of the hormone at birth, they did receive exposure to T during the postnatal period. This exposure to T, postnatally, is not equivalent to the exposure to T experienced by normal male animals and, thus, may not produce the same effects on responsiveness to AMPH. Moreover, the TPFEMALE animals did not receive subsequent exposure to T during later developmental time-points that may be important for development of the adult behavior pattern (during puberty, for example). If this later exposure to T is necessary for the full development of the behavior shown by adult male animals, then this could account for the finding that TPFEMALE animals remain more responsive to AMPH than MALE animals.

It is also apparent that the effect of T-exposure on overall levels of AMPH-induced activity was stronger on the test day for sensitization than during the pre-exposure period.

This can be attributed to the greater stereotypy shown by NO-T animals during the pre-exposure period which would, in turn, decrease levels of locomotor activity (c.f., Segal & Schuckit, 1983). Given the low dose of AMPH used on the test day for sensitization, stereotypy would not be expected to occur even in animals pre-exposed to AMPH. The confounding variable of stereotypy in response to the 1.5 mg/kg dose used during the pre-exposure period makes it difficult to accurately assess the effects of neonatal T-exposure on changes in AMPH-induced locomotor activity with repeated injections of the drug.

The sex of the animal was also important in determining the response of the animal to AMPH. Female animals, regardless of neonatal T-exposure, showed increased locomotor scores in response to AMPH in comparison to male animals during the pre-exposure period. This suggests that factors other than neonatal T-exposure contribute to the greater locomotor responsiveness of female animals to AMPH.

One explanation is that the exposure of the female animal to ovarian hormones over the life-span influences subsequent responsiveness to AMPH. As discussed previously, there are a number of studies that have demonstrated that exposure to ovarian hormones over the life-span influences adult female behavior (e.g., Denti & Negroni, 1975; Gerall et al., 1973; Stewart & Cygan, 1980). Furthermore, it has been found that if females that have been treated with small doses of TP in the neonatal period retain their ovaries throughout development, they show more female-like behavior in adulthood than do TP-treated females that have also been ovariectomized at birth (Blizard & Denef, 1973; Hendricks & Duffy, 1974; but see Hendricks & Weltin, 1976). Although neonatal TP treatment alters reproductive function in the female, the ovaries of these animals produce large quantities of estrogens in adulthood (Weisz & Lloyd, 1965). It should be noted, however, that the doses of TP used in these previous experiments were lower than that used in the present experiment, and the time of administration of the hormone was somewhat later in the perinatal period. It is possible, however, that exposure to ovarian hormones throughout development contributes to the greater responsiveness of the female

animal to AMPH in adulthood.

As was observed in Experiment 2, there was a small sex difference in overall levels of AMPH-induced activity in animals that were tested following gonadectomy in adulthood, however, in neither experiment was the sex difference as large as that observed for the intact animals tested in Experiment 1. In addition, in neither experiment did the female animals show the large increases in AMPH-induced locomotor activity with repeated injections that were observed for intact females.

Finally, neonatal exposure to T, alone, cannot account for the sex difference in the magnitude of sensitization observed for intact animals. Although neonatal T-exposure reduced both the acute and the sensitized response to AMPH on the test day for sensitization, it did not significantly alter the magnitude of the sensitization effect. In the present experiment, however, the smallest sensitization effect was shown by group MALE. Thus, it appears that neonatal exposure to T contributes to the sex difference in AMPH-induced behavior, but this factor alone cannot account for the behavior of intact male and female animals.

EXPERIMENT 4

The results of Experiment 3 indicate that at least part of the adult sex difference in responsiveness to AMPH can be attributed to a sexual dimorphism produced by exposure of the male animal to T during the early neonatal period. When animals were tested with a low dose of AMPH in the absence of circulating gonadal hormones, animals exposed to T at birth (MALE + TPFEMALE) were less active than animals not so exposed (FEMALE + GDXMALE). As in Experiment 2, however, female animals ovariectomized in adulthood did not show large increases in AMPH-induced activity over days of testing, and the sex difference between females and males gonadectomized as adults was clearly smaller than that observed in Experiment 1 for intact animals. These differences appear to be mainly due to differences between intact and ovariectomized female animals. Although previous experiments investigating sex differences in sensitization of AMPH-induced behaviors have not found a role for ovarian hormones (Camp & Robinson, 1988b; Robinson, 1984), circulating ovarian hormones have been found to enhance the acute responsiveness to AMPH in adult female animals (Becker, 1990b; Becker & Beer, 1986; Becker & Cha, 1989; Camp et al., 1986).

The present experiment was carried out to determine whether circulating estradiol at the time of testing would enhance the sex difference in responsiveness to AMPH between male and female rats and, further, whether any facilitatory effect of estradiol would be influenced by neonatal exposure to T.

Method

Subjects

Subjects were 36 male and 36 female New Colony Wistar rats obtained from eight litters born at Concordia University. Details of housing conditions, breeding, neonatal hormonal manipulations, and adult surgeries were similar to those described for Experiment 3 with some exceptions. The animals were moved from one housing facility to another when they were 59 to 63 days of age. From PN25 to the time of the transfer, animals were housed in same-condition groups (two to three individuals per group). Upon arrival in the new facility, animals were housed individually. Adult surgical procedures were undertaken when the animals were 81 to 86 days of age.

Apparatus

The apparatus used in this experiment was identical to that described for Experiment 1 except that the testing room had additional illumination provided by two, overhead red light bulbs (25-watts each).

Procedure

Locomotor testing began 15 to 17 days following the adult surgeries. The procedures for subject assignment and testing of locomotor activity for the pre-exposure period were similar to those described for Experiment 3 except that 30 to 35 minutes prior to receiving the AMPH or SAL injection and being placed into the activity boxes, all animals received 5.0 μg estradiol benzoate (EB; Sigma) in 0.1 ml peanut oil (sc) as they were removed from the home cage. This dose of EB has been shown to facilitate AMPH-induced rotational behavior and to produce an increase in AMPH-induced, extracellular striatal DA in ovariectomized female animals when AMPH is administered 30 minutes after the hormone injection (Becker, 1990b). On the test day, all animals again received EB, 30

to 35 minutes prior to receiving 0.75 mg/kg AMPH (ip).

The last day of the pre-exposure period was videotaped and stereotypy was scored as described in Experiment 3.

Locomotor activity and stereotypy data were analyzed in an identical fashion to that described for Experiment 3.

Results

Pre-Exposure Period

Due to a mechanical failure of the horizontal photocells during the second hour of one activity session on pre-exposure day 5, some locomotor data for three AMPH subjects (one GDXMALE, one TPFEMALE, and one MALE animal) were lost. The missing data for each of the three subjects were estimated with the mean horizontal photocell counts recorded by the other eight members of their respective groups during this time period.

As in Experiment 3, AMPH significantly increased activity for all groups (drug: $F(1,64) = 179.78$, $p < .0005$), and neonatally T-exposed animals showed lower activity scores than did animals not exposed to T (T-exposure: $F(1,64) = 14.17$, $p < .001$; see Figure 9). There was a tendency for the effect of T-exposure to be larger for the animals receiving AMPH (T-exposure x drug: $F(1,64) = 2.91$, $p = .09$).

There were again significant differences in activity between female (FEMALE + TPFEMALE) and male (MALE + GDXMALE) animals (sex: $F(1,64) = 10.67$, $p < .005$; sex x drug: $F(1,64) = 6.40$, $p < .02$). Post-hoc analyses revealed that the sex difference was significant only for animals treated with AMPH ($p < .01$). The three-way interaction was not significant (T-exposure x sex x drug: $F(1,64) < 1$; again, FEMALE > GDXMALE and TPFEMALE > MALE).

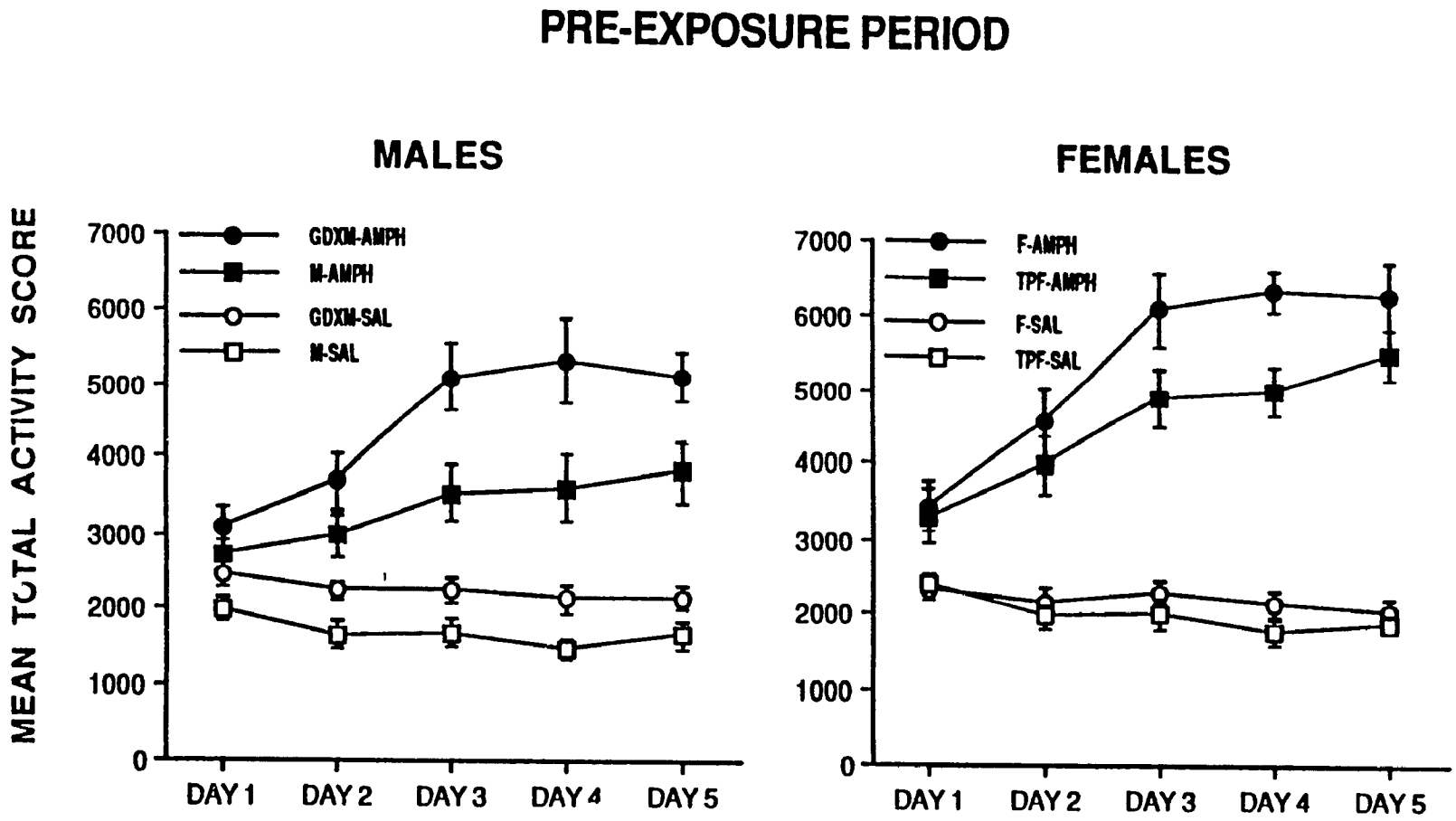


Figure 9. Mean total locomotor activity scores (± SEM) in response to either AMPH (1.5 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period when EB was given prior to each session. Data for males (MALE and GDXMALE) and females (FEMALE and TPFEMALE) are shown in the left and right panels of the figure, respectively.

Animals treated with AMPH showed sensitization in the pre-exposure period as evidenced by an increase in activity across days (see Figure 9). This change in activity was greater in NO-T (GDXMALE + FEMALE) animals than in T (MALE + TPFEMALE) animals (day x T-exposure x drug: $F(4,256) = 3.17$, $p < .05$). Although the groups did not differ in AMPH-induced locomotor activity on the first day of testing ($p > .01$), by day 3 of the pre-exposure period the NO-T animals showed significantly greater AMPH-induced activity than the T animals ($ps < .01$). There were no significant differences as a function of T-exposure group or days in the SAL-treated groups. The change in activity across days for AMPH-treated animals was also greater in females (FEMALE + TPFEMALE) than in males (MALE + GDXMALE; day x sex x drug: $F(4,256) = 2.49$, $p = .05$). Although there was no significant sex difference on the first day of testing ($p > .01$), by pre-exposure day 3 females showed significantly greater AMPH-induced activity than males ($ps < .01$). Again, there were no differences in the SAL-treated groups. The four-way interaction was not significant ($F(4,256) < 1$).

The increase in activity across days may be due to lower stereotypy scores for animals tested with AMPH in the presence of circulating estradiol. Figure 10 shows the frequency distribution of stereotypy ratings for the four groups of AMPH-treated animals on the last day of the pre-exposure period. The median scores of the four groups did not differ significantly from each other ($H(3) = 3.67$, $p = .299$).

Test for Sensitization

When animals pre-exposed to AMPH or SAL in the presence of EB were challenged with AMPH in the presence of EB, animals pre-exposed to AMPH again showed higher levels of activity than did animals receiving the drug for the first time (pre-exposure drug: $F(1,64) = 13.97$, $p < .001$; see Figure 11). Animals exposed to T, neonatally, showed lower locomotor activity scores in comparison to those animals that were not (T-exposure: $F(1,64) = 42.22$, $p < .0005$). As in Experiment 3, neonatal

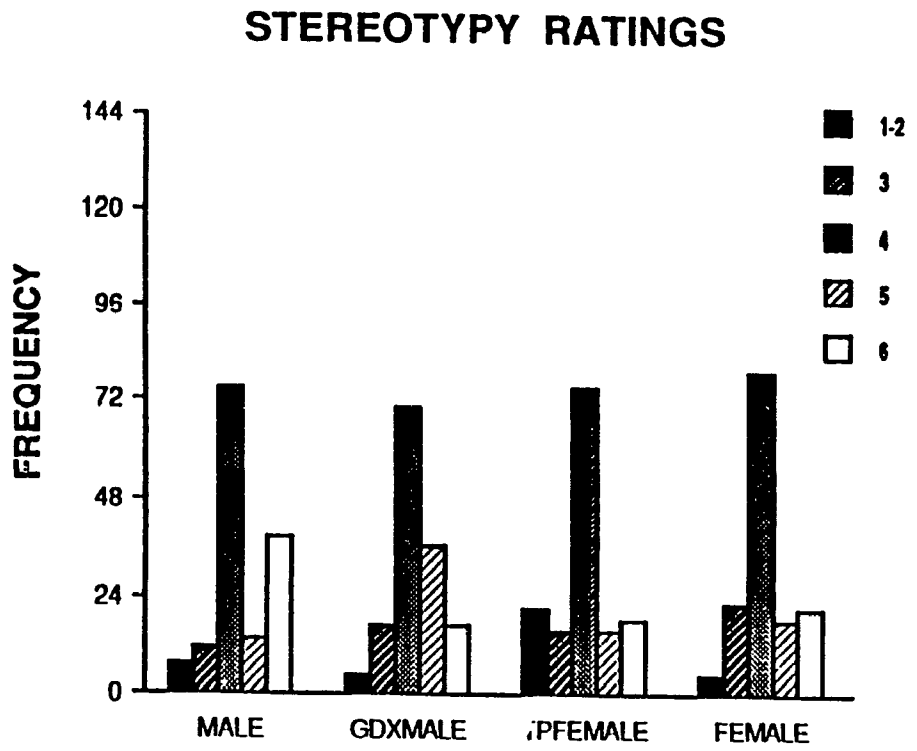


Figure 10. Frequency distribution of stereotypy ratings on pre-exposure day 5 for animals receiving AMPH in the presence of EB.

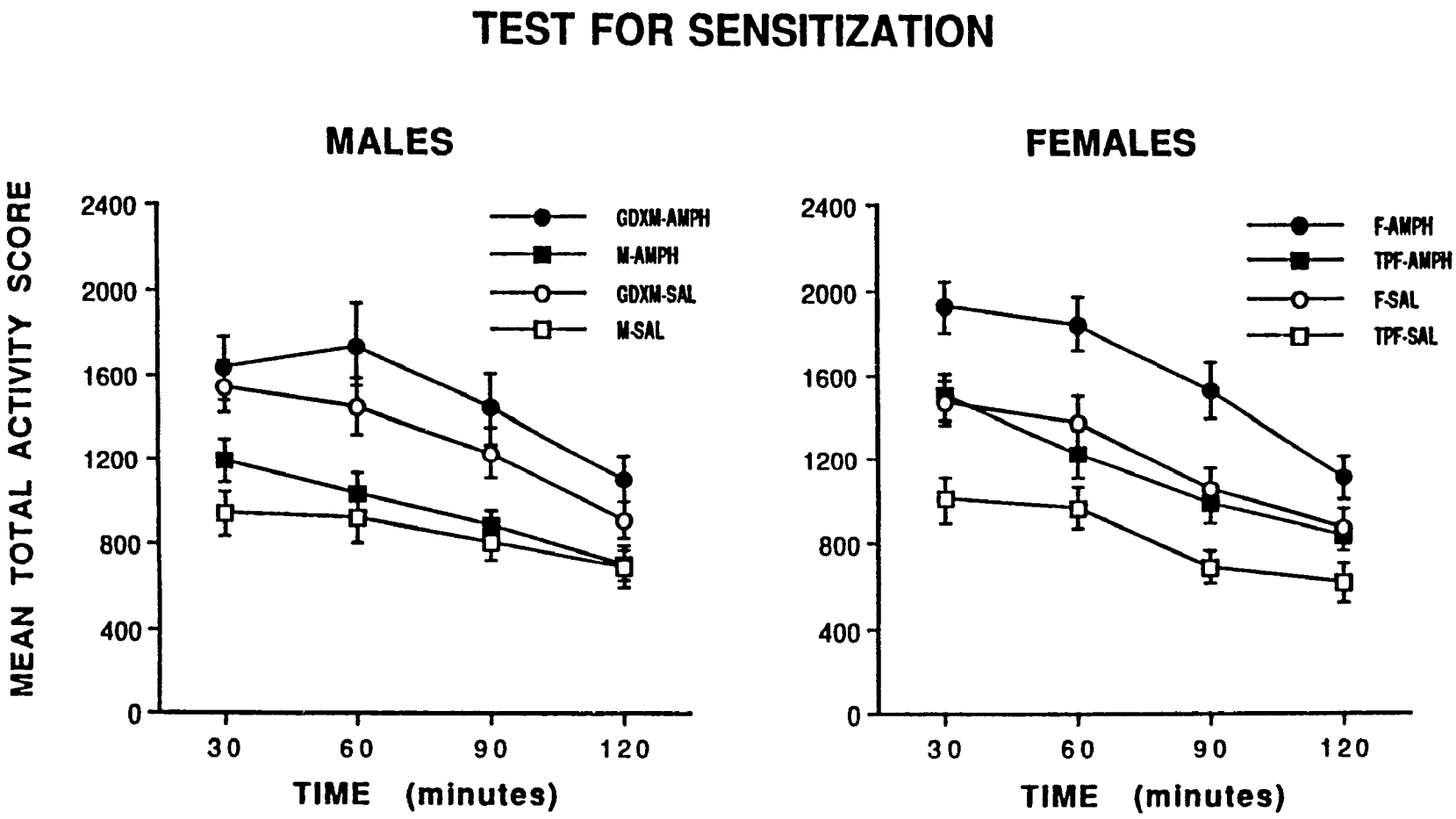


Figure 11. Mean total locomotor activity scores (± SEM) during each 30-minute time-block on the test day for sensitization, when all animals received 0.75 mg/kg AMPH in the presence of EB. Data for males (MALE and GDXMALE) and females (FEMALE and TPFEMALE) are shown in the left and right panels of the figure, respectively.

T-exposure reduced both the acute and sensitized response to the challenge injection of AMPH, but did not reduce the degree of sensitization as measured by the difference between AMPH and SAL pre-exposed animals (T-exposure x pre-exposure drug: $F(1,64) < 1$).

The main effect of time-block was significant ($F(3,192) = 127.6$, $p < .0005$), and thus, activity scores again declined across the two-hour test session for all groups. This effect varied by T-exposure group (time-block x T-exposure: $F(3,192) = 7.3$, $p < .0005$) and sex (time-block x sex: $F(3,192) = 3.03$, $p < .05$). NO-T animals and females showed a greater decline in activity scores across the session than did T animals and males, respectively ($ps < .01$).

Discussion

The results of the present experiment confirm the findings of Experiment 3; exposure to T during the neonatal period decreased responsiveness to AMPH in the adult animal, but was not sufficient to eliminate the sex difference. In the present study, in particular, the mean AMPH-induced activity scores of groups GDXMALE and TPFEMALE fell between those of groups FEMALE and MALE during the pre-exposure period. This finding provides strong support for the hypothesis that, although T-exposure in the neonatal period decreases AMPH-induced behavior, other hormonal factors contribute to the sex difference observed between intact male and female animals.

In the present experiment, the effect of neonatal exposure to T appeared to be magnified when the animals were tested with AMPH in the presence of EB. This magnification of the difference between T and NO-T groups was due to the increased effectiveness of AMPH in the NO-T groups treated with EB both during the pre-exposure period and during the test for sensitization when all animals were treated with AMPH. Clearly neonatal T-exposure rendered the animals less sensitive to the effects of estradiol as

adults. Furthermore, if the results of Experiments 3 and 4 are compared, when animals were tested in the presence of circulating estradiol the NO-T animals showed greater sensitization to AMPH than the T animals, as measured by increases in AMPH-induced activity over the pre-exposure days. These findings suggest that the pattern of behavior observed for intact female animals in Experiment 1, and thus the sex difference in response to repeated drug treatments, depends on the presence of circulating estradiol at the time of testing.

As noted in the general introduction, estradiol has been shown to have complex effects on DA function depending on the dose of the hormone used and the time after administration that the effect is measured (Van Hartesveldt & Joyce, 1986). Given that low doses of estradiol appear to enhance the release of DA shortly after the hormone is given (Di Paolo et al., 1985; Morissette et al., 1990, Becker, 1990a, 1990b; Castner et al., 1993), it might be supposed that EB in combination with the intermediate dose of AMPH used in the pre-exposure period would act like a higher dose of AMPH to induce greater stereotypy, and concomitantly, decrease locomotor activity (c.f., Kuczenski & Segal, 1988; Segal & Schuckit, 1983). This would also be expected given the evidence that intact, adult females show greater levels of stereotypy than intact, adult males (Camp & Robinson, 1988a, 1988b) in response to high doses of AMPH. Instead, in the present experiment in which a relatively moderate dose of AMPH was used, locomotor activity was increased and stereotypy was decreased in the presence of EB.

Estradiol has also been shown to have longer-term effects on the midbrain DA systems and on behaviors dependent on these systems (e.g., Becker & Beer, 1986; Becker, Snyder, Miller, Westgate & Jenuwine, 1987; Chiodo & Caggiula, 1983; Joyce et al., 1984). Therefore, although a single, low dose of estradiol may either enhance or suppress DA-dependent behaviors, acutely, long-term effects induced by each injection of EB may have contributed to the results obtained in this experiment, in that the animals were receiving multiple exposures to a low dose of the hormone throughout the pre-exposure

period. In fact, the injection regime used in the present experiment approximates the pulsatile estradiol peaks experienced by the intact female during the normal estrous cycle (Feder, 1981; Freeman, 1988).

Finally, neither neonatal exposure to T, nor sex (grouped across conditions), significantly altered the magnitude of sensitization to AMPH when the measure of sensitization was the difference in response to AMPH between animals pre-exposed to AMPH and those receiving the drug for the first time. This indicates that neither of these two variables, alone, can account for the sex difference in sensitization observed in Experiment 1. On the other hand, group MALE again showed the smallest sensitization effect on the test day, and the effect shown by both groups MALE and FEMALE was similar to that observed for the intact male and female animals tested in Experiment 1.

In summary, exposure of the male animal to T during the early neonatal period for sexual differentiation contributes to the sex difference in AMPH-induced activity in adult animals. This exposure to T, postnatally, appears to decrease overall levels of AMPH-induced activity, but is not sufficient to explain the sex difference observed for intact animals. Exposure of both sexes to gonadal hormones at other times in development also appears to be involved. In particular, the presence of circulating estradiol in the intact female animal contributes to the greater activity, and perhaps sensitization, exhibited by this group. Neonatal exposure to T would seem to reduce sensitivity to estradiol in the adult animal.

EXPERIMENT 5

In Experiments 3 and 4, animals that were exposed to T for a limited time in the perinatal period (males gonadectomized at birth and females administered TP at birth; groups GDXMALE and TPFEMALE) showed levels of AMPH-induced locomotor activity intermediate between those of normal males and females. This finding led to the suggestion that exposure to T both pre and postnatally produces a greater suppression of AMPH-induced activity in the adult male than exposure at either one of these times alone.

The present experiment tested this hypothesis by manipulating exposure to T during both the pre and postnatal periods in male animals. Because T has both androgenic properties (as T or its androgenic metabolite, dihydrotestosterone) and estrogenic properties (as its estrogenic metabolite, estradiol), prenatal exposure to T was manipulated by administration of either flutamide (FLUT; an androgen receptor blocker) or 1,4,6-androstatrien-3,17-dione (ATD; which blocks the aromatization of T to estradiol) to the pregnant dams during gestation.

Method

Subjects

Subjects were 72 male New Colony Wistar rats obtained from 18 litters born in the laboratory at Concordia University. Colony conditions were as described previously. All testing took place during the light cycle.

Breeding. Procedures for breeding and housing of pregnant females were as described for Experiment 1.

Prenatal Hormonal Manipulations. On E14, females appearing to be pregnant by visual inspection were randomly assigned to receive one of three treatments: 5.0 mg FLUT

(Schering), 5.0 mg ATD (Steraloids), or 0.1 ml of the propylene glycol vehicle (PROP). The doses of FLUT and ATD used have been shown to produce significant effects on the development of male and female sexual behavior (e.g., Clemens & Gladue, 1978; Gladue & Clemens, 1982). FLUT and ATD were suspended in PROP and then re-suspended by vortexing immediately prior to injection. All injections were given in the thigh (im). Treatments were administered on a daily basis up to and including E22.

Neonatal Hormonal Manipulations. Beginning on E21, the breeding cages were checked every few hours for the presence of pups. Following the birth (PN0), the mother was removed from the breeding cage and the pups were transported to another room. There the litter was sexed and culled to a maximum of 12 pups, including at least three female animals. Male animals were either gonadectomized under hypothermic anaesthesia (GDXMALE) or were subjected to a sham operation in which the skin of the abdomen and the abdominal wall were opened and then sutured closed (MALE). A full sham operation was necessary because the FLUT-treated animals could not be sexed externally. Following these manipulations the pups were returned to the nest, and after a few minutes, the mother was re-introduced.

The litters remained undisturbed except for weekly cage cleaning procedures until PN21 when weaning took place. On PN25 to 26, all animals were housed in same-condition groups (two to three individuals per group) in standard, wire-mesh hanging cages. Animals remained in these groups until they were 60 to 64 days of age at which time they were individually housed.

Adult Surgical Procedures. When animals were 81 to 87 days of age they were either gonadectomized (MALE) or sham-operated (GDXMALE) under Metofane anaesthesia. Animals received 0.1 ml penicillin G (im) after this surgery.

Apparatus

Locomotor activity was measured in the same activity boxes described in Experiment 1.

Procedure

Subject Assignment. One pair of MALE animals and one pair of GDXMALE animals were randomly chosen from each of eighteen litters (six litters per prenatal treatment condition). One member of each pair was randomly assigned to receive either AMPH or SAL, creating a 2 (MALE vs. GDXMALE) x 3 (ATD vs. FLUT vs. PROP) x 2 (AMPH vs. SAL) design with six subjects in each group. Assignment of animals to the activity boxes was again counterbalanced both within and between testing squads.

Activity Testing. Activity testing began 15 to 16 days following the adult surgeries. Procedures and drug doses for the pre-exposure period and the test for sensitization were as described for Experiment 3. The last day of the pre-exposure period was videotaped, and stereotyped behavior was scored as described in Experiment 3.

Data Analyses. Total photocell counts recorded for each animal on each day of the pre-exposure period were analyzed by a four-way repeated measures ANOVA with prenatal treatment (ATD vs. FLUT vs. PROP), postnatal treatment (MALE vs. GDXMALE), and drug (AMPH vs. SAL) as the between-subjects factors, and pre-exposure day as the within-subjects factor. Total photocell counts recorded for each animal during each 30-minute time-block across the two-hour session on the test day for sensitization were analyzed by means of a four-way repeated measures ANOVA with prenatal treatment, postnatal treatment and pre-exposure drug as the between-subjects factors and time-block as the within-subjects factor. Post-hoc analyses of significant interaction effects were made using the method described in Experiment 1.

Median stereotypy scores were analyzed as described in Experiment 3.

Results

Pre-Exposure Period

Mean total locomotor activity scores for each group on each pre-exposure day are shown in Figure 12. As expected, AMPH significantly increased activity for all groups (drug: $F(1,60) = 82.81$, $p < .0005$). There were no significant effects of either prenatal or postnatal treatment. For male animals, there are clearly no differences in AMPH-induced activity between the three prenata. treatment groups (see Figure 12). For GDXMALE animals, however, there appears to be a tendency for prenatal androgen blockade (FLUT animals) to enhance AMPH-induced activity, and prenatal aromatase inhibition (ATD animals) to decrease AMPH-induced activity relative to those animals treated with vehicle prenatally.

There was a small increase in AMPH-induced locomotor activity across the five days of the pre-exposure period (day x drug: $F(4,240) = 7.80$, $p < .0005$). Visual inspection of the locomotor scores for individual animals made it clear, that as observed in Experiment 3, not all animals showed an increase in locomotor activity in response to AMPH with successive injections. Thus, although there was a tendency for some groups to show an enhancement of AMPH-induced locomotor activity with days of testing (e.g., group GDXMALE-FLUT), the effects of day in the ANOVA did not vary as a function of either prenatal or postnatal treatment.

Figure 13 shows the frequency distribution of stereotypy ratings for the six groups of AMPH-treated animals on day 5 of the pre-exposure period. Analyses of these data revealed a trend towards a significant effect of prenatal treatment ($H(2) = 5.64$, $p = .06$). ATD animals showed greater median stereotypy scores than either group FLUT ($U = 36$, $p < .05$) or group PROP ($U = 39$, $p = .06$), and the latter two groups did not differ from each other ($U = 69$, $p = .887$). There were no differences as a function of postnatal treatment when the data were pooled across prenatal treatment conditions, and although

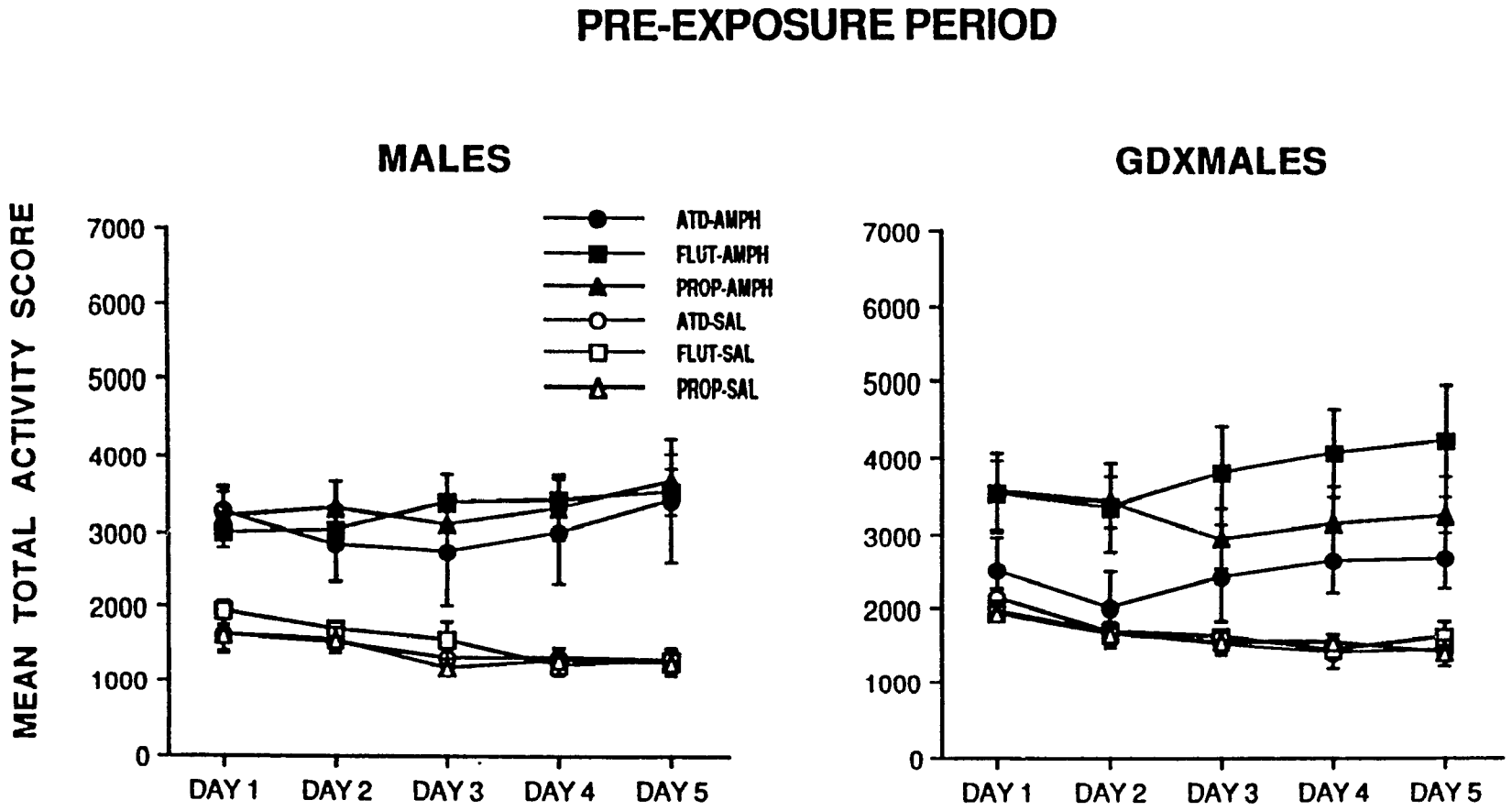


Figure 12. Mean total locomotor activity scores (± SEM) for MALE (left panel) and GDXMALE (right panel) animals in response to either AMPH (1.5 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period.

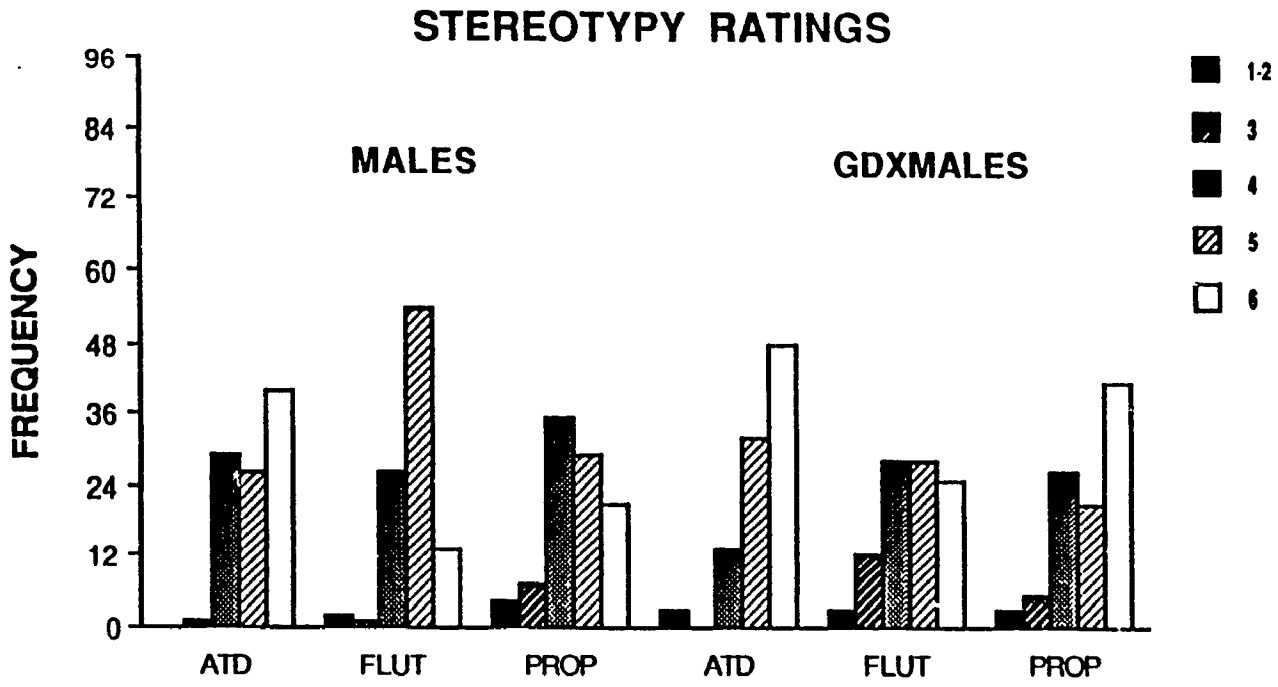


Figure 13. Frequency distribution of stereotypy ratings on pre-exposure day 5 for animals receiving AMPH.

group MALE-PROP tended to show lower median stereotypy scores than group GDXMALE-PROP, this effect did not reach significance ($p > .10$).

Test for Sensitization

Figure 14 shows the mean total locomotor scores for each group at each 30-minute time-block across the two-hour session on the test for sensitization when all animals received 0.75 mg/kg AMPH prior to the behavioral test. In order to simplify the data presentation, the activity of the SAL groups was averaged across prenatal treatment conditions for MALE and GDXMALE animals, respectively. These average values are plotted in each panel of Figure 14 for comparison to the AMPH pre-exposed groups.

Animals previously exposed to AMPH showed greater activity in response to the low-dose challenge than animals that received the drug for the first time (pre-exposure drug: $F(1,60) = 11.83$, $p < .005$). There were no significant effects of prenatal treatment (prenatal treatment: $F(2,60) < 1$; prenatal treatment x pre-exposure drug: $F(2,60) < 1$), however, GDXMALE animals showed greater levels of activity than did MALE animals (postnatal treatment: $F(1,60) = 8.00$, $p < .01$). This effect did not vary as a function of pre-exposure drug (postnatal treatment x pre-exposure drug: $F(1,60) < 1$), and therefore, although postnatal exposure to T decreased locomotor activity in response to the low, challenge dose of AMPH in both animals pre-exposed to AMPH and animals pre-exposed to SAL, postnatal treatment did not influence the magnitude of sensitization as defined by the difference between animals pre-exposed to AMPH and those pre-exposed to SAL.

Activity for all groups declined across the two-hour test session (time-block: $F(3,180) = 65.41$, $p < .0005$), but this effect did not vary as a function of prenatal treatment, postnatal treatment, or pre-exposure drug.

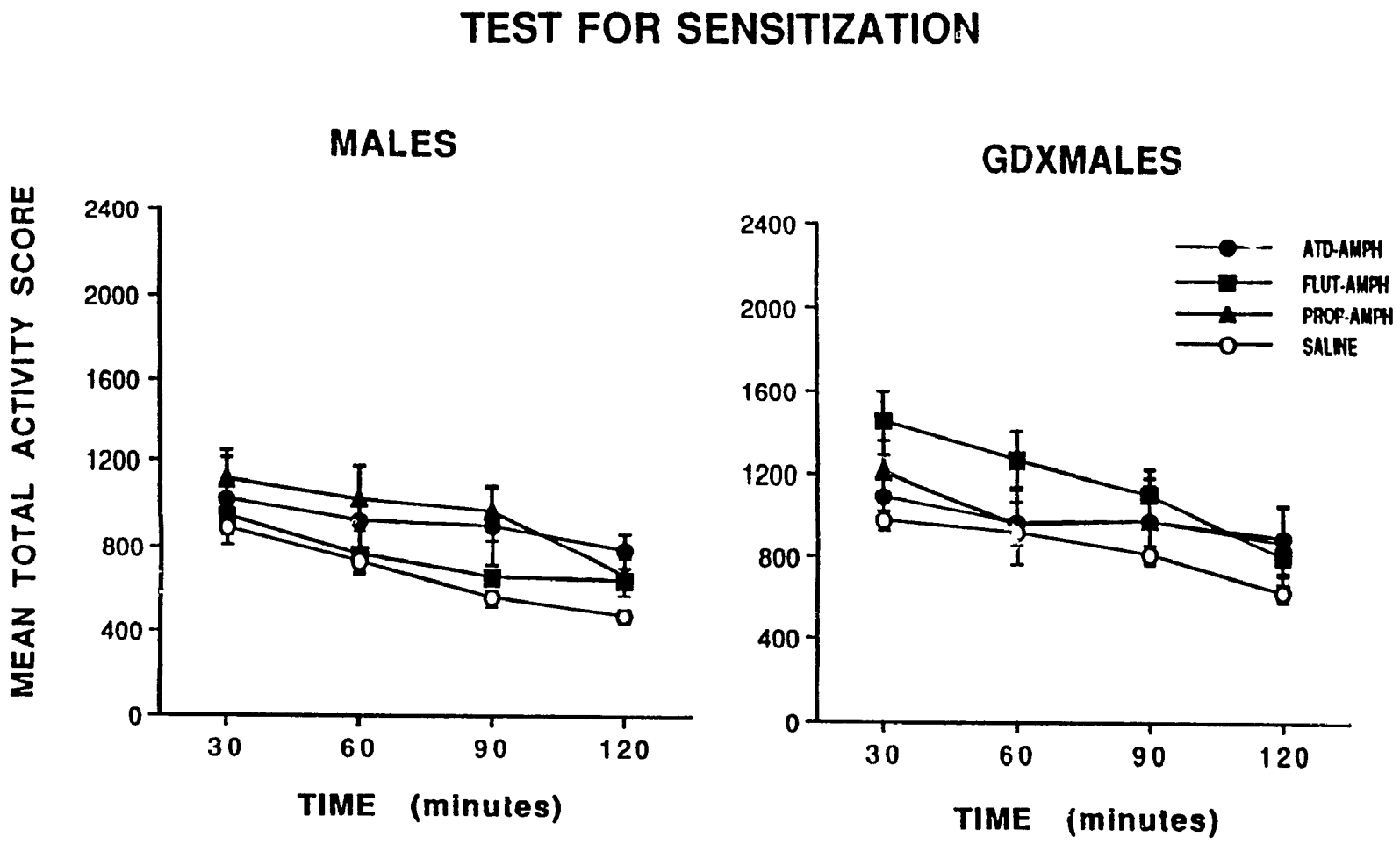


Figure 14. Mean total locomotor activity scores (± SEM) for MALE (left panel) and GDXMALE (right panel) animals during each 30-minute time-block on the test day for sensitization when all animals received 0.75 mg/kg AMPH.

Discussion

In the present experiment, prenatal exposure to T was found not to influence the AMPH-induced locomotor activity of the adult male animal. Neither blockade of androgen receptors (group MALE-FLUT), nor blockade of the conversion of T to estradiol (group MALE-ATD), prenatally, rendered male animals more responsive to AMPH than the untreated control animals (group MALE-PROP). Thus, postnatal exposure to T appears to be the perinatal factor responsible for the reduced sensitivity to AMPH in the adult male. On the other hand, the finding that prenatal androgen blockade tended to enhance AMPH-induced locomotor activity in male animals not exposed to T, postnatally (group GDXMALE-FLUT), suggests some role for androgenic stimulation, prenatally. The fact, however, that prenatal treatments were without effect in animals that retained their testes throughout development suggests that exposure to T, either in the immediate postnatal period or at some other time during development, is sufficient to decrease sensitivity to AMPH in the adult male.

When MALE and GDXMALE animals were tested in the absence of circulating gonadal hormones, they did not show significantly different overall levels of AMPH-induced locomotor activity during the pre-exposure period, but MALE animals did show a decreased response to the low dose of AMPH on the test day for sensitization in comparison to GDXMALE animals (see Experiment 3). This finding provides further support for the hypothesis that exposure to T in the postnatal period contributes to the lower AMPH-induced activity exhibited by adult male animals.

The inability to demonstrate a clear effect of postnatal T-exposure during the pre-exposure period may be due to differences in stereotypy. Unlike in Experiment 3, however, stereotypy was not found to differ significantly as a function of postnatal treatment group. This may be due to the fact that the effect of postnatal treatment differed within prenatal treatment groups. For example, in FLUT-treated animals, group MALE

tended to show higher median stereotypy scores than group GDXMALE, whereas the opposite relationship was observed for PROP-treated rats. Interestingly, prenatal blockade of estrogenic stimulation (groups ATD-MALE and ATD-GDXMALE) tended to enhance stereotyped behaviors in response to AMPH. This greater stereotypy in ATD-treated animals may have contributed to the tendency for this group to show the lowest levels of activity during the pre-exposure period.

In Experiments 3 and 4, group TPFEMALE showed greater activity than group MALE and showed less activity than group FEMALE. Based on the results of the present experiment, there is little evidence to suggest that this greater activity was due to the lack of exposure to T, prenatally. The present findings do not rule out the possibility that T-exposure at a later developmental time, such as puberty, contributed to the lower AMPH-induced activity of group MALE, or the possibility that the presence of ovaries in group TPFEMALE throughout development increased responsiveness to AMPH relative to that of group MALE.

In Experiments 3 and 4, group GDXMALE was found to be more active than group MALE, but not as active as group FEMALE. The greater activity of group GDXMALE in comparison to group MALE can be attributed to the lack of exposure to T during the postnatal period and, perhaps, to the lack of exposure to T at later times during development. If these were the only factors involved, however, group GDXMALE would be expected to show levels of AMPH-induced activity similar to those of group FEMALE. Based on the behavior of group GDXMALE-FLUT-AMPH in the present experiment, it might be argued that the lack of androgenic stimulation, prenatally, combined with a lack of exposure to T, postnatally, was responsible for the enhanced response of female animals to AMPH in adulthood. Thus, the lack of exposure to T both pre and postnatally in normal female animals may contribute to the sex difference in AMPH-induced activity observed in Experiment 1.

EXPERIMENT 6

In Experiments 3 and 4, females were found to show greater responsiveness to AMPH than males during the pre-exposure period, regardless of neonatal T-exposure. This finding led to the suggestion that prior exposure of the female animal to ovarian hormones throughout development may enhance their responsiveness to AMPH in adulthood. Furthermore, when group FEMALE was tested in the presence of circulating estradiol, the pattern of activity across pre-exposure days was more like that of intact females. This finding led to the suggestion that sex differences in circulating estradiol contribute to the sex difference in responsiveness to AMPH observed for intact male and female animals. The present experiment was conducted to investigate these possibilities.

Responsiveness to repeated treatment with AMPH was assessed in female animals that were ovariectomized prior to puberty or as adults. In adulthood, animals were tested either in the presence of estradiol, or in the absence of circulating gonadal hormones. Initially, two separate experiments were carried out, one in which animals were ovariectomized on PN0 and the other in which animals were ovariectomized post-weaning (on PN25). The results of the two experiments were in some aspects dissimilar, however, and this appeared to be due to inter-experiment variability of overall levels of AMPH-induced locomotor activity in animals ovariectomized in adulthood, unrelated to time of ovariectomy in early development. Because there was no evidence to suggest that time of ovariectomy in early development had an effect on responsiveness to AMPH in the adult, the data from the two experiments were combined (see Appendix for a complete discussion).

Method

Subjects

Subjects were 144 female New Colony Wistar rats obtained from 22 litters born in the laboratory at Concordia University. Colony conditions were as described previously. All testing took place during the light cycle.

Breeding. Procedures for breeding and housing of pregnant females were as described for Experiment 1.

Neonatal Hormonal Manipulations. Beginning on E21, the breeding cages were checked every few hours for the presence of pups. In the case of 12 of the litters, following the birth (PN0), the mother was removed from the breeding cage and the pups were transported to another room. There the litter was sexed and culled to a maximum of 12 pups, including at least four male animals. Female animals were either ovariectomized under hypothermic anaesthesia or were subjected to the anaesthetic procedure alone. Following these manipulations the pups were returned to the nest, and after a few minutes, the mother was re-introduced.

In the case of the remaining 10 litters, following the birth (PN0), the mother was removed from the cage and the litters were sexed and culled to a maximum of 12 pups, at least four of which were males. Following these manipulations the pups were returned to the nest, and after a few minutes, the mother was re-introduced.

Litters remained undisturbed except for weekly cage cleaning procedures until PN21 when weaning took place.

Prepubertal Hormonal Manipulations. On PN25 to 26, all animals were housed in same-condition groups (two to three individuals per group) in standard, wire-mesh hanging cages. On PN25, the animals that were left unmanipulated at birth were either ovariectomized or sham-operated under Metofane anaesthesia. Animals received 0.05 ml penicillin G (im) after this surgery. They were then housed as above. Animals remained in

these groups until they were between 61 and 69 days of age at which time they were individually housed.

Adult Surgical Procedures. When animals were 81 to 89 days of age a second surgical procedure was performed using Metofane anaesthesia. Animals that had been sham-operated on PN0 or PN25 were ovariectomized (ADULT), whereas animals that had been ovariectomized on PN0 or PN25 were sham-operated (EARLY). Thus, all animals were gonadectomized at the time of testing in adulthood. Animals received 0.1 ml penicillin G (im) after this surgery.

Apparatus

Locomotor activity was measured in the same activity boxes described in Experiment 1.

Procedure

Subject Assignment. Within each litter, animals of each ovariectomy group (ADULT or EARLY) were randomly assigned to receive either EB or OIL (see below) and either AMPH or SAL. Wherever possible, one animal from each litter was assigned to each condition (i.e., when a litter contained eight female animals). Thus, a 2 (ADULT vs. EARLY) x 2 (EB vs. OIL) x 2 (AMPH vs. SAL) design was created, with 18 subjects in each group. Assignment of animals to the activity boxes was counterbalanced both within and between testing squads.

Activity Testing. Activity testing began 15 to 16 days following the adult surgeries. The procedures used for the pre-exposure period and the test for sensitization were as described for Experiment 3. In addition, animals received a sc injection of either 5.0 μg of EB or 0.1 ml of peanut oil (OIL), 30 to 35 minutes prior to each activity session. The last day of the pre-exposure period was videotaped and stereotyped behavior was scored as described in Experiment 3.

Data Analyses. Total photocell counts recorded for each animal on each day of the pre-exposure period were analyzed by a four-way repeated measures ANOVA with time of ovariectomy (ADULT vs. EARLY), hormone (EB vs. OIL), and drug (AMPH vs. SAL) as the between-subjects factors and pre-exposure day as the within-subjects factor. Total photocell counts recorded for each animal during each 30-minute time-block across the two-hour session on the test day for sensitization were analyzed by means of a four-way repeated measures ANOVA with time of ovariectomy, hormone, and pre-exposure drug as the between-subjects factors and time-block as the within-subjects factor. Post-hoc analyses of significant interaction effects were made using the method described in Experiment 1.

Median stereotypy scores were again analyzed using a Kruskal-Wallis ANOVA to detect overall between group differences and Mann-Whitney U tests for subsequent pairwise comparisons. Given the large number of subjects involved in some of the comparisons, the Mann-Whitney U approximates a normal distribution, and thus, Z scores are reported for these comparisons (Siegel, 1956).

Results

Pre-exposure Period

The mean total locomotor scores for each group on each day of the pre-exposure period are shown in Figure 15. AMPH again significantly increased activity for all groups (drug: $F(1,136) = 301.04$, $p < .0005$). Animals that were ovariectomized prepubertally were less active than animals that were ovariectomized in adulthood (time of ovariectomy: $F(1,136) = 10.76$, $p < .005$). The effect of time of ovariectomy varied with drug group (time of ovariectomy x drug: $F(1,136) = 4.95$, $p < .05$). Post-hoc analyses revealed that early ovariectomy decreased AMPH-induced activity ($p < .01$) but there was no effect in the SAL-treated animals.

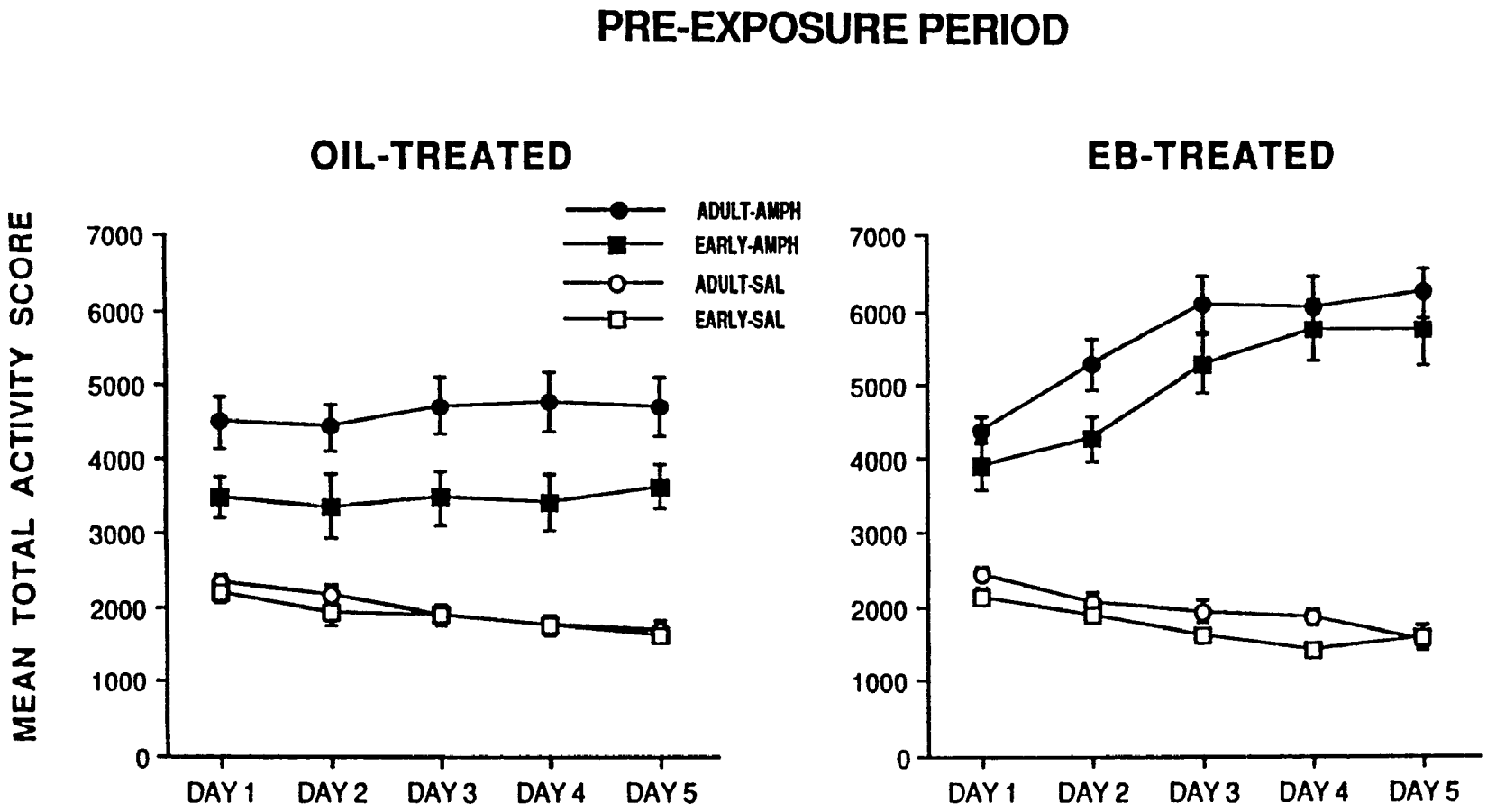


Figure 15. Mean total locomotor activity scores (± SEM) for OIL-treated (left panel) and EB-treated (right panel) animals in response to either AMPH (1.5 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period.

Animals that received EB at the time of testing showed significantly greater activity than animals that received OIL (hormone: $F(1,136) = 13.55$, $p < .0005$), and this effect also depended on drug group (hormone x drug: $F(1,136) = 17.13$, $p < .0005$). EB enhanced AMPH-induced locomotor activity ($p < .01$), but there was no effect of hormone in SAL-treated animals. The three-way interaction of time of ovariectomy x hormone x drug was not significant ($F(1,136) = 1.04$, $p > .05$), and thus, ovariectomy prior to puberty decreased the responsiveness of female animals to AMPH, regardless of the presence of EB at the time of testing (see Figure 15).

The enhancement of locomotor activity induced by AMPH increased with days of testing (day x drug: $F(4,544) = 38.22$, $p < .0005$), and as is evident in Figure 15, this effect depended on hormonal conditions at the time of testing (day x hormone x drug: $F(4,544) = 12$, $p < .0005$). Locomotor activity in response to AMPH increased over days in animals tested with EB ($p < .01$), but did not change significantly over days for animals treated with OIL. Although there was no effect of hormone on the first day of the pre-exposure period, animals treated with EB showed significantly greater activity than animals treated with OIL by day 3 of the pre-exposure period ($ps < .01$). There were no differences as a function of day or hormone in SAL-treated rats.

The lack of change in behavior over days of testing in OIL-treated rats may be due to the development of stereotyped behaviors in these animals. Figure 16 shows the frequency distribution of stereotypy ratings for animals treated with AMPH on day 5 of the pre-exposure period. The Kruskal-Wallis ANOVA revealed a significant effect of group ($H(3) = 11.61$, $p < .01$), with group EARLY-OIL-AMPH showing the highest median stereotypy scores and group ADULT-EB-AMPH showing the lowest median stereotypy scores. Subsequent pairwise comparisons revealed that animals treated with OIL had greater median stereotypy scores than animals treated with EB ($Z = 2.84$, $p < .01$). Furthermore, there was a tendency for ovariectomy prior to puberty to increase median stereotypy scores ($Z = 1.88$, $p = .06$), however, when the effect of time of

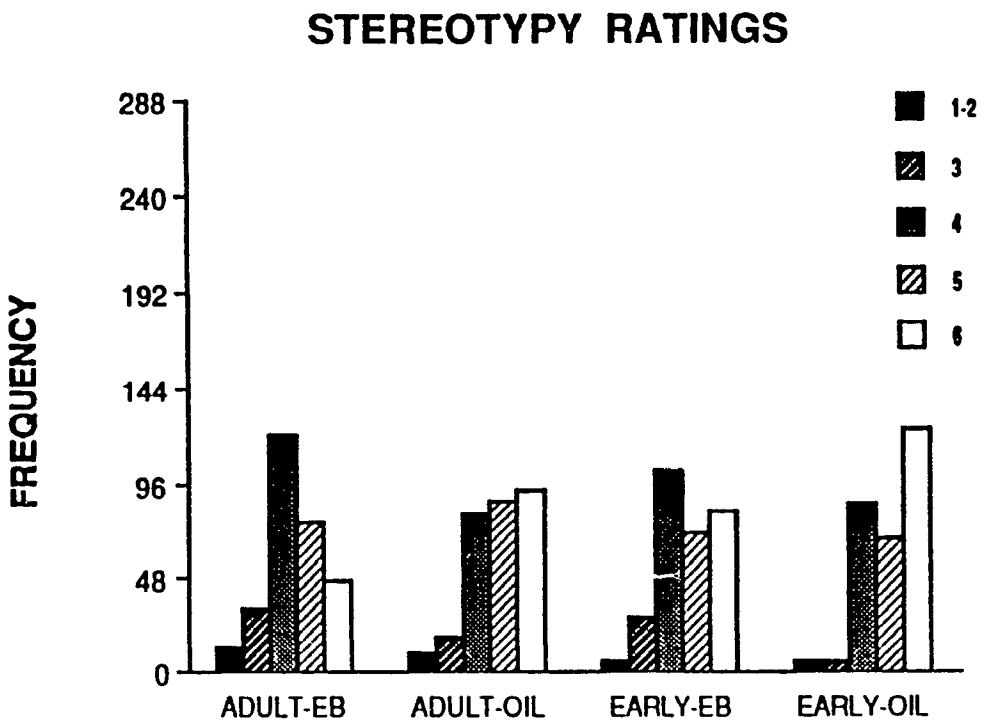


Figure 16. Frequency distribution of stereotypy ratings on pre-exposure day 5 for animals receiving AMPH.

ovariectomy was examined separately for each hormone condition, there were no significant differences between groups EARLY and ADULT for either animals receiving EB or OIL.

Test for Sensitization

Mean total locomotor activity scores for each group, at each 30-minute time-block across the two-hour test for sensitization, are shown in Figure 17. The data for one animal in the EARLY-OIL-SAL condition were lost due to an faulty AMPH injection. As expected, animals pre-exposed to AMPH showed greater activity than animals receiving the drug for the first time (pre-exposure drug: $F(1,135) = 28.38$, $p < .0005$). Animals that received EB at the time of the test showed greater activity scores than animals that received OIL (hormone: $F(1,135) = 44.03$, $p < .0005$). As can be seen in Figure 17, EB at the time of testing increased both the acute and the sensitized response to the challenge injection of AMPH, but did not significantly enhance the degree of sensitization as measured by the difference between AMPH and SAL pre-exposed animals (i.e., the hormone x pre-exposure drug interaction was not significant: $F(1,135) < 1$). There were no significant effects of time of ovariectomy.

Activity scores for all animals declined across the two-hour test session (time-block: $F(3,405) = 194.35$; $p < .0005$). This effect varied by hormone condition (hormone x time-block: $F(3,405) = 13.08$, $p < .0005$). The effect of hormone was largest during the early part of the test session, and scores declined more quickly in OIL-treated rats ($ps < .01$).

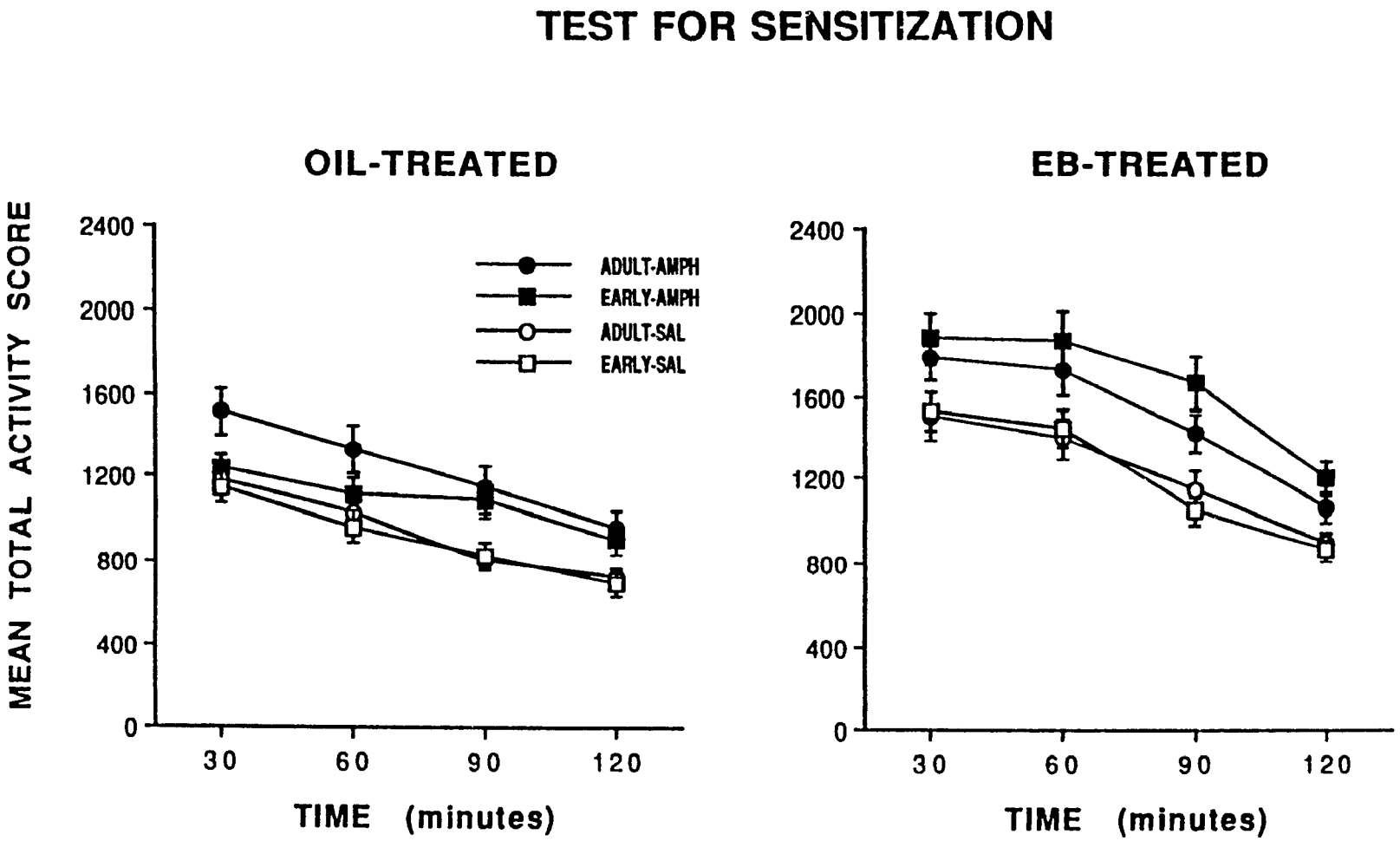


Figure 17. Mean total locomotor activity scores (± SEM) for OIL-treated (left panel) and EB-treated (right panel) animals for each 30-minute time-block on the test day for sensitization when all animals received 0.75 mg/kg AMPH.

Discussion

In the present experiment, ovariectomy prior to puberty decreased overall levels of AMPH-induced activity during the pre-exposure period regardless of the presence of estradiol at the time of testing, but did not alter the pattern of behavior change exhibited over pre-exposure days. Thus, exposure of the female animal to ovarian hormones over the life-span does make a significant contribution to responsiveness to AMPH in adulthood. Two hypotheses can be put forward to account for this effect.

First, it is possible that a period subsequent to PN25 constitutes a critical period for the effect of ovarian hormones on the development of female-typical responsiveness to AMPH. Such a critical period has been suggested by Becker and Ramirez (1981a) to explain the effect of prepubertal ovariectomy on the development of the female-typical response of AMPH-induced DA release from striatal slices. Other researchers have suggested that exposure of the female animal to ovarian hormones in the postnatal period can influence both reproductive and non-reproductive behaviors exhibited by the adult female animal (e.g., Gerall et al., 1973; Stewart & Cygan, 1980). The time from PN10 to PN20 has been suggested to constitute a critical period for the effects of ovarian hormones on the development of open-field behavior in the female rat (Stewart & Cygan, 1980). Because, in the present experiment, there was little difference between females ovariectomized at birth or post-weaning (see Appendix), the critical period for the effects of early exposure to ovarian hormones on AMPH-induced activity, if any, must be later than that described for open-field behavior.

A second hypothesis is that the reduced responsiveness to AMPH in animals ovariectomized prior to puberty is due to the long-term absence of ovarian hormones and not to the lack of ovarian hormones at any one "critical" point during development. As noted in the general introduction, Hendricks (1992) has suggested that many of the effects of early ovariectomy can be attributed to the fact that long-term absence of ovarian

hormones, even in the adult, decreases responsiveness to circulating gonadal hormones (e.g., Beach & Orndoff, 1974). This suggestion is based on the observation that after repeated testing in the presence of hormone replacement in adulthood, females ovariectomized early in development come to show sexual behavior similar to that of females ovariectomized as adults (Hendricks, 1992). There is some evidence to support this suggestion in the present experiment, as there was no effect of time of ovariectomy on the test day for sensitization in animals that had been given repeated exposure to EB during the pre-exposure period. The fact, however, that time of ovariectomy had a significant effect in OIL-treated rats during the pre-exposure period (and not on the test day) does not support the hypothesis that the effect is due solely to decreased responsiveness to circulating estradiol.

Whatever the underlying mechanism, the present data lend further support to the hypothesis that long-term exposure of the female animal to ovarian hormones over the life-span enhances later responsiveness to AMPH and contributes to the sex difference observed between normal male and female animals. The lack of life-long exposure to these hormones might have contributed to the reduced activity of group GDXMALE in comparison to group FEMALE observed in Experiments 3 and 4.

A second finding of the present experiment is that estradiol enhanced AMPH-induced locomotor activity. Animals treated with EB at the time of each test showed greater levels of AMPH-induced locomotor activity, and greater sensitization over days of the pre-exposure period, than animals treated with OIL. Again, the evidence points to an important role for circulating estradiol in the sex difference in sensitization of AMPH-induced locomotor activity, as measured by the change in behavior with repeated injections. Furthermore, as in Experiment 4, EB-treated animals were found to show lower levels of stereotypy than OIL-treated animals.

The magnitude of sensitization on the test day did not vary significantly as a function of hormonal treatment at the time of testing, although animals pre-exposed to

AMPH in the presence of EB showed greater activity than animals pre-exposed to AMPH in the presence of OIL. Again, the behavior of EB-treated animals on the test day for sensitization was similar to that of the intact female animals tested in Experiment 1.

EXPERIMENT 7

In Experiment 6, ovariectomy prior to puberty was found to reduce locomotor activity in response to AMPH in the adult female rat. In addition, circulating estradiol at the time of testing was found to make an important contribution to the enhanced responsiveness of the adult female, pointing again to the importance of this hormone in the female-typical pattern of AMPH-induced activity. In Experiments 3 and 4, TPFEMALE animals showed enhanced AMPH-induced activity in comparison to MALE animals, despite the fact that both received exposure to T, postnatally. The results of Experiment 5 suggest that this effect is probably not due to differences in the exposure of the groups to T, prenatally. In light of the results of Experiment 6, however, it remains possible that life-long exposure to ovarian hormones in group TPFEMALE enhanced their AMPH-induced activity in adulthood. Furthermore, in Experiment 4, the effect of circulating estradiol was reduced in animals exposed to T at birth, leading to the suggestion that exposure to T in the neonatal period decreases responsiveness to circulating estradiol in the adult animal.

These possibilities were addressed in the present experiment. Exposure to ovarian hormones during development was manipulated in female animals that were also exposed to T at birth. In adulthood, all animals were tested either in the absence of circulating gonadal hormones or in the presence of EB.

Method

Subjects

Subjects were 60 female New Colony Wistar rats obtained from nine litters born in the laboratory at Concordia University. Colony conditions were as described previously. All testing took place during the light cycle.

Breeding. Procedures for breeding and housing of pregnant females were as described for Experiment 1.

Neonatal Hormonal Manipulations. Beginning on E21, the breeding cages were checked every few hours for the presence of pups. When pups were found (PN0), the mother was removed from the breeding cage and the pups were transported to another room. There the litter was sexed and culled to a maximum of 12 pups, including at least three male animals. Female animals were either ovariectomized under hypothermic anaesthesia (EARLY) or were subjected to the anaesthetic procedure alone (ADULT). In addition, the female pups were administered 200 µg TP in 0.05 ml peanut oil on this and the following day. The volume of the injection was reduced from that used in Experiments 3 and 4 in order to avoid leakage of the oil into the site of ovariectomy. Following these manipulations the pups were returned to the nest, and after a few minutes, the mother was re-introduced. The pups were removed briefly from the nest 24 hours later on PN1 to administer a second TP treatment.

Litters remained undisturbed except for weekly cage cleaning procedures until PN21 when weaning took place. On PN25 to 26, all animals were housed in same-condition groups (two to three individuals per group) in standard, wire-mesh hanging cages. Animals remained in these groups until they were between 60 and 66 days of age at which time they were individually housed.

Adult Surgical Procedures. When animals were 81 to 87 days of age they were either ovariectomized (ADULT) or sham-operated (EARLY) under Metofane anaesthesia. Animals received 0.1 ml penicillin G (im) after this surgery.

Apparatus

Locomotor activity was measured in the same activity boxes described in Experiment 1. As in Experiment 4, the testing room was illuminated by two additional 25-watt, red light bulbs.

Procedure

Subject Assignment. Subject assignment was as described for Experiment 6, creating a 2 (ADULT vs. EARLY) x 2 (EB vs. OIL) x 2 (AMPH vs. SAL) design. ADULT groups contained seven subjects each, whereas EARLY groups contained eight subjects each. Assignment of animals to the activity boxes was counterbalanced both within and between testing squads.

Activity Testing. Activity testing began 15 to 16 days following the adult surgeries. The procedures used for the pre-exposure period and the test for sensitization (including drug and hormone doses) were as described for Experiment 6.

Total photocell counts for each animal on each day of the pre-exposure period and for each 30-minute time-block across the two-hour session on the test for sensitization were analyzed in an identical fashion to that described for Experiment 6.

The last day of the pre-exposure period was videotaped and stereotyped behaviors were scored and analyzed as described for Experiment 3.

Results

Pre-exposure Period

The data for three animals (two from group EARLY-EB-AMPH and one from group EARLY-EB-SAL) were discarded from the experiment when a post-experiment necropsy revealed evidence of incomplete neonatal ovariectomy. Thus, for group EARLY-EB-AMPH, $n = 6$, and for group EARLY-EB-SAL, $n = 7$. In addition, due to problems with the horizontal photocells, data for three subjects were lost during one session of the pre-exposure period (two EARLY-OIL-AMPH, one ADULT-OIL-AMPH). The missing data were estimated by the group mean for the remaining subjects of their respective groups for the activity session in question.

Figure 18 shows the mean total locomotor activity scores for each group on each day of the pre-exposure period. As expected, AMPH increased activity for all groups (drug: $F(1,49) = 127.74$, $p < .0005$). This effect, however, did not vary by either time of ovariectomy (time of ovariectomy x drug: $F(1,49) = 1.60$, $p > .05$) or hormone condition (hormone x drug: $F(1,49) < 1$), although it can be seen from Figure 18 that among EB-treated groups, animals ovariectomized in adulthood tended to show greater AMPH-induced activity than animals ovariectomized in early development.

In contrast to the results obtained in previous experiments, EB did not increase AMPH-induced locomotor activity, and in fact, OIL-treated rats were slightly more active than EB-treated rats. This effect was significant on the first and second days of the pre-exposure period (day x hormone: $F(4,196) = 3.14$, $p < .05$; post-hoc comparisons: $ps < .01$). Furthermore, there was no significant change in AMPH-induced activity over days of the pre-exposure period (day x drug: $F(4,196) = 1.04$, $p > .05$; see Figure 18).

Similar results were obtained in a second group of 36 rats treated with TP at birth, ovariectomized on PN25 or in adulthood, and tested in the presence of either EB or OIL (data not shown). In this replication, however, animals showed a small increase in AMPH-induced activity with days of testing (day: $F(4,128) = 7.64$, $p < .001$).

The lack of change in behavior over days of testing may be a function of increased stereotypy. Figure 19 shows the frequency distribution of stereotypy ratings for the four groups of AMPH-treated animals on the last day of the pre-exposure period. The median scores of the four groups did not differ significantly from each other ($H(3) < 1$).

Test for Sensitization

Mean total locomotor activity scores for each group at each 30-minute time-block on the test day for sensitization are shown in Figure 20. Sensitization was observed for all groups; animals pre-exposed to AMPH showed greater activity than animals receiving the

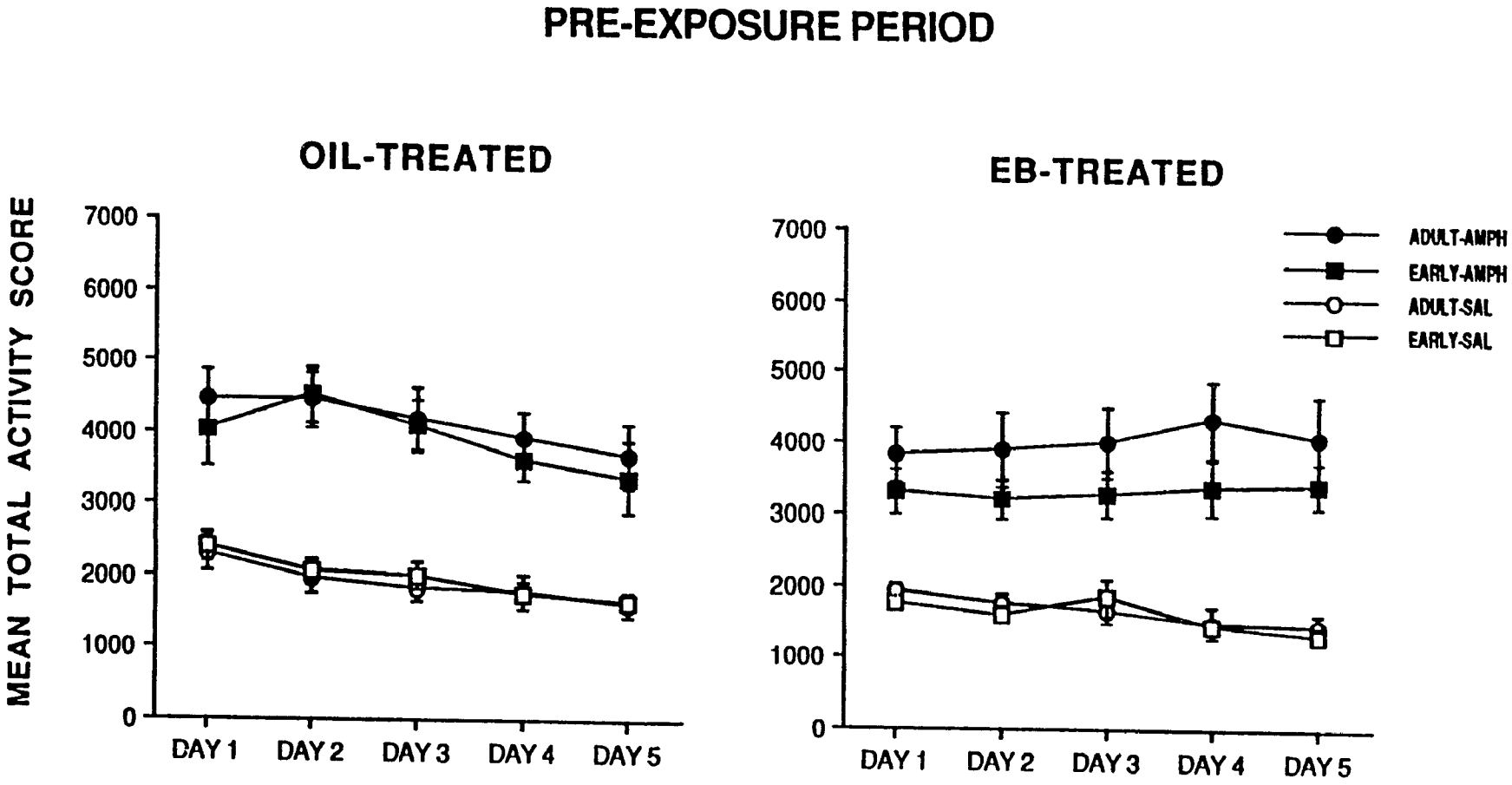


Figure 18. Mean total locomotor activity scores (± SEM) for OIL-treated (left panel) and EB-treated (right panel) animals in response to either AMPH (1.5 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period.

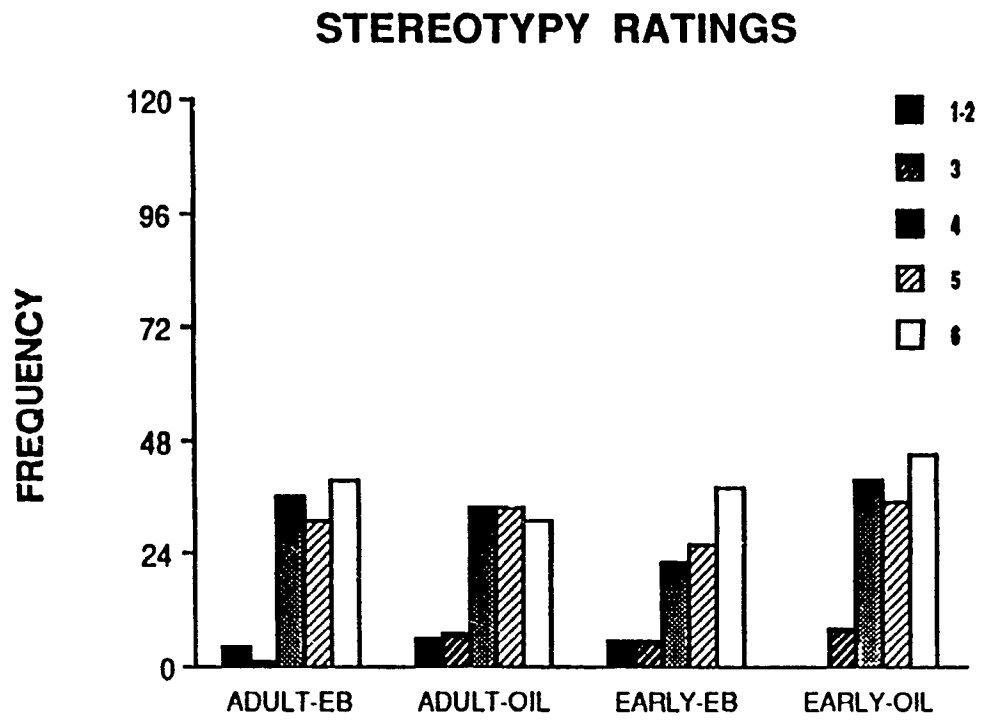


Figure 19. Frequency distribution of stereotypy ratings on pre-exposure day 5 for animals receiving AMPH.

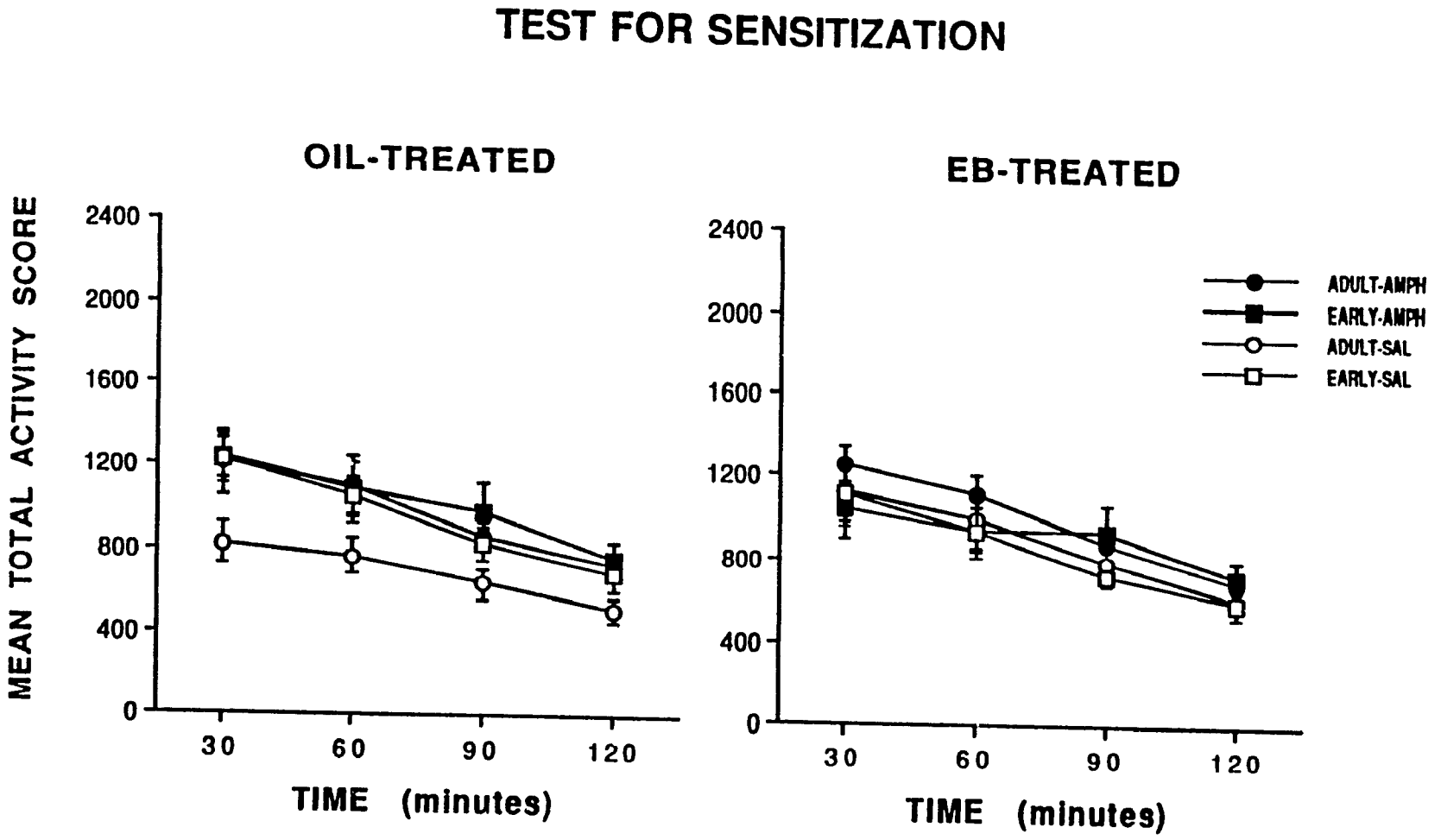


Figure 20. Mean total locomotor activity scores (± SEM) for OIL-treated (left panel) and EB-treated (right panel) animals during each 30-minute time-block on the test day for sensitization when all animals received 0.75 mg/kg AMPH.

drug for the first time (pre-exposure drug: $F(1,49) = 4.14$, $p < .05$), however, this effect did not vary as a function of hormone or time of ovariectomy.

In the second group of 36 rats, described above, levels of activity in response to the AMPH challenge on the test day for sensitization were somewhat greater than those of the AMPH pre-exposed animals shown in Figure 20, although there were no significant differences as a function of hormone condition or time of ovariectomy. As SAL groups were not included in this replication, however, the magnitude of sensitization in these animals cannot be assessed.

There was a main effect of time-block ($F(3,147) = 75.53$, $p < .0005$), and thus, activity scores for all groups declined across the two-hour test session. This effect did not vary as a function of pre-exposure drug, hormone, or time of ovariectomy.

Discussion

In this experiment in which all females were exposed to T at birth, early ovariectomy had no significant effect on the response to AMPH in adulthood. Furthermore, estradiol did not enhance activity in response to AMPH, nor did it produce a significant change in activity with repeated injections in the pre-exposure period. Again, neonatal exposure to T appeared to alter the ability of estradiol to produce a facilitatory effect on AMPH-induced activity. These findings make it unlikely that the higher levels of activity seen in Experiment 4 in group TPFEMALE compared to group MALE were due to lifelong exposure to ovarian hormones, and thus, the possibility remains that exposure of the male animal to testicular hormones at other time-points during development contributes to their decreased responsiveness to AMPH as adults.

In Experiment 4, however, group TPFEMALE appeared to show greater responsiveness to estradiol than in the present experiment. It is not clear what accounts for this discrepancy. Animals in Experiment 4, tested with circulating estradiol, showed

greater changes in activity over days of the pre-exposure period, and a somewhat larger effect on the test day for sensitization, than did animals in the present experiment, in spite of the fact that overall levels of activity were comparable in the two experiments.

GENERAL DISCUSSION

The experiments presented in this thesis were conducted to establish the nature of the sex difference in locomotor activity in response to acute and repeated injections of AMPH in adult rats, and to investigate systematically the contributions of gonadal hormones to this sex difference. Intact females were found to show greater responsiveness to an acute injection of AMPH, and greater sensitization following repeated administrations of the drug, than were intact males. Both circulating ovarian and testicular hormones in the adult, and differential exposure to these gonadal hormones in early development, were found to contribute to these sex differences.

A summary of the major findings to emerge from this set of experiments will be presented in the following pages. Each finding will be considered, in turn, and some of the data from each of the experiments that are relevant to the issues will be represented in summary form. In this discussion, "male" and "female" will refer to those animals that did not receive exogenous manipulations of gonadal hormones in early development; those that did will be referred to by their treatment condition (e.g., GDXMALE or TPFEMALE).

The Acute Response to AMPH

Intact, adult females showed greater locomotor activity in response to an acute injection of AMPH than did intact, adult males. The hormonal basis of this sex difference emerges from an analysis of the data from several of the experiments, and it becomes clear that exposure to different gonadal hormones at more than one time-point contributes to this sex difference in the acute effect of AMPH. It can be noted that there are two occasions in each experiment when the acute response to AMPH can be observed. One is the first day of the pre-exposure period when animals received drug for the first time. The second is the test day for sensitization when animals given saline throughout the pre-exposure period

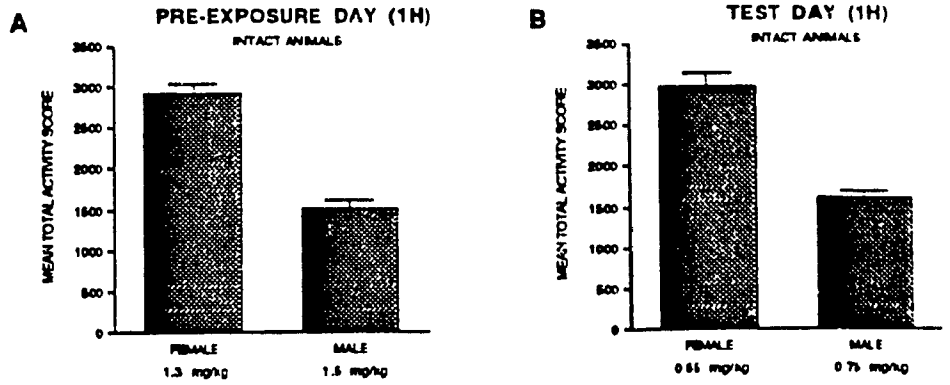
received AMPH for the first time. The responses of the intact animals tested in Experiment 1 on these two occasions are shown in Figure 21, Panel 1 (A and B). Data are shown for the first hour of each session, during which the effect of AMPH was most clearly seen.

Testicular Hormones in the Perinatal Period. Exposure to T during the perinatal period decreased the acute response to AMPH in adults. This effect was most evident in the response of animals previously exposed to SAL to the low dose of AMPH on the test days for sensitization in Experiments 3 and 4 . Thus, this effect was present in animals tested both in the absence of circulating gonadal hormones (Figure 21, Panel 2) and in the presence of estradiol (Figure 21, Panel 3). Gonadectomy at birth enhanced the acute response of males to AMPH (see also Experiment 5), whereas TP given at birth decreased the acute response of females.

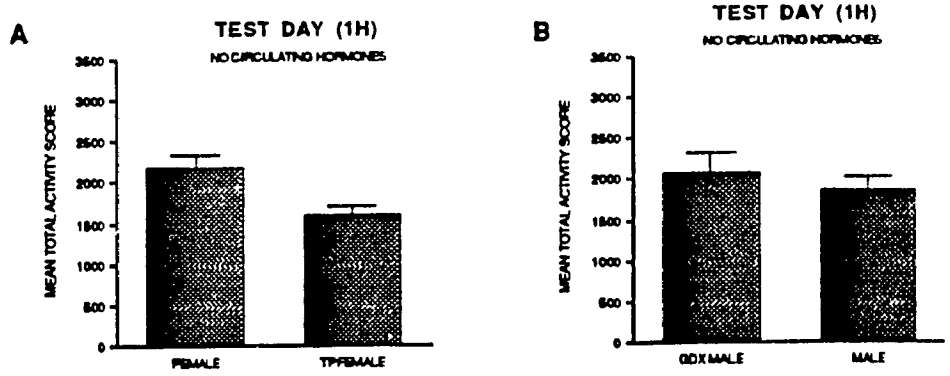
The reduction in the acute response to AMPH produced by neonatal exposure to T is also reflected in the small sex difference observed in many of the experiments on the first day of the pre-exposure period regardless of the hormonal conditions at the time of testing (e.g., see Experiment 2, Figures 3 and 4, and Experiment 3, Figure 6). Thus, as has been shown for other non-reproductive behaviors (e.g., activity in the open-field, Stewart et al., 1975; Stewart, Vallentyne, & Meaney, 1979 and maze-learning, Stewart et al., 1975; F'oof & Havens, 1992) one major contributor to the decreased responsiveness of the intact male to the acute effects of AMPH compared to that of the intact female is exposure to T in the perinatal period.

Circulating Estradiol. A second major contributor to the sex difference in response to an acute injection of AMPH was the presence of circulating estradiol in the adult female. The response of females tested in the presence or absence of EB (i.e., from Experiments 3 and 4) is shown in Panel 2 of Figure 22 (see also Experiment 6, Figures 15 & 17). Note that on the test day for sensitization, the level of AMPH-induced activity in the EB-treated females (Panel 2B) equaled that of the intact females (Panel 1B). Thus, circulating estradiol clearly contributes to the greater responsiveness of females to AMPH.

Panel 1: EXPERIMENT 1



Panel 2: EXPERIMENT 3



Panel 3: EXPERIMENT 4

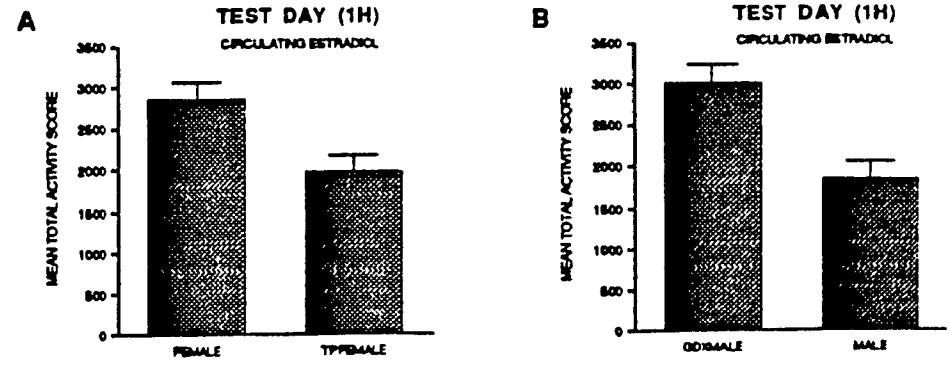
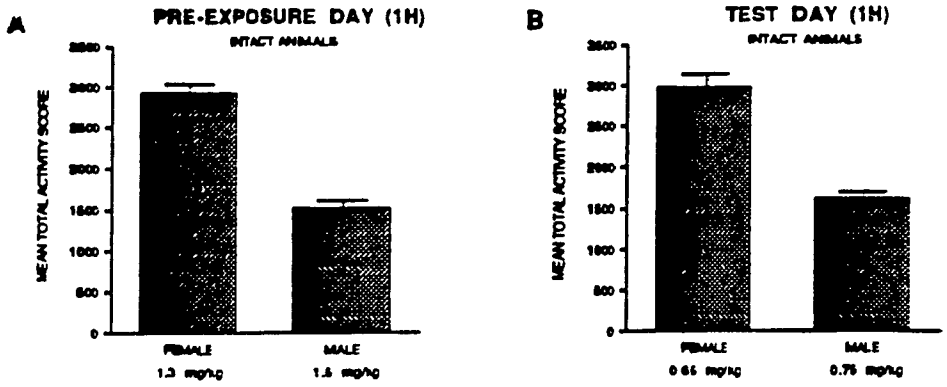


Figure 21. Mean activity scores for the first hour following an acute injection of AMPH. **Panel 1.** Intact males and females on (A) pre-exposure day 1 and (B) the test day. **Panel 2.** Effect of perinatal T-exposure in SAL pre-exposed (A) females and (B) males on the test day (no circulating hormones). **Panel 3.** Effect of perinatal T-exposure in SAL pre-exposed (A) females and (B) males on the test day (circulating estradiol).

Panel 1: EXPERIMENT 1



Panel 2: EXPERIMENTS 3 AND 4

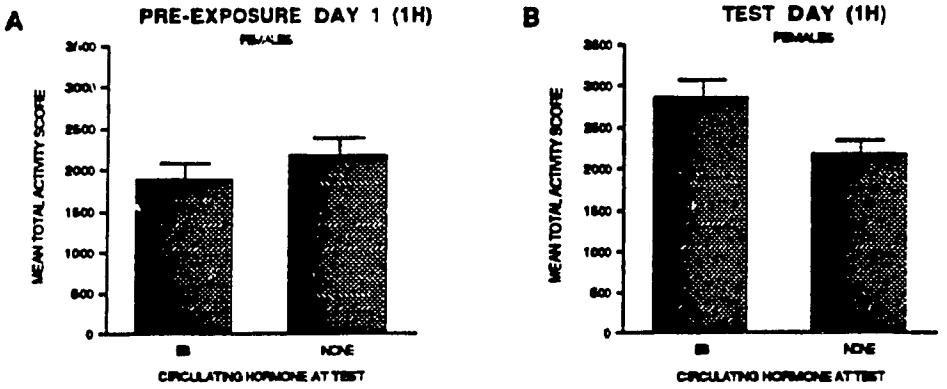


Figure 22. Mean activity scores for the first hour following an acute injection of AMPH. **Panel 1.** Intact males and females on (A) pre-exposure day 1 and (B) the test day. **Panel 2.** SAL pre-exposed females tested in the absence (NONE; Experiment 3) or presence (EB; Experiment 4) of circulating estradiol on (A) pre-exposure day 1 and (B) the test day.

Because EB was administered 30 minutes prior to each test session, it can be suggested that its effects on the locomotor response to an acute injection of AMPH are due to some immediate effect on the DA systems, as has been shown for other AMPH-induced behaviors (e.g., Becker, 1990b). If this were the case, however, then the same enhancement should have been observed on the first day of the pre-exposure period. It is clear that EB did not enhance the acute locomotor responsiveness of the female rat on the first day of the pre-exposure period in the present experiments (see Panel 2A of Figure 22, and Experiment 6, Figure 15).

One explanation for this discrepancy is that the prior exposure of the female animal to estradiol during the pre-exposure period altered the response to the acute injection of AMPH on the test day for sensitization. This effect would also account for the greater response of the intact female animals on the first day of the pre-exposure period, given that they have received prior exposure to endogenous ovarian hormones. Thus, the data from these experiments point to an important role for repeated or long-term exposure to estradiol in determining its enhancing effects on AMPH-induced locomotion in the female rat.

The Interaction Between Perinatal T and Circulating Estradiol. The response of animals to the enhancing effects of estradiol on the acute locomotor response to AMPH was found to be a function of exposure to T in the perinatal period. Exposure to T, neonatally, reduced the response of animals to AMPH in the presence of EB (see Panel 3, Figure 21). Thus, neonatal gonadectomy of males increased the response to AMPH, whereas neonatal TP-treatment of females reduced the response to AMPH. Furthermore, the levels of activity in response to an acute injection of AMPH shown by GDXMALE rats, in comparison to male animals, was greater when animals were tested with EB than when they were tested in the absence of circulating gonadal hormones (compare Panel 2B with Panel 3B, Figure 21). Thus, the greater responsiveness to an acute injection of AMPH in the intact female, compared to that of the intact male, appears to be accounted for in large part by an interaction between the lack of exposure to T, perinatally, and the presence of

circulating estradiol.

The fact that neonatal exposure to T appears to reduce sensitivity to estradiol in the adult animal has been found previously for other behaviors (e.g., female sexual behavior). The adult female rat, in the presence of estradiol (and progesterone), will show a characteristic behavioral pattern (proceptive behaviors and lordosis) in response to copulatory stimulation from the male. Exposure to T at birth drastically decreases the incidence of these receptive behaviors in adulthood, despite the presence of circulating estradiol and progesterone (e.g., Gerall & Kenney, 1970).

Circulating Testosterone. Castration of the male rat in adulthood enhanced the locomotor response to an acute injection of a moderate dose of AMPH as measured on the first day of the pre-exposure period. This effect was evident in Experiment 2, in which male animals tested in the presence of T were less responsive to the first injection of AMPH than were male animals tested in its absence. Thus, circulating T appears to contribute to the lower response of the intact male rat to an acute injection of the drug. In contrast to the effects observed on the first day of the pre-exposure period, however, little difference in AMPH-induced locomotor activity between males tested in the presence or absence of circulating T was found on the test day for sensitization. It is not clear what would account for this discrepancy.

Although circulating T reduced responsiveness to an acute injection of AMPH in males, it did not reduce responsiveness to an acute injection of AMPH in females on either the first day of the pre-exposure period or on the test day for sensitization (compared to females tested without T; see Experiment 2). Neither did it enhance responsiveness of the female to an acute injection of AMPH on the test day for sensitization which might have been expected if T were acting through its metabolite, estradiol (see Discussion of Experiment 2). It should be noted, however, that T was administered in a continuous fashion by means of a silastic capsule and not by the intermittent injection regime used to administer EB in all other experiments. A similar lack of effect of estradiol on the response

of the female animal to an acute injection of cocaine was observed when estradiol was administered in a continuous fashion by silastic implant (Peris, Decambre, Coleman-Hardee, & Simpkins, 1991). Thus, the pattern of estradiol administration may be an important variable.

Ovarian Hormones in Early Development. The response of the female animal to an acute injection of AMPH was also enhanced by exposure to ovarian hormones in early development, making it likely that exposure to ovarian hormones throughout development contributes to the female-typical response to AMPH. This effect was observed, however, only during the pre-exposure period and did not appear to interact with circulating estradiol.

The Response to Repeated Injections of AMPH

Both intact male and intact female animals showed significant sensitization to the locomotor effects of AMPH across days of the pre-exposure period. This effect was greater in intact females. Like the acute response to AMPH, this sex difference in sensitization was largely attributable to an interaction between exposure to T during the perinatal period and the presence of circulating estradiol at the time of testing.

The Interaction Between Perinatal T and Circulating Estradiol. Animals not exposed to T in the perinatal period and tested with EB as adults showed the greatest sensitization over days of the pre-exposure period (Experiment 4, and also the findings for female animals in Experiments 6 and 7). Under these conditions, GDXMALE animals showed greater sensitization than males, whereas TPFEMALE animals showed less sensitization than females. In the absence of circulating estradiol, none of the treatment groups in any of the experiments showed significant changes in AMPH-induced locomotor activity over days of testing (e.g., see Figures 6, 9, and 15). Thus, circulating estradiol is a major contributor to the greater sensitization to AMPH seen in intact female animals.

One problem that arises with this interpretation, however, is that stereotyped behaviors may have obscured changes in AMPH-induced locomotor activity in those

animals that were tested in the absence of estradiol. The data collected on pre-exposure day 5 are consistent with the idea that the animals tested under these conditions showed significantly greater stereotypy than the animals tested with EB. Furthermore, the fact that the effect of neonatal T on responsiveness to AMPH emerged in response to the lower dose of AMPH on the test day for sensitization in animals tested without EB, is a further indication that differences in stereotypy contributed to the results observed in the pre-exposure period. Van Hartesveldt and Joyce (1986) have proposed that estradiol has different effects on the mesoaccumbens and mesostriatal DA systems. Given that these two systems are differentially involved in AMPH-induced stereotypy and locomotor activity (e.g., Sharp et al., 1987; Kelly et al., 1975), estradiol may have altered the expression of stereotypy versus locomotor activity.

Regardless of this possibility, it is clear that, in the presence of circulating estradiol, both male and female animals showed greater increases in locomotor activity over days of testing than they did when tested without circulating hormones. The fact that in males treated with EB the change in locomotor activity over days more closely resembles that observed in intact males, or males treated with T, points to the possible role for T or its metabolite, estradiol, in the regulation of DA function in the male rat (see Mitchell & Stewart, 1989). This effect of T was not seen in female animals; that is, females tested in the presence of circulating T did not show increases in activity with days of testing, again suggesting that the pattern of hormone administration in females may be critical. On the other hand, Peris et al. (1991) found female animals that received estradiol replacement by means of a silastic implant showed greater sensitization to cocaine (greater response to the drug on the last day of testing) than animals tested without hormone replacement.

Other Hormonal Factors. It is evident from the results of Experiment 4, in particular, that exposure to T in the perinatal period did not eliminate the sex difference in the change in activity over days of testing in response to a moderate dose of AMPH. The change in activity over days of testing in the pre-exposure period in GDXMALE and

TPFEMALE animals did not differ and was intermediate between that shown by male and female animals. Prenatal exposure to testicular androgens did not appear to make a large contribution to this sex difference in behavior (see Experiment 5), at least in animals that also received exposure to T, postnatally. Exposure to T, prenatally, may have influenced the behavior of the GDXMALE animals, however (i.e., decreasing it relative to females).

The reduced levels of activity shown by GDXMALE animals during the pre-exposure period may also have been due, in part, to the lack of exposure to ovarian hormones over the life-span (see Experiment 6). Interestingly, male rats castrated at birth also show somewhat lower levels of female sexual behavior (i.e., lordosis) as adults than do normal female rats, and implantation of these neonatally-castrated males with ovaries, prepubertally, enhances their female sexual behavior in adulthood (Gerall et al., 1973; Hendricks & Duffy, 1974). There was no evidence that exposure to ovarian hormones could account for the enhanced behavior of TPFEMALE animals in comparison to males (see Experiment 7). Rather, it seems likely that an additional hormonal influence, perhaps exposure of the male animal to testicular hormones over the life-span, contributes to the reduced responsiveness of male animals compared to TPFEMALE animals.

Alternatively, the finding that manipulations of T in the perinatal period did not abolish the differences between male and female animals may indicate that the course of development of the two sexes is fundamentally different. Given the data of Reisert and colleagues (e.g., Beyer et al., 1991; Engele et al., 1989; Ovtscharoff et al., 1992; see Reisert & Pilgrim, 1991 for review), it is possible that the two sexes undergo differential development prior to any influence of gonadal hormones. Subsequently, however, the sexes receive differential exposure to gonadal steroids. The exposure, or lack of exposure, to T prenatally may alter the subsequent responsiveness of the nervous system to gonadal hormones, postnatally. It is likely, therefore, that administration of exogenous T to the female will have effects different from those of postnatal T-exposure in the normal male animal. Thus, developmental changes that occur in the female prior to receiving the

exogenous T may render particular neural system(s) less sensitive to the effects of postnatal exposure to T.

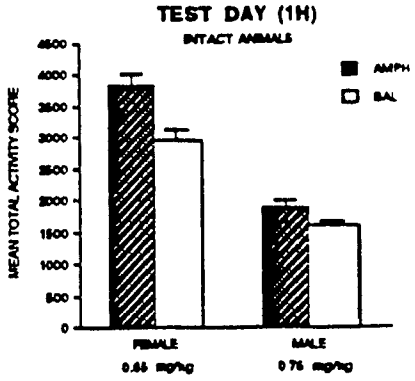
The Response to a Challenge Injection of AMPH

Intact female animals, pre-exposed to AMPH, showed higher levels of activity on the test day for sensitization than intact male animals in response to the low-dose challenge injection of the drug. More importantly, sensitization, as measured by the difference in AMPH-induced activity between animals pre-exposed to SAL and those pre-exposed to AMPH, was significantly greater in intact females than males (see Panel 1 of Figure 23).

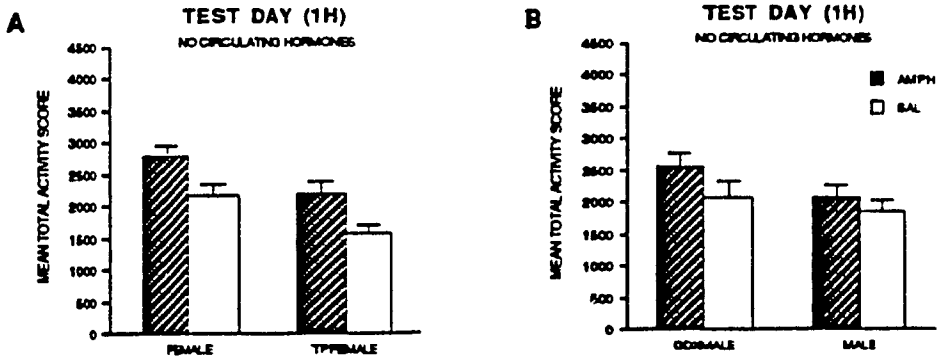
The Effects of Perinatal T-Exposure. In Experiments 3 and 4, animals exposed to T neonatally, had lower levels of activity in response to AMPH on the test day for sensitization than those animals that were not exposed to T. Perinatal manipulations of T, however, did not alter the magnitude of sensitization (see Panels 2 and 3 of Figure 23). Regardless of whether animals were tested in the presence of estradiol, or in the absence of circulating gonadal hormones, the magnitude of the sensitization effect shown by TPFEMALE animals was more like that of females than males (but see Experiment 7), even though the overall levels of activity were similar to those of males. Likewise, the magnitude of sensitization in GDXMALE animals was similar to that of male animals (but see Experiment 3), even though overall levels of activity were more like those shown by females.

The Effects of Circulating Estradiol. When tested in the presence of circulating estradiol, both male and female animals showed sensitization effects that were virtually identical to those shown by intact animals (compare Panel 1 with Panels 3A and 3B of Figure 23). Thus, some minimal level of circulating hormones seems to be required for the full expression of the sex difference in sensitization, pointing again to the possible importance of circulating estradiol to the behavior of both male and female animals. The fact remains, however, that regardless of the actual magnitude of the sensitization effect,

Panel 1: EXPERIMENT 1



Panel 2: EXPERIMENT 3



Panel 3: EXPERIMENT 4

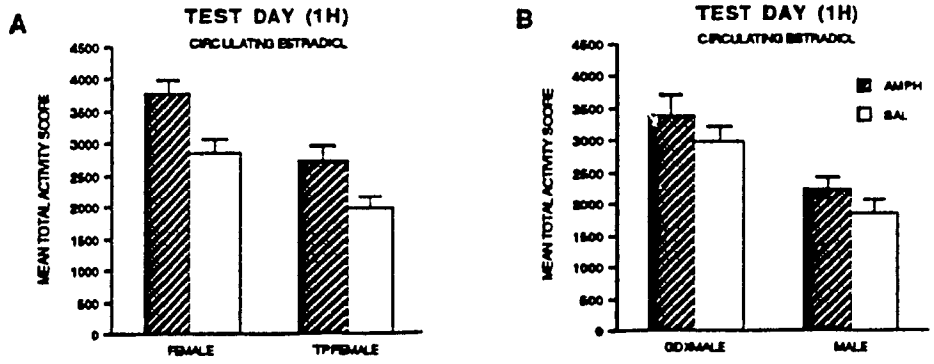


Figure 23. Mean activity scores for animals that were pre-exposed to AMPH or to SAL during the first hour following the challenge injection of AMPH. **Panel 1.** Intact males and females. **Panel 2.** Effect of perinatal T-exposure in (A) females and (B) males (no circulating hormones). **Panel 3.** Effect of perinatal T-exposure in (A) females and (B) males (circulating estradiol).

females tested in the absence of circulating gonadal hormones showed somewhat greater sensitization than males, indicating that there is some additional influence on this sex difference.

Other Issues. The effects of the different hormonal manipulations on the magnitude of sensitization, as measured on the test day for sensitization, were variable across experiments. For example, there was little evidence for a sex difference in the magnitude of sensitization in the animals tested without circulating gonadal hormones in Experiment 2 (CHOL animals), whereas females tested under similar conditions in Experiment 3 showed slightly greater sensitization than males. Similarly, in both Experiments 3 and 4, the sensitization effect shown by TPFEMALE animals was like that of females. In Experiment 7, however, the sensitization shown by TP-treated females was more like that shown by males, at least in some groups.

One explanation for this variability is that manipulation and handling of animals at birth, in itself, contributes to individual differences in responsiveness to AMPH in adulthood (e.g., see discussion in the Appendix). In light of this suggestion, it is interesting to note that unlike those in all other experiments, the animals tested in Experiment 2 were not raised in the laboratory at Concordia University. The male animals in that experiment showed greater sensitization than any other male group tested. A similar tendency towards greater sensitization can be observed for male animals that were born at the University, but received different levels of manipulation at birth. Males not cooled at birth tended to show greater sensitization than those that were, when tested in the presence of EB (see Figure 25, Appendix). Thus, it is possible that the degree of sensitization is influenced by these factors.

Issues for Further Research

It can be concluded from the present experiments that intact male and female animals exhibit a marked sex difference in the acute locomotor response to AMPH and in

the sensitization of this behavior in response to repeated administrations of the drug. In the male, exposure to testicular hormones during the early perinatal period, and in the female, the presence and pattern of circulating estradiol were found to be important contributors to these sex differences. These factors in themselves, however, cannot account for all of the effects observed in the present set of experiments. Several unanswered questions remain.

First, additional hormonal influences may be involved in the sex difference in AMPH-induced behavior. Exposure to ovarian hormones at puberty may enhance the responsiveness of the adult female to AMPH, as seen in the experiment in which female were ovariectomized at birth or on PN25. The design of the present experiments, however, does not preclude the possibility that long-term ovariectomy itself decreases responsiveness to the drug. In order to examine this hypothesis it would be necessary to manipulate exposure to ovarian hormones at specific times during development. This might be accomplished by employing a modification of the methodology used by Gerall et al. (1973). Thus, animals would be ovariectomized at different times during development, left without circulating gonadal hormones for a time, and undergo hormone replacement via the use of ovarian transplants. Although such an experiment would be difficult from a practical standpoint, it would provide an elegant test of the hypothesis that there is a critical period for the effects of early ovarian hormones on responsiveness of the adult female rat to AMPH.

Related to the effects of gonadal hormones during puberty is the observation that TPFEMALE animals remain more responsive to AMPH than males. Previous research provides evidence for the ability of exogenous T to masculinize the open-field behavior of the female rat when administered peripubertally (Stevens & Goldstein, 1983). However, this treatment was not found to potentiate the effects of T at birth (i.e., animals treated with TP at birth were not different from those treated with T at puberty, or those that were treated at both times). It remains possible, however, that exposure to testicular androgens beyond the perinatal period contributes to the responsiveness of the adult male animal to

AMPH. Again, a combination of castration with hormone replacement at different time-points during development of the male animal would be required to investigate this possibility. It would be of interest to examine the effects of peripubertal T-exposure in the neonatally TP-treated female as well.

A second major issue that emerges from the present thesis is that estradiol enhances the locomotor activity and suppresses the stereotypy elicited by a moderate dose of AMPH. In the present experiments, however, stereotypy was not measured on each day of the pre-exposure period. Therefore, the exact relationship between these two behaviors over days of testing is not known. This would be particularly important to determine for animals tested in the absence of EB, but it would also be of interest to know whether stereotypy decreased over days of testing in animals that were tested in the presence of the hormone. Before the contribution of estradiol to the sensitization of locomotor activity in the female animal can be made with certainty, this issue must be resolved. Apart from measuring both stereotypy and locomotor activity on each pre-exposure day, the use of a lower dose of AMPH during the pre-exposure period might be helpful.

In the present experiments, the enhancing effects of estradiol on the locomotor activity produced by an acute injection of AMPH in the female animal appeared to be dependent on prior exposure to the hormone. Furthermore, the possibility was raised that the pattern of hormone exposure is an important variable in this effect. The effects of prior exposure to estradiol on AMPH-induced behaviors have been investigated in several studies (e.g., Becker et al., 1984; Becker & Beer, 1986; Joyce et al., 1984). Estradiol administration prior to AMPH treatment, however, has seldom been manipulated so as to mimic the pattern of repeated, intermittent exposure to the hormone experienced by the cycling female. Furthermore, animals pre-exposed to EB have usually been compared to animals without hormone replacement, rather than animals receiving EB for the first time (but see Joyce et al., 1984).

Exposure to EB was seen to enhance the change in locomotor activity over days of

testing in the female rat. The possibility exists that the repeated, intermittent fashion of EB administration contributed to this effect. This could be approached by studying the development of sensitization in female rats given EB replacement by silastic implant versus intermittent injections. Furthermore, to determine whether this effect is unique to animals that receive each injection of AMPH in the immediate presence of EB, it would be of interest to test intact females, repeatedly, at the same stage of the estrous cycle and compare their behavior to that of similarly-treated, randomly cycling females.

Sexually Dimorphic Responses to Amphetamine in Perspective

The behavioral response to acute and repeated injections of AMPH is dependent on the mesocorticolimbic and nigrostriatal DA systems, including both the DA neurons themselves and the targets that they innervate (e.g., striatum and NAS; Clarke et al., 1988; Kelly et al., 1975). It is reasonable to assume, therefore, that the sex differences in this behavioral response are mediated by the influence of gonadal steroids on these systems, both during development and in adulthood. This assumption allows one to speculate on the nature of these sex differences and how they arise.

The DA neurons have been shown to be different in male and female animals early in embryonic development (see Reisert & Pilgrim, 1991). These differences appear to indicate the existence of a primary, genetic sex difference in the structure of the developing DA cells. Building upon this fundamental sex difference, the differential exposure of the male and female animal to T and its metabolite, estradiol, during the perinatal period would serve to augment the sex differences in the DA cells. These hormones, acting at steroid receptors and having genomic effects, could produce changes in dendritic and axonal growth and differentiation, synaptic connectivity, and neuronal survival, all of which have been shown to be produced by these steroids in other brain regions (see Toran-Allerand, 1984). Thus, the physical structure of the developing DA cells may be rendered further sexually dimorphic by the differential influence of the gonadal steroids during this period.

These sex differences could, in turn, have profound effects on the development of the target regions that the DA cells come to innervate (e.g., see Kalsbeek et al., 1987; 1989). Furthermore, given that the messenger RNA for the estrogen receptor has been found to be expressed in the developing striatum and mPFC (e.g., Toran-Allerand et al., 1992), it is likely that the gonadal steroids have direct actions on the cells postsynaptic to the DA neurons, and thus, produce further sex differences in the functional effects of transmission in the DA systems. Finally, as discussed in the general introduction, the growth and development of the DA systems, like other higher brain regions (e.g., hippocampus and cortex) does not end until well after birth. Structures having prolonged periods of plasticity may, therefore, be influenced by the presence of the gonadal steroids in the late postnatal and prepubertal periods. In particular, exposure to estradiol during these later periods could have subtle effects on the structure and function of the DA systems, particularly in the female animal.

As has been shown in the present studies, the DA systems of the male and female animal are differentially responsive to the effects estradiol and to the interaction between the effects of estradiol and AMPH, and it appears that the functioning of the DA systems can be modified in the female animal by the presence and pattern of circulating estradiol in adulthood. In the adult female, estradiol has been shown to have numerous affects on both the DA neurons and their postsynaptic targets (e.g., striatum), altering release of DA (e.g., Becker 1990a, 1990b; Castner et al., 1993), DA receptors (e.g., Becker, Bazzett, Rice, & Rybak, 1992; Lévesque & Di Paolo, 1988), cell firing (e.g., Chiodo et al., 1983), and a number of cellular characteristics (e.g., ion channels, second messengers and G-proteins; Maus et al., 1989; Maus, Homburger, Bockaert, Glowinski, Prémont, 1990; Mermelstein, Becker, & Surmeier, 1993). Because many of these effects are extremely rapid, and receptors for estradiol have not been found in the adult midbrain or striatum, the genomic mechanism for the actions of steroids would not seem to account for these effects. The steroid hormones have been found to have a number effects directly on the membrane of

the cell or by interacting with receptors for other ligands (e.g., see McEwen, 1991 for review). Thus, the functioning of the DA system in the adult animal can be modulated rapidly by the presence of the hormone in the system. Based on the data in the present thesis, it appears however, that there is also a role for repeated or long-term effects of estradiol on the DA systems. This raises the possibility that even in the absence of classic estradiol receptors, estradiol can produce long-term effects on the DA systems. Given the data suggesting that estradiol can produce structural changes in the adult brain (e.g., Woolley & McEwen, 1992), a final intriguing possibility is that subtle changes in neural structure may be produced in the adult female animal during the normal estrous cycle.

The above scenario, although speculative, represents a plausible framework for understanding how sex differences in the nervous system, and thus, in behavior are produced. By this view, the sex differences in AMPH-induced behaviors represents the combined influence of a number of different events, both hormonal and non-hormonal, over the life-span that produce a divergence in the development of the male and female nervous systems. As adults, this differential development has altered the capacity of the sexes to respond not only to the modulatory signals of the circulating gonadal hormones, but also potentially, to the effects of environmental stimuli, other endogenous and exogenous signals, exogenous chemicals, stressors and aging.

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APPENDIX

Inter-experiment Variability in Responsiveness to Amphetamine

Two separate experiments were done in Experiment 6 on the role of life-long exposure to ovarian hormones. In the first, females were ovariectomized at birth or given sham surgery under hypothermic anaesthesia. When tested as adults, it was noted that the sham animals tested with EB had relatively low scores compared to similarly treated animals in Experiment 4 (group FEMALE); their relatively low scores resulted in a lack of difference between neonatally ovariectomized and sham groups. In the second experiment in which ovariectomy was done on PN25, the sham-operated animals tested with EB had scores comparable to those of group FEMALE from Experiment 4 when tested as adults, and the early ovariectomy groups had low scores, almost identical to those of the neonatal ovariectomy groups in the first experiment.

It was hypothesized, therefore, that the hypothermic anaesthesia given to neonates in the first experiment may have played a role in producing the low scores in the sham-operated animals receiving EB at the time of testing in adulthood. In order to test this hypothesis an experiment was conducted to compare the behavior of male and female animals that were either sham-cooled at birth or were left unmanipulated (kept warm under a lamp for the approximately 60 minute period that the pups were removed from the nest). Animals were raised as described in the other experiments, and in adulthood were all gonadectomized under Metofane anaesthesia. Activity testing began 15 to 16 days following these adult surgeries and the procedures (including drug and hormone doses) were as described for Experiment 4. That is, in the pre-exposure period all animals received an injection of EB, 30 to 35 minutes prior to receiving an AMPH (1.5 mg/kg) or SAL injection and being placed into the activity box. Three days after the last pre-exposure day all animals received 0.75 mg/kg AMPH in a test for sensitization.

The results of this experiment are shown in Figures 24 and 25. It is clear that cooling the animals at birth, alone, did not alter their responsiveness to AMPH in the presence of estradiol. The results do show, however, that overall levels of AMPH-induced

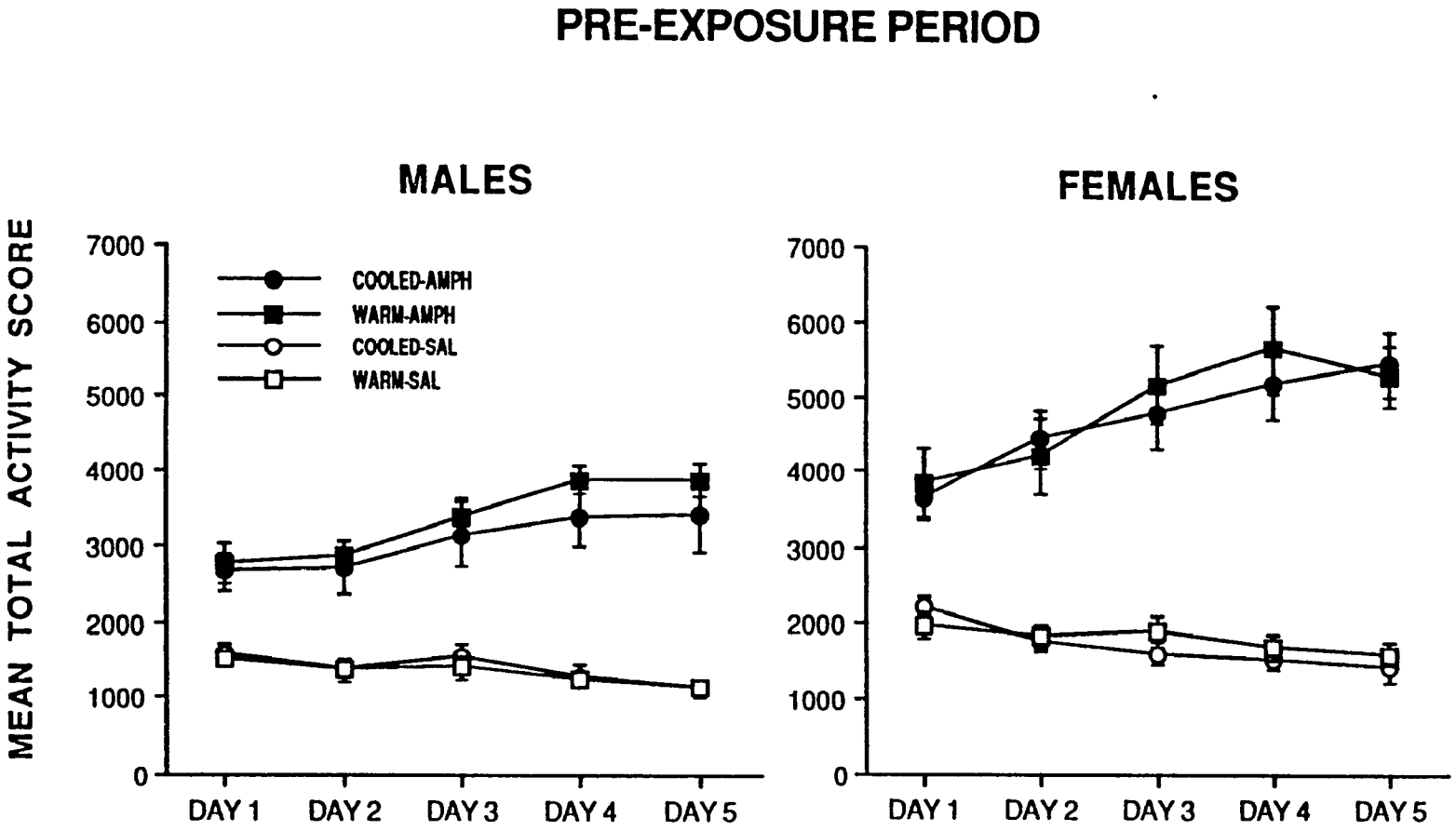

Figure 24. Mean total locomotor activity scores (± SEM) for males (left panel) and females (right panel) in response to either AMPH (1.5 mg/kg) or SAL for the two-hour session on each day of the pre-exposure period.

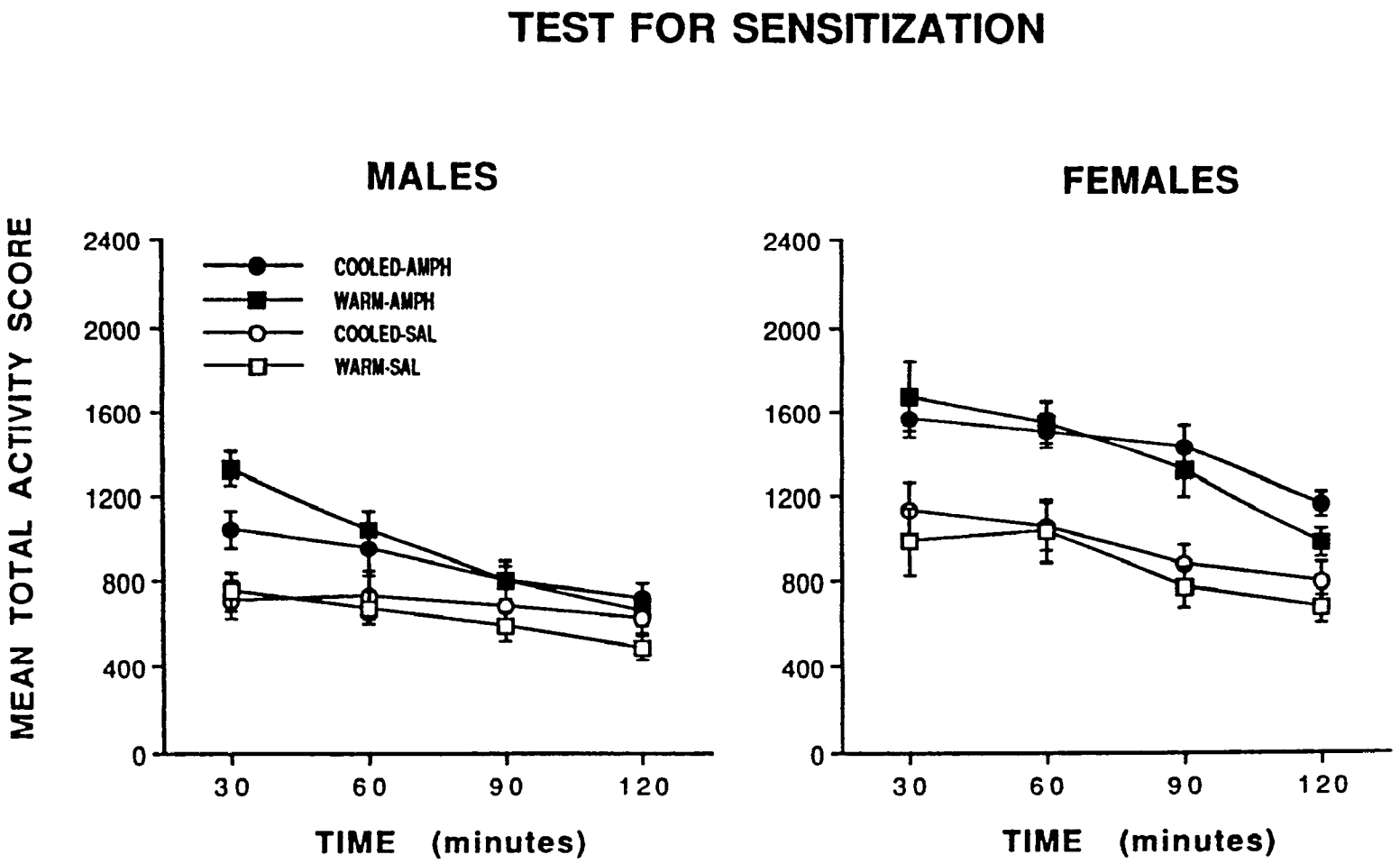

Figure 25. Mean total locomotor activity scores (± SEM) for males (left panel) and females (right panel) during each 30-minute time-block on the test day for sensitization when all animals received 0.75 mg/kg AMPH.

activity of females in this experiment were again, relatively low, although females treated with AMPH showed the expected increase in AMPH-induced activity over days of testing, and the expected sex differences were found (day x sex x drug: $F(4,252) = 3.25$, $p < .05$). Thus, although manipulation at birth, itself, may contribute to overall levels of activity in response to AMPH in the adult animal, it does not appear to alter the responsiveness of female animals to estradiol per se.

Based on these results, a final experiment was conducted to replicate the original four ADULT groups. Littermate pairs of female animals were removed from the nest on PN0 and either sham-cooled or kept warm. Animals were kept out of the nest for approximately 60 minutes. On PN25, animals that had been kept warm at birth underwent a sham operation under Metofane anaesthesia. Animals were ovariectomized in adulthood, two weeks prior to activity testing. Activity testing was identical to that described for Experiment 6. Thus, there were four experimental groups: SHAM0-EB-AMPH, SHAM0-OIL-AMPH, SHAM25-EB-AMPH, SHAM25-OIL-AMPH. The results of this experiment are shown in Figure 26. It is clear that both groups of animals show enhanced activity in the presence of estradiol (hormone: $F(1,32) = 4.55$, $p < .05$; day x hormone: $F(4,128) = 5.88$, $p < .005$), however, the overall levels of AMPH-induced activity are intermediate to those observed in the other experiments.

Taken together, it is clear that overall levels of AMPH-induced activity vary in adult female animals. Figure 27 shows all the groups of female animals that were ovariectomized in adulthood just prior to the activity test. The combined groups of animals ovariectomized in early development (EARLY groups from Experiment 6) are also included for comparison. As can be seen from these data, there is also some variability in the overall levels of activity in the animals tested in the absence of circulating gonadal hormones.

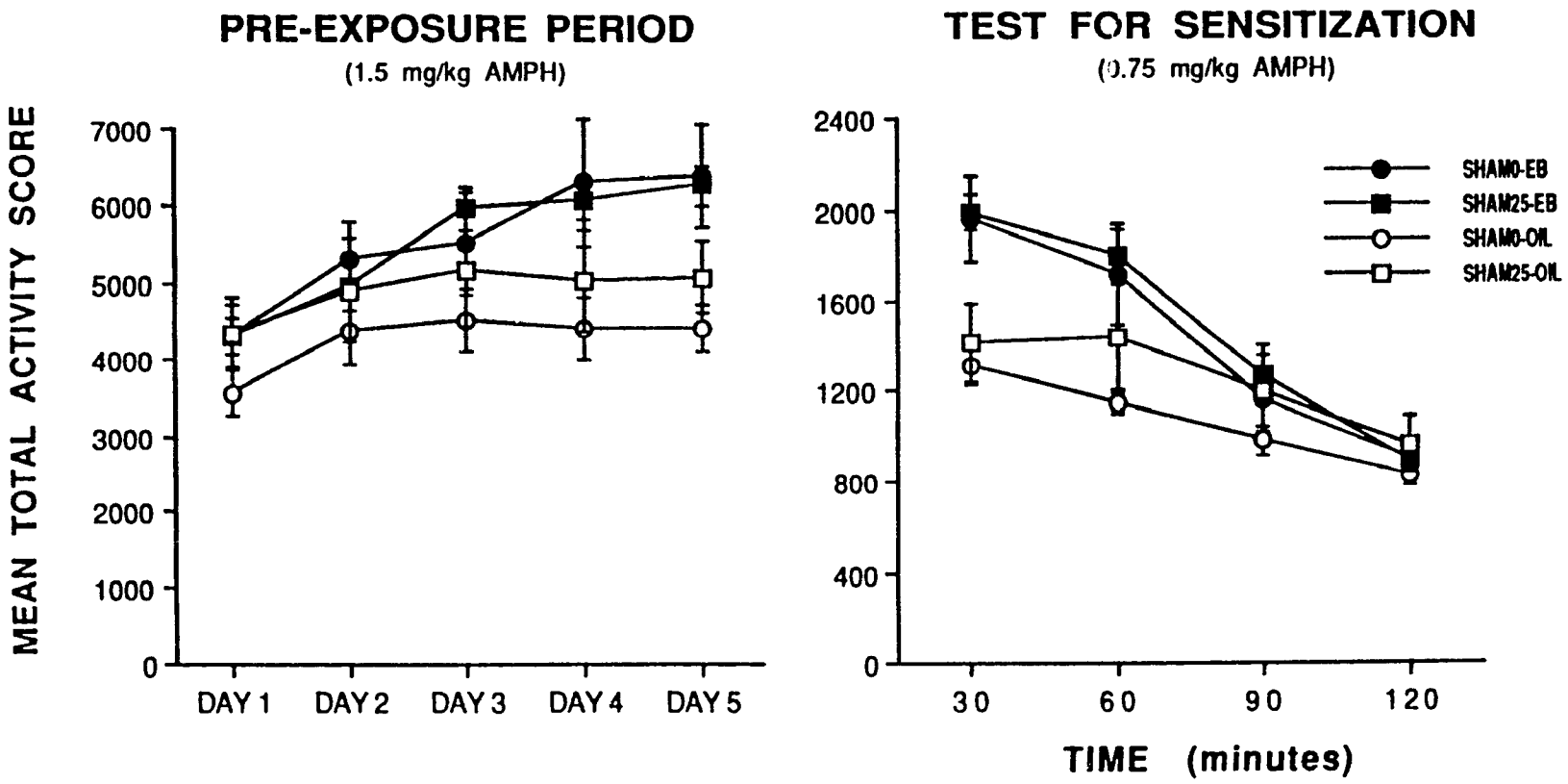

Figure 26. Mean total locomotor activity scores (± SEM) for the two-hour session on each day of the pre-exposure period (left panel) and during each 30-minute time-block on the test day for sensitization (right panel) for females sham-operated during early development, ovariectomized in adulthood, and given either OIL or EB at the time of each test.

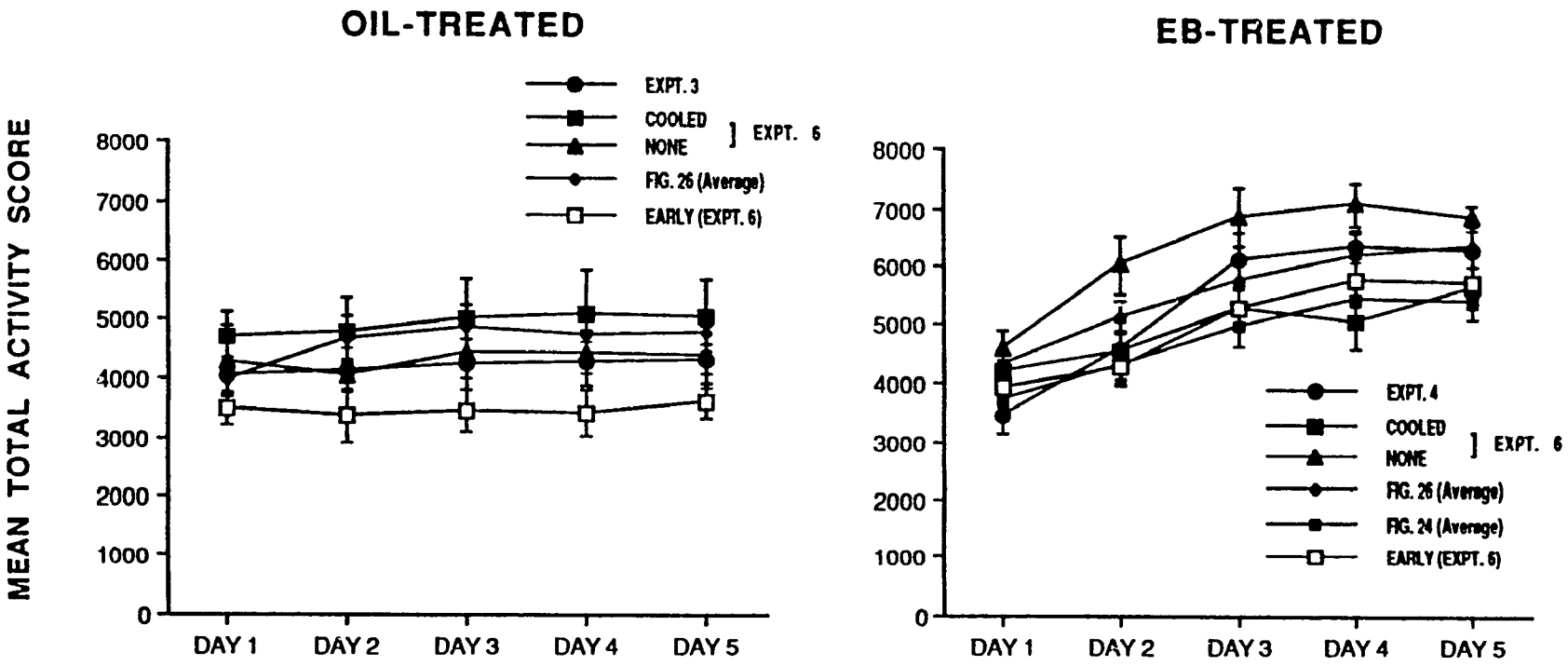

Figure 27. Mean total locomotor activity scores (± SEM) in response to AMPH (1.5 mg/kg) for the two-hour activity session on each day of the pre-exposure period for different groups of OIL-treated (left panel) and EB-treated (right panel) female animals tested in the present thesis. Filled symbols: animals ovariectomized in adulthood. Open squares: animals ovariectomized prior to puberty.

Because of the observed inter-experiment variability, and because there was no evidence to suggest that the effect of early ovariectomy on responsiveness to AMPH in the adult female varied between experiments, the decision was made to combine the original two experiments into a single data set. This data set allows the question of whether ovariectomy prior to puberty decreases responsiveness to AMPH in the adult female to be addressed, taking into account inter-experiment variability.