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dissertation entitled

Developmental Toxicities of Polychlorinated Biphenyls on Female Sexual Behavior and Incertohypothalamic Dopaminergic Neurons: Its Implication on the Neural Control of Sexual Behavior of Rats

presented by

Yu-Wen Chung

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Zoology-Neuroscience

Lynnwood R. Clemens
Major professor

Date Jan 26, 2000

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DEVELOPMENTAL TOXICITIES OF POLYCHLORINATED BIPHENYLS ON SEXUAL BEHAVIOR AND INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS: ITS IMPLICATION ON THE NEURAL CONTROL OF SEXUAL BEHAVIOR OF RATS

By

Yu-Wen Chung

A DISSERTATION

Submitted to

Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology and Neuroscience Program

2000

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ABSTRACT

DEVELOPMENTAL TOXICITIES OF POLYCHLORINATED BIPHENYLS ON SEXUAL BEHAVIOR AND INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS: ITS IMPLICATION ON THE NEURAL CONTROL OF SEXUAL BEHAVIOR OF RATS

By

Yu-Wen Chung

Polychlorinated biphenyls (PCBs) are long-lasting environmental pollutants that have been reported to disrupt development and reproduction in many species. However, very little is known about the effect of PCBs on sexual behavior. I examined the effect of two commercial PCBs, Aroclor 1221 (A1221) and Aroclor 1254 (A1254), on the development of sexual behavior and specific brain dopaminergic neurons in Long-Evans rats. A1221 and A1254 were chosen for their distinct difference in estrogenicity. Three treatment paradigms were used: perinatal treatment on gestation day 14, neonatal day 1 and day 10; neonatal treatment from day 1 to day 7; adult treatment from day 67 to day 72. Animals were tested for sexual behavior in adulthood.

Permanent changes were seen in female sexual behavior after developmental treatment with PCB mixtures. Different PCB mixtures appeared to have different critical periods. The change of female sexual behavior in neonatally A1254 treated rats was associated with the disruption of the developing A13 dopaminergic system.

Closer examination of the incertohypothalamic dopaminergic systems revealed a dynamic brain asymmetry wherein the presence of some dopaminergic cells was only seen following context-dependent copulatory stimulation. Copulation differentially

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induced more detectable tyrosine hydroxylase (TH) immunoreactive (IR) and/or FOS/TH-IR cells in the A11 and A13 regions. The testing situation, i.e. female-paced or male-paced, also affected the timing of TH-IR and FOS/TH-IR induction.

Copulation also induced more detectable FOS-IR cells in the medial preoptic nucleus compared to the noncopulated control. A striking difference in FOS-IR in the sexually dimorphic nucleus of the preoptic area of the hypothalamus (SDN-POA) appeared when females received one ejaculatory series of sexual stimulation from the male under different testing situations. The possible roles of SDN-POA in female reproduction might be to terminate the estrus and female cyclicity for pregnancy.

Males were not as susceptible to PCB exposure as female rats since I only observed minor changes in their sexual behavior subsequent to developmental exposure to PCBs. However, I found a strong correlation between male sexual behavior and cell number in the major pelvic ganglia (MPG). The number of the MPG cells showed sexually allomorphism among control females, male copulators and noncopulators. Neonatal PCB treatment resulted in a heterogeneous population of rats with wide spectra in MPG neuronal development.

The medial preoptic area, zona incerta, A11 dopaminergic neurons, spinal cord and pelvic ganglia formed a coordinated network involved in the control of sexual behavior. PCB exposure during development of these systems might disrupt the balance between central and peripheral nervous systems and result in the alteration of sexual behavior.

To my parents,

Hsiu-Ching Chung and Hsiu-Lien Lin

and

those who helped me navigate

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ACKNOWLEDGEMENTS

For their advice and influence into learning about the beauty of science, I express my sincere gratitude to my major professor Dr. Lynwood G. Clemens and the members of my dissertation committee, Drs. W Richard Dukelow, Kay E. Holekamp, John I. Johnson and Antonio A. Nunez.

I also thank Drs. Anthony E. Ackerman, Gary M. Lange, Kevin Sinchak and Liang-Yo Yang. They taught me experimental techniques I learned during my first three years at MSU. Dr. Jiming Fang is highly appreciated for his technical advice and support. I would like to acknowledge several undergraduate students who helped me with the cell counting: Rose Sydlowski (in the pelvic ganglia), Kimberly Roedel and Jamie A. Johansen (in the medial preoptic area). I also thank other lab associates, Dr. Brenda Alston-Mills, Anne M. Corrigan, Mattew Martin, Kristin Pikul, and Dan Thornhill for their support and company.

I would especially like to thank my parents, Hsiu-Ching Chung and Hsiu-Lien Lin, for their encouragement and support. I thank Dr. Kalle Karu, who designed the computer program for recording the behavior tests and has been there for me during these years. Especially warm thanks are extended to many friends who have helped to cheer me during the rough times and to celebrate with me during the happy times.

Finally, I thank the faculty and staff in the Department of Zoology and the Neuroscience Program for their support. The work in this dissertation was supported by a NIEHS Superfund grant (P42ES04911-09) and a NSF grant (IBN9728883) to Dr. Lynwood G. Clemens.

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HVA
IF
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IRL
L
LAAD
LH
LHRH
LQ
MAO
MF
ML
MP
MPG
MPOA
MPON
NC
NE
NO
NPY

LIST OF ABBREVIATIONS

3MT	3-methoxytyramine
%IL	percentage of intromission leave
%ML	percentage of mount leave
A1221	Aroclor 1221
A1254	Aroclor 1254
Ach	acetylcholine
Ah	aryl hydrocarbon
AHH	aryl hydrocarbon hydroxylase
AL	approach latency
CBs	chlorinated biphenyls
COMT	catechol-O-methyl transferase
DA	dopamine
DOPA	L-dihydroxy-phenylalanine
DOPAC	dihydroxyphenyl acetic acid
E	ejaculatory series
EL	ejaculation layency
EROD	ethoxyresorunfin-O-deethylase
FOS-IR	FOS-immunoreactive or FOS immunoreactivity
FP	female-paced
GABA	γ-aminobutyric acid
HVA	homovanillic acid
IF	intromission frequency
III	inter-intromission interval
IR	intromission rate
IRL	intromission return latency
L	lumbar
LAAD	L-amino acid decarboxylase
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
LQ	lordosis quotient
MAO	monoamine oxidase
MF	mount frequency
ML	mount latency
MP	male-paced
MPG	major pelvic ganglia
MPOA	medial preoptic area of the hypothalamus
MPON	medial preoptic nucleus of the hypothamalus
NC	noncopulated
NE	noradrenaline
NO	nitric oxide
NPY	neuropeptide Y

OHCBs
PCB
PCBs
PRL
S
SDN-POA
T
TCDD
TH
TH-IR

VCS
VIP
ZI

OHCBs	hydroxylated chlorinated biphenyls
PCB	polychlorinated biphenyl
PCBs	polychlorinated biphenyls
PRL	prolactin
S	sacral
SDN-POA	sexually dimorphic nucleus of the preoptic area
T	thoracic
TCDD	tetrachlorodibenzo-*p*-dioxin
TH	tyrosine hydroxylase
TH-IR	tyrosine hydroxylase-immunoreactive or tyrosine hydroxylase immunoreactivity
VCS	vaginocervical stimulation
VIP	vasoactive intestinal polypeptide
ZI	zona incerta

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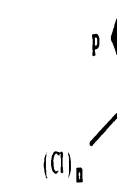
CHAPTER 1: INTRODUCTION

Although the effects of polychlorinated biphenyls (PCBs, Figure 1) on development and reproduction are well documented, very little is known about the effect of PCBs on sexual behavior. Since PCBs have neurotoxic effects on dopaminergic neurons (Safe *et al.*, 1985; Seegal *et al.*, 1990, 1991; Shain *et al.*, 1991; Brouwer *et al.*, 1995), and since dopamine plays a role in controlling sexual behavior (Everitt *et al.*, 1974; Carter & Davis, 1977; Foreman & Moss, 1979; Foreman & Hall, 1987; Grierson *et al.*, 1988; Mani *et al.*, 1994), it is possible that PCBs can disrupt sexual behavior by their actions on dopaminergic neurons. PCBs also have estrogenic and antiestrogenic effects on the development of systems controlling reproduction (Brouwer *et al.*, 1995). In this dissertation, two commercial PCB mixtures, Aroclor 1221 (estrogenic) and Aroclor 1254 (non-estrogenic) were used to examine the possible risks of PCB exposure during pregnancy and/or lactation, and adulthood. The effects of PCB exposure on sexual behavior and development of incertohypothalamic dopaminergic systems provide the main focus for this analysis. In the next section, I review the characteristics of PCBs, the study subjects, and the concepts of sexual differentiation of copulatory behavior.

Polychlorinated Biphenyls (PCBs)

Since their introduction for industrial use in 1929, PCBs (Figure 1) have been used for over 50 years in a wide variety of industrial applications such as manufacture of adhesives, paints, carbonless reproducing paper, printing inks, elastomers, wax extenders, flame retardants and as general fillers (Hutzinger *et al.*, 1972, 1974; Fishbein, 1974;



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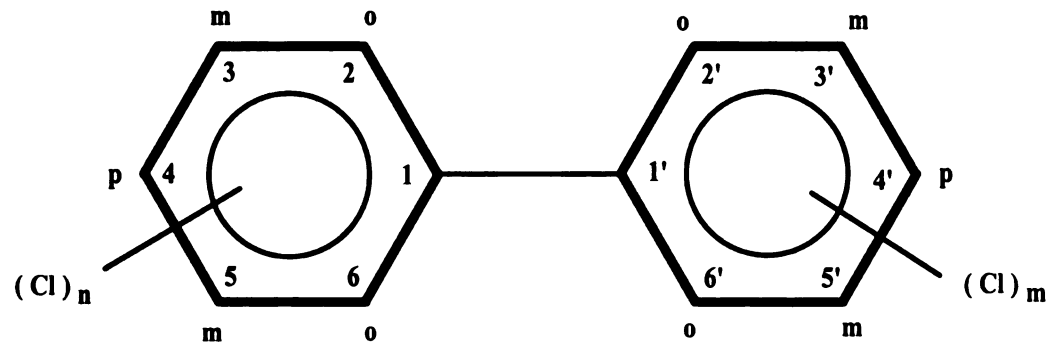

Figure 1. Molecular structure of polychlorinated biphenyls. O: ortho, m: meta, p: para position.

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Peakall, 1980). Their excellent dielectric properties make them useful as electric insulators and coolants in transformers. Their heat stability, high boiling point and low vapor pressure makes them ideal as lubricants, vacuum diffusion pump oil, heat transfer fluids, plasticizers and in high pressure hydraulic fluids (Dustman *et al.*, 1971; Fishbein, 1974). It was not until 1966 that PCBs were detected as pollutants in environmental samples (Jensen, 1966). In the following years, PCBs were found to be universal contaminants of aquatic and terrestrial ecosystems (Risebrough *et al.*, 1968; Heath *et al.*, 1970; Stickel, 1972; Roberts *et al.*, 1978). PCB residues have been identified in almost every component of the global ecosystem including rivers and lakes, the atmosphere, fish, wildlife, human adipose tissue, blood and breast milk (Risebrough *et al.*, 1968; Fishbein, 1972; Jensen & Sundström, 1974; Musial *et al.*, 1974; Holdrinet *et al.*, 1977; Cordle *et al.*, 1978; Wasserman *et al.*, 1979; Ballschmiter *et al.*, 1981; Buckley, 1982; Safe, 1982; Jacobson *et al.*, 1984, 1989; Koopman-Esseboom *et al.*, 1994; Moore *et al.*, 1997). The manufacture and use of PCBs in the United States was banned in 1977 (U.S. Environmental Protection Agency, 1978). Although the banning of PCBs in the industrially developed world has resulted in decreasing environmental levels (Schmitt *et al.*, 1990), bioaccumulation and biomagnification of the remaining PCBs still result in significant concentrations in upper trophic level (position in the food chain) species such as fish-eating waterbirds and mammals such as seals and humans who eat PCB-contaminated seafood (Dewailly *et al.*, 1989; Haraguchi *et al.*, 1992; Jansson *et al.*, 1993). The relative abundance of individual chlorinated biphenyls (CBs) in environmental samples varies with the location and source of contamination, age of the samples, and environmental matrix such as the trophic level of the organism. In general,

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fish, wildlife and human samples are dominated by persistent penta- to hepta-CBs, due to their resistance to biodegradation relative to the more readily metabolized mono-CBs to tetra-CBs (Sunström *et al.*, 1976) that possess vicinal hydrogens in the 3,4 position of one of the phenyl rings.

There are 209 PCB congeners that differ both in the number and positions of chlorines on the biphenyl moiety (Figure1; Barlow & Sullivan, 1982; Mullin *et al.*, 1984) and in the kinds of toxic responses they elicit (Parkinson & Safe, 1987; Safe, 1994). Approximately 120 PCBs have been identified in the environment (Bush *et al.*, 1985). On the basis of their ability to induce aryl hydrocarbon hydroxylase (AHH) activity in rat hepatoma cells, Safe (1990) has classified PCB congeners into three main classes: (I) coplanar dioxin-like congeners that interact at the aryl hydrocarbon (Ah) receptor, induce associated hepatic enzymes, and share many toxicological properties with the dioxins (Figure 2) and dibenzofurans (Figure 3); (ii) di-ortho-substituted congeners that neither interact with the Ah receptor nor induce the associated hepatic enzymes, and (iii) mono-ortho-substituted congeners, which are intermediate in activity between the two above-described classes of congeners (Seegal *et al.*, 1997).

Coplanar PCBs

Only PCBs that are substituted in the para and meta positions will exhibit maximum coplanar conformational character and approximate the relatively flat structure of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Moreover, of the 20 possible coplanar PCBs, it is apparent from several studies that AHH-inducing activity is only observed for compounds substituted at both para positions (Goldstein *et al.*, 1977; Poland & Glover,

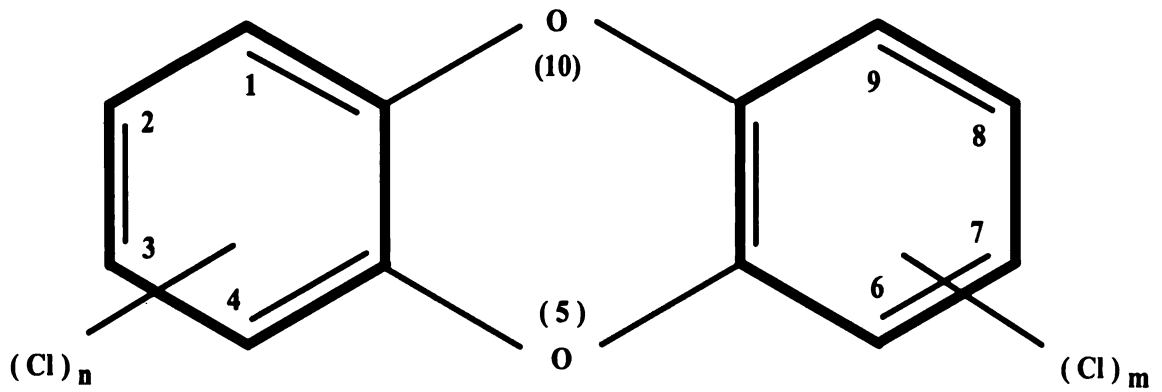

Figure 2. Molecular structure of polychlorinated dibenzo-*p*-dioxins.


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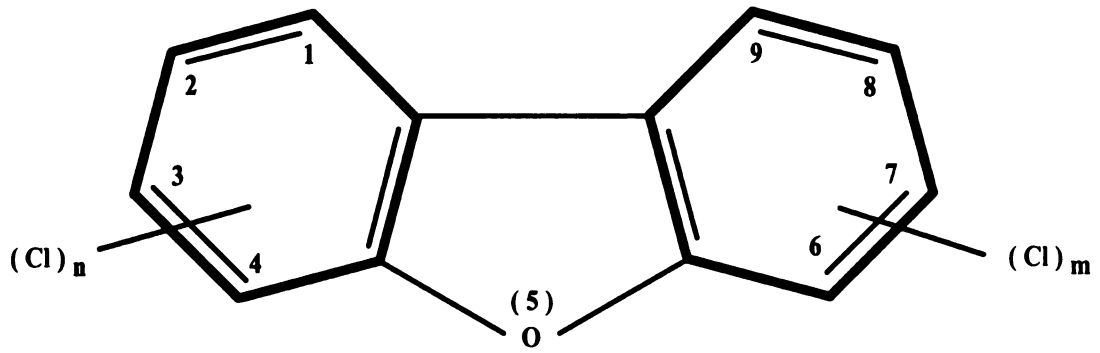

Figure 3. Molecular structure of polychlorinated dibenzofurans.

1977; Parkinson *et al.*, 1981). Active coplanar PCBs elicit toxic responses typical of 2,3,7,8-TCDD including weight loss after exposure, hepatonecrosis, porphyria (hereditary abnormalities of porphyrin metabolism), thymic atrophy and reproductive toxicity (McKinney *et al.*, 1976; Goldstein *et al.*, 1977; Poland & Glover, 1980; Biocca *et al.*, 1981; Marks *et al.*, 1981; Parkinson *et al.*, 1983).

Mono-ortho-substituted PCBs

The introduction of a single ortho-chloro substituent into the biphenyl ring results in decreased coplanarity between two phenyl rings due to steric interactions between the bulky ortho-chloro and hydrogen substituents. At least five of the mono-ortho analogs of the coplanar PCBs elicit toxic effects which resemble (qualitatively) 2,3,7,8-TCDD and several of these compounds have been identified in commercial PCBs and as residues in human tissues (Jensen & Sundström, 1974; Kuroki & Masuda, 1977; Mullin *et al.*, 1981).

Di-ortho-substituted PCBs

The di-ortho coplanar PCBs exhibit low binding affinities for the cytosolic Ah receptor protein (Bandiera *et al.*, 1983) and are relatively inactive as AHH/EROD (ethoxyresorunfin-O-deethylase) inducers in rat hepatoma H-4-II-E cells (Sawyer & Safe, 1982). The toxicity of the di-ortho coplanar PCBs has not been systematically investigated; However, two members of this group, 2,2',3,3'4,4'- and 2,2',3,4,4',5'-hexachlorobiphenyls are porphyrinogenic in rats after long-term feeding studies (Stonard & Grieg, 1976). Both of these compounds are among the most active di-ortho coplanar PCB inducers of rat hepatic microsomal enzymes AHH and cytochrome P-450C (Safe *et*

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al., 1985). It is evident that the most active PCB compounds are approximate isostereomers of 2,3,7,8-TCDD and there is an excellent correlation between the cytosolic receptor binding affinities, AHH induction potencies and toxicities of these compounds.

Davis and Safe (1990) further classified PCBs into: (a) class I: coplanar PCBs substituted at para and/or meta positions, (b) class II: mono-ortho coplanar PCBs, (c) class III: mono-ortho coplanar PCBs minus a single para-chloro group, (d) class IV: di-ortho coplanar PCBs, (e) class V: tri-ortho coplanar PCBs, and (f) tetra-ortho-substituted PCBs. The structure of these compounds again determines their receptor binding abilities and possible toxic mechanisms (Safe *et al.*, 1985; Kannan *et al.*, 1988; Korach *et al.*, 1988; Davis & Safe, 1990; Shain *et al.*, 1991).

Coplanar vs. Ortho-substituted PCBs

Coplanar congeners with chlorine substitutes only in the meta- and para-positions are ligands of the Ah receptor and induce the gene family I (CYP1A1, CYP4A1) whereas poly-ortho-substituted PCBs induce gene family II (Borlakoglu *et al.*, 1992). The two types of PCB congeners may also interfere differentially with the nervous system. Coplanar 3,4,3',4'-tetrachlorobiphenyl (1 mg/Kg body weight from gestation day 7 to day 18) exposed rats had increased descent latencies in a catalepsy test and impairments of passive avoidance behavior (Weinand-Härer *et al.*, 1997). On the other hand, metabolites of the coplanar 3,4,3',4'-tetrachlorobiphenyl are known to block the binding sites of vitamin A and thyroid hormones at their plasma transport protein complex, thereby leading to a decrease in total and free T_4 levels and a decrease in plasma retinol

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(Brouwer, 1991). Since thyroid hormones are important regulators of neuronal development (Porterfield & Hendrich, 1993), behavioral effects in PCB-treated animals may also be mediated by this mechanism.

Coplanar congeners, in addition to their ability to interact at the Ah receptor, also alter estrogenic function, either by enhancing the metabolism of estrogens to hydroxy- and catechol estrogens (Gierthy *et al.*, 1988) or by downregulating estrogen receptors (Safe *et al.*, 1991) and may interfere with DNA binding (Harris *et al.*, 1990; Zacharewski *et al.*, 1991). Because coplanar PCBs are the inducers of Phase I and Phase II metabolic detoxification enzymes which reduce the estrogen-dependent expression of proteins by enhancing the enzymatic conversion of latent 17β-estradiol to less active metabolites, they can elicit an antiestrogenic response (Spink *et al.*, 1992). Thus, given the well-known interactions between estradiol and dopamine, particularly during development (Stumpf *et al.*, 1983; Simerly, 1989), the elevations in dopamine and metabolite concentrations following perinatal exposure to coplanar PCBs may in part be due to coplanar-induced alterations in steroidal function during development (Seegal *et al.*, 1997).

In addition to the potential role of the parent congener, Morse *et al.* (1995) have demonstrated that perinatal exposure to 3,4,3',4'-tetrachlorobiphenyl results in significantly greater accumulation of metabolites of this congener (particularly 3,5,3',4'-tetrachloro-4-biphenylol) in the fetus and offspring than in the dam. In turn, certain hydroxylated biphenyls have been shown to be estrogenic (Korach *et al.*, 1988). Thus, the neurochemical changes may be due to the estrogen-like activity of its metabolites during critical periods of development, rather than to the continuing presence of the

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Ortho-substituted PCBs are neuroteratogens and developmental exposure altered dopamine function and the change in neurochemical function persisted in the absence of PCBs in brain at adulthood (Seegal, 1992). The ortho-substituted PCBs have been shown to reduce dopamine levels in the brain and in PC-12 cells, while the coplanar PCBs display reduction (Agrawal *et al.*, 1981), elevation (Seegal *et al.*, 1997) or no effect (Seegal *et al.*, 1990; Shain *et al.*, 1991). Interestingly, the dose response curve of commercial PCBs is sometimes biphasic (Chishti *et al.*, 1996) because commercial PCBs usually contain mixtures of both ortho-substituted and coplanar PCBs. Aroclor 1254 (A1254), one commercial PCB mixture, has been shown to decrease dopamine at low doses and increase dopamine at much higher doses (Chishti *et al.*, 1996). The changes of dopamine levels resulting from PCB treatment (Figure 4) are due to the inhibition of dopamine synthesizing enzymes, tyrosine hydroxylase or L-amino acid decarboxylase, or to the vesicular storage or release of dopamine or vesicular monoamine transporter (Chishti *et al.*, 1996; Augus *et al.*, 1997; Choksi *et al.*, 1997).

Exposure to ortho-substituted congeners, such as 2,4,2',4'-tetrachlorobiphenyl, affects phosphoinositide metabolism and translocation of protein kinase C (Kodavanti *et al.*, 1994). Alteration in the above biochemical endpoints could affect either the phosphorylation state of tyrosine hydroxylase or the affinity of tyrosine hydroxylase for its cofactor, tetrahydrobiopterin (Weiner, 1978; Nagatsu *et al.*, 1994). Both of these factors play important roles in regulating dopamine synthesis (Zigmond *et al.*, 1989) and

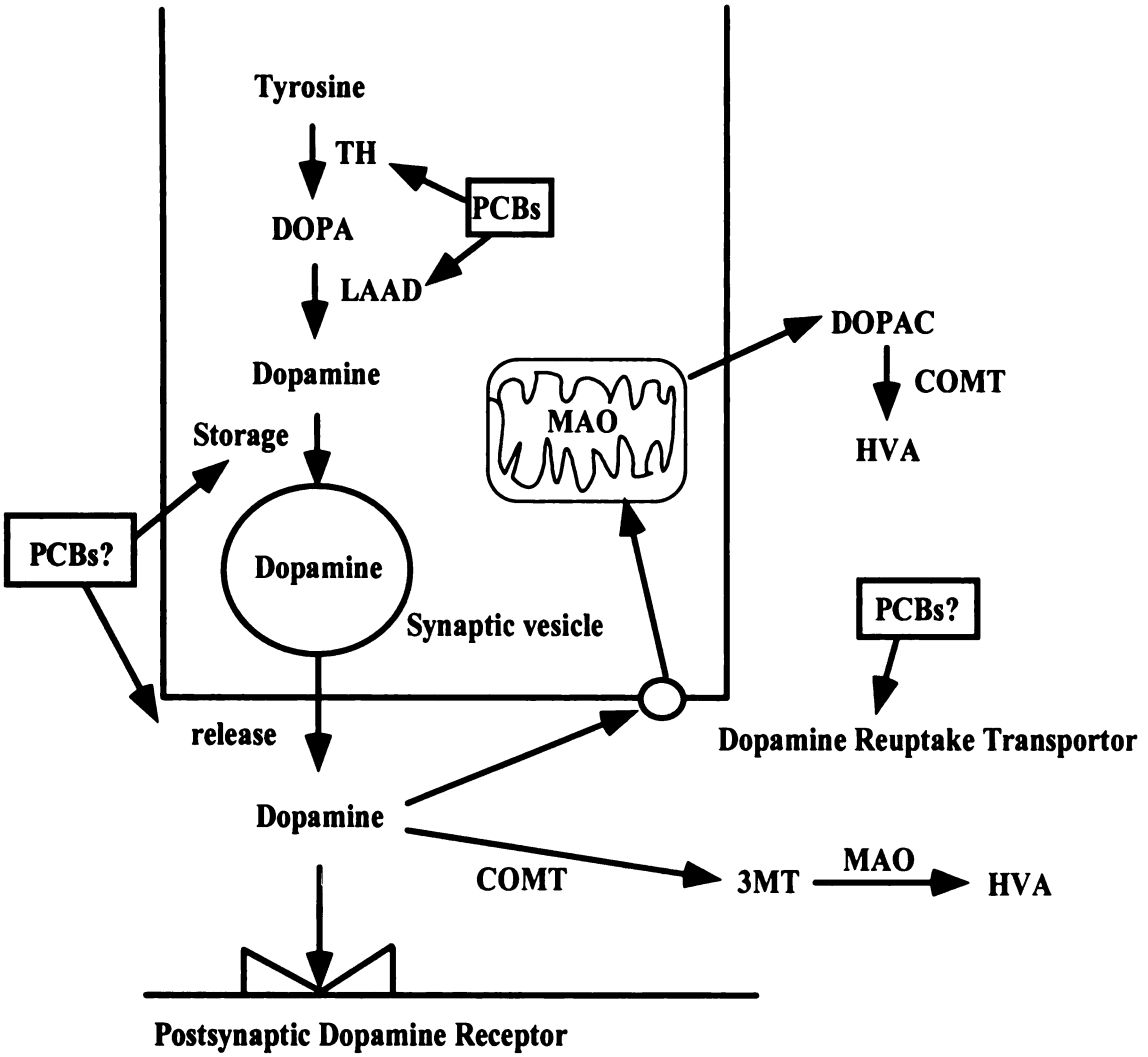

Figure 4. Possible mechanisms of PCB effects on dopamine concentration. COMT: catechol-O-methyl transferase; DOPA: L-dihydroxy-phenylalanine; DOPAC: dihydroxyphenyl acetic acid; HVA: homovanillic acid; LAAD: L-amino acid decarboxylase; MAO: monoamine oxidase; 3MT: 3-methoxytyramine; TH: tyrosine hydroxylase.

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may be involved in the reductions in brain dopamine seen in 2,4,2',4'-tetrachlorobiphenyl-exposed animals (Seegal *et al.*, 1997).

In addition to changes in brain dopamine function, postnatal day 10 exposure to individual congeners (including ortho-substituted congeners and coplanar 3,4,3',4'-tetrachlorobiphenyl) resulted in long-term changes in behavior and regional brain muscarinic and nicotinic receptor densities (Eriksson *et al.*, 1991; Eriksson & Fredriksson, 1996). The persistent changes in cholinergic receptor function may alter the normal dopaminergic/cholinergic balance, resulting in altered brain function (Consolo *et al.*, 1992; Marchi *et al.*, 1992; Di Chiara & Morelli, 1993).

The reductions in brain dopamine concentrations are a consequence of ortho-substituted PCB congener-induced inhibition of the synthesis of dopamine during critical periods of development, acting in concert with PCB-induced changes in cholinergic receptor function. On the other hand, the persistent elevations in brain dopamine and metabolite concentrations following perinatal exposure to coplanar 3,4,3',4'-tetrachlorobiphenyl may be mediated by alterations in steroid hormone function during key developmental periods (Seegal *et al.*, 1997).

To summarize, coplanar PCBs possess estrogenic or antiestrogenic activity and probably act through Ah receptors, resulting in alteration of thyroid hormones and retinoid functions. The interaction of estrogenic effects of coplanar PCBs and dopaminergic systems probably cause the elevation of dopamine contents in the brain. On the contrary, ortho-substituted PCBs probably act upon the signal transduction pathway to disrupt phosphoinositide metabolism and translocation of protein kinase C and result in the decrease of dopamine content in the brain.

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Metabolism of PCBs

PCBs undergo oxidative alteration via cytochrome-requiring P450 enzymes that catalyze the formation of intermediate arene oxides that lead to hydroxyl or methyl sulphone metabolites (Bergman *et al.*, 1994). Hydroxylated chlorinated biphenyls (OHCBs) and their sulfate and glucuronide conjugates are typically excreted via feces or urine. However, organisms may retain OHCBs because these compounds are capable of binding to plasma proteins, particularly transthyretin (Bergman *et al.*, 1994), or to lipids. OHCBs have been identified in blood of marine mammals, fish-eating birds and humans at concentrations in some cases exceeding those of the unmetabolized PCBs. A number of toxic effects are produced exclusively by metabolites of PCBs. OHCBs have been associated with alterations in vitamin A and thyroid hormone metabolism (Brouwer & Van den Berg, 1986; Brouwer *et al.*, 1990), while methylsulphone-PCB metabolites have been implicated as possible toxic agents in the lung and adrenals and in fetuses of mice (Brandt *et al.*, 1985; Lund *et al.*, 1985; Darnerud *et al.*, 1986). Toxic effects associated with the metabolite are related to the effects of the parent compound through Ah-receptor-mediated PCB metabolism (Brouwer, 1991).

Toxicities of PCBs

PCBs have been shown to have a variety of effects on reproduction. Some PCB-isomers have estrogenic activity in the rat uterus (Bitman *et al.*, 1972; Fishbein, 1974; Gellert, 1978). Other effects on reproduction attributed to PCBs include a decrease in fertility of sea lions (Olsson *et al.*, 1974; Helle, 1976), reduction in the number of female mink that complete pregnancy (Bleavins *et al.*, 1980), disturbances in the estrous cycle of

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female mice, reduced litter size due to a reduction of implanted ova and the increase in degeneration rate of embryos *in vitro* and *in utero* (Örberg and Kihlström, 1973; Kholkute *et al.*, 1994) possiblly due to altered biochemical environment of the ovaries and uterus during oogenesis or pregnancy (Török *et al.*, 1976). It has been shown that dietary administration of PCB compounds during decidualized pseudogestation produced acute alterations in uterine and ovarian biochemistry (Spencer, 1982). PCBs also affect male fertility. Organ weights in the male reproductive system were depressed in PCB-treated male rats. Cauda epididymal sperm numbers were also reduced (Sager *et al.*, 1987).

PCBs have the potential to disrupt reproduction through various pathways. They can affect endocrine systems that are essential for reproduction (Colborn *et al.*, 1993). For example, testicular and seminal vesicular functions were disrupted after PCB treatment in male mice (Sanders *et al.*, 1977); androgen metabolism was altered in male rats (Derr and Dekker, 1979; Haake-McMillan and Safe, 1991), and estrous cycles and progesterone synthesis were disrupted in female rats (Jonsson *et al.*, 1976).

Estrogen plays a key role in the sexual differentiation of rats (MacLusky and Naftolin, 1981). Some PCBs bind to estrogen receptors, exhibit estrogenic activity, cause precocious puberty, persistent vaginal estrus and premature reproductive aging (Bitman and Cecil, 1970; Örberg and Kihlström, 1973; Gellert, 1978; Korach *et al.*, 1988). Other PCBs, on the contrary, exhibits minimal binding to estrogen receptors, and their hydroxylated metabolites may even have antiestrogenic activity (Moore *et al.*, 1997). However, other hydroxy-PCBs may exert estrogenic effects as well. A dramatic example was seen in turtles where the estrogenic effect of hydroxy-PCBs reversed the

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temperature-dependent sex determination (Bergeron *et al.*, 1994; Crews *et al.*, 1995). Reports of abnormal sexual development in reptiles (Guillette *et al.*, 1994, 1995) or birds (Fry, 1995) as well as feminized responses in male fish (Jobling & Sumpter, 1993; White *et al.*, 1994; Jobling *et al.*, 1995) have also strengthened the notion that environmental chemicals can function as estrogens. The weak estrogenic activity of commercial PCB mixtures was recognized early in studies of PCB toxicity (Bitman & Cecil, 1970); however, only the less chlorinated Aroclor mixtures (≤ 48% Cl by weight) exhibited estrogenicity, consistent with the hypothesis that lesser chlorinated OHCB metabolites were the active compounds (Nelson, 1974).

Another issue related to PCB toxicities is their potential to alter the behavior of exposed animals or humans. Epidemiological studies indicate that PCBs may produce neurological and behavioral dysfunctions in perinatally exposed human infants including deficits in visual recognition memory and decreased intelligence scores (Jacobson *et al.*, 1985; Rogan *et al.*, 1986, 1987, 1988; Gladen & Rogan, 1988; Gladen *et al.*, 1988). Similar findings were noted in laboratory studies of nonhuman primates (Bowman *et al.*, 1978; Bowman & Heironmus, 1981; Schantz & Bowman, 1983; Mele *et al.*, 1986; Schantz *et al.*, 1989).

Mice exposed to tetrachlorobiphenyls during gestation have been reported to exhibit a long-lasting neurobehavioral syndrome consisting of stereotypic head movements, rotational behavior, increased motor activity, impaired neuromuscular strength and coordination, and learning deficits (Tilson *et al.*, 1979). Other investigators (Lucier *et al.*, 1978; Chou *et al.*, 1979) have also noted that *in utero* exposure to tetrachlorobiphenyls produces hyperactivity possibly resulting from the interference with

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PCBs also exhibit toxic effects on homeostatic mechanisms. PCBs produce ultrastructural lesions in thyroid follicular cells and a reduction in serum levels of thyroid hormones in neonatal rats (Collins and Capen, 1980; Gray *et al.*, 1993). Many other toxicities of PCBs have also been documented including hepato-, immuno- and dermal toxicities, teratogenicity as well as carcinogenicity (Fein *et al.*, 1984; Overmann *et al.*, 1987; Brouwer *et al.*, 1995).

The effects of different PCB congeners are also species-specific. Certain ortho-substituted PCB congeners (e.g., 2,2'-dichlorobiphenyl) can inhibit tyrosine hydroxylase activity and dopamine synthesis in both Sprague-Dawley and Long-Evans hooded rats. However, the ortho-meta-substituted PCB congener 2,2',5,5'-tetrachlorobiphenyl inhibited tyrosine hydroxylase activity only in Spraque-Dawley rats (Choksi *et al.*, 1997).

Basic Biology of Long-Evans Rats

The Long-Evans rats used in our laboratory are derived from wild brown rats (*Rattus norvegicus*). This species breeds throughout the year in most situations. The annual litter rate varies from 2.2 for small rats to 8.2 for large ones. The mean embryo count is 9.3±2.3 per litter. The 50% point for initiation of ovulation is at 153 g (body weight) and 144 mm (body length). The 50% point for vaginal perforation is 102 g and 143 mm. The 50% point for testis decent is 105±45 g, and spermatozoa are present in 50% of males at 200±70 g. The Long-Evans rat is polyestrous all the year round, and ovulation is spontaneous, occurring near the end of estrus. Estrus lasts about 20 hours, and the cycle from 4 to 6 days. Estrus usually begins between 7 and 8 pm, with 75%

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beginning between 4 pm and 10 pm and less than 1% between 3 am and 11 am. The average duration of the first estrus is slightly less than that of later ones, the mean being 9.1 hours. In the normal estrous period the female is most receptive during the first 3 hours. The usual time of ovulation is 8 to 11 hours after the beginning of the estrus. The corpus luteum formed after rupture of the follicle is physiologically inactive unless the cervix has been stimulated mechanically or by coitus. The interval between the estrous periods thus represents postestrum, and its length is determined by the time required to ripen new follicles. When the cervix is stimulated, prolactin is released from the anterior pituitary, enabling the corpus luteum to secrete progesterone. This continues for about 14 days, during which time the rat is pseudopregnant. New follicles do not ripen, estrus does not occur, and the uterus undergoes various changes, such as growth of the glands and sensitization of the endometrium to trauma. The regression of the corpus lutea is followed by the ripening of new follicles, estrus, and ovulation (Asdell, 1964).

The gestation period is 22 days with very little variation. If the mother is suckling six or more young, implantation in a new pregnancy is delayed, and the pregnancy is consequently prolonged. In the absence of pregnancy, lactation causes the corpus lutea of the postparturition ovulation to persist. Removal of the young is followed by estrus 4 days later (Asdell, 1964).

Sexual Differentiation in Rats

In many species marked sex differences in the control of endocrine function and behavior by the central nervous system are the normal outcome of sexual differentiation, including the recognition of the sexual partner, mating pattern, and the subsequent

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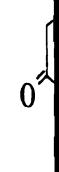
production and rearing of young (Maclusky & Naftolin, 1981). Other sexually dimorphic functions include the play and social behavior, agonistic, learning, posture during urination, scent marking behavior, vocalization, regulation of food intake, and body weight (Hutchison , 1997; Fitch & Denenberg, 1998).

The brain is critically involved in the regulation of reproduction (Kalra & Kalra, 1983), and the process of sexual differentiation in the brain is comparable to that of the internal reproductive organs or the genitalia (Gorski, 1985; Parker *et al.*, 1999a,b). The period of sexual differentiation varies from species to species and may be prenatal, perinatal, or postnatal, depending upon the level of maturation of the central nervous system at birth (Gorski, 1983). Even in the same species the sexual differentiation of a given function may differ temporally, in terms of hormonal sensitivity and the specific gonadal steroid responsible for differentiation (Christensen & Gorski, 1978; Gorski & Jacobson, 1981). There may be evidence for the sexual differentiation of a given function in one species but not in another (Gorski, 1985).

During perinatal periods of brain development, there are definable phases of hormonal sensitivity when steroid hormones influence maturing neuronal mechanisms underlying both the neuroendocrine system and behavior in rats. However, the way in which these phasic effects of steroids interact with the changing fetal and neonatal environment to bring about behavioral development is not fully understood (Hutchison, 1997). There is little doubt now that the steroid hormones, androgen and its estrogen metabolites (Figure 5) are involved in the sexual differentiation of behavioral development by direct action on the brain. Current ideas on the mode of action of steroids in the brain suggest that a sexually differentiated phenotype develops as a



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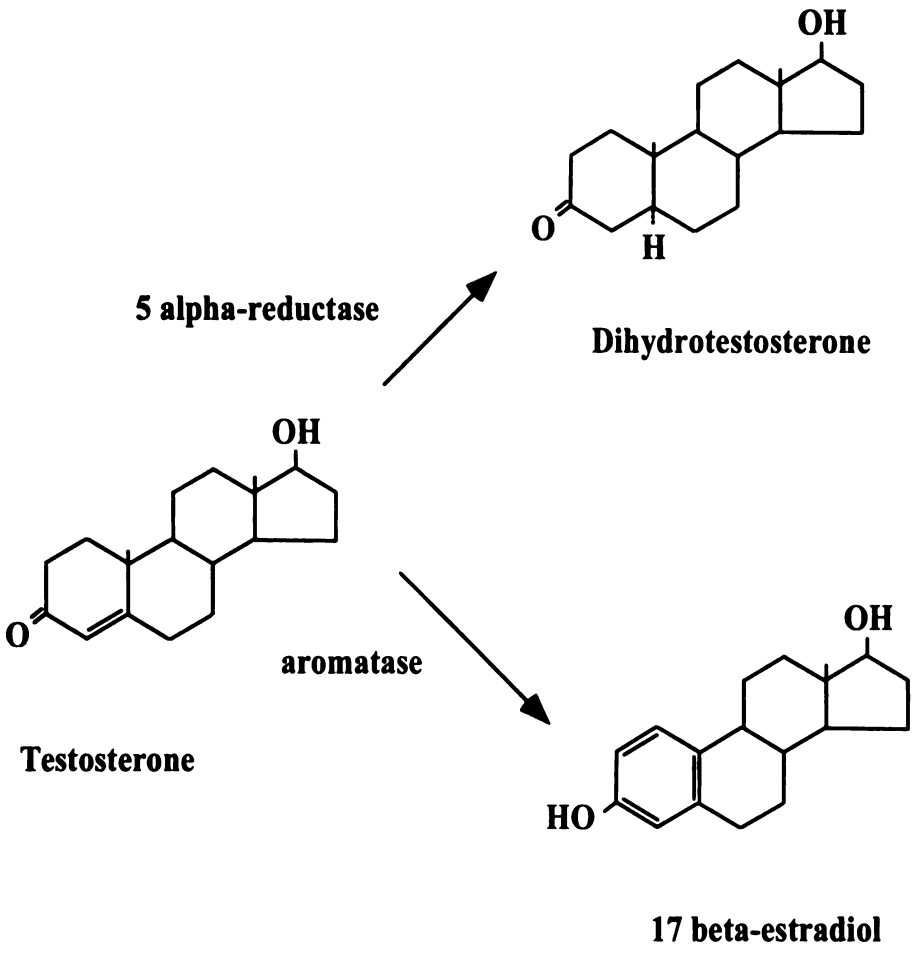

Figure 5. Pathways for *in situ* metabolism of testosterone by neural tissue.

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consequence of gonadal steroid action on an undifferentiated bipotential substrate (Adkins-Regan *et al.*, 1997; Arnold, 1997; Fitch & Denenberg, 1998). Theoretically, the distinction can be made between permanent organizing effects of steroid, which are irreversible, and the transient activational effects of steroids required for adult behavior (Goy & McEwen, 1980; Arnold & Gorski, 1984).

Current ideas concerning the developmental action of hormones on behavior are derived partly from early embryonic work (Jost, 1972; Jost & Magre, 1984). There appear to be three stages in sexual development from an undifferentiated primordium common to genetic males and females. First, gonadal sex is determined by the presence or absence of the male testis-determining gene on the Y chromosome, SRY, and its related genes SF-1, WT1, Dax-1, and SOX9 (Goodfellow & Camerino, 1999; Koopman, 1999; Ohno, 1999; Parker *et al.*, 1999; Scherer, 1999); second, the reproductive tract and genital morphology are differentiated hormonally (Arnold, 1997; Hutchison, 1997; Gorski, 1999); third, sexual differentiation of the brain and behavior occurs as the final stage, also under hormonal control (Arnold, 1997; Hutchison, 1997). The general principle is that in mammals the male phenotype develops when a specific hormonal signal (androgen) is present. In the absence of this signal, reproductive tissues differentiate into the female type.

The early organization hypothesis can be summarized as follows. (1) In the heterogametic sex, the appropriate hormone of that sex irreversibly organizes brain mechanisms of behavior during a critical period early in development. (2) The organizational hypothesis implies that there is a continuum in behavioral development from female to male that may depend, in mammals, on how much androgen is present

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during the critical period of development. This notion has important implications because the amount of androgen in the developing mammalian brain could determine the degree of behavioral masculinization. (3) There is no requirement for a defeminization process. (4) Organizational effects are irreversible; they occur in perinatal development during a limited critical period before the brain and other target tissues have finally matured.

There are a number of major difficulties with the organizational hypothesis; the first relates to the site of action of androgen. While gonadal hormones are known to act on the brain during development, it is unclear to what extent they influence the peripheral nervous system. The second difficulty lies with the supposition that sexual differentiation is a unitary event involving a one-hormone androgenic effect. The orthogonal model of sexual differentiation (Whalen, 1974; Yahr, 1985) establishes that in contrast to the classical theory, in which sexual differentiation occurs along a continuum, masculine and feminine aspects of sexual behavior are differentiated at separate stages in development and probably independently. A third difficulty is whether hormonal activation of sexual behavior in adulthood and organization during perinatal development are separable processes (Hutchison, 1997). Two aspects of the organizational hypothesis, therefore, have to be reconsidered. First, there appears to be no strict time limit to the critical period for the differentiating effects of estrogen. Second, given the right conditions, mechanisms underlying behavior can be organized by steroids in the adult animal (Hutchison & Hutchison, 1985; Schumacher & Balthazart, 1985). Perhaps the greatest challenge for the steroids trigger sexual differentiation hypothesis comes from the studies in non-mammalian species. The disjunction of gonadal and neural phenotypes and the lack of effects of steroids on the phenotype of the sexually dimorphic plumage in birds have led

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us to speculate upon alternatives for this hypothesis (Arnold, 1997).

The neural basis for mammalian sex differences is characterized by sexual dimorphism in central and peripheral nervous systems. There are sex differences in the number of synapses (Raisman & Field, 1973) in the medial preoptic area, the volume of the sexually dimorphic nucleus of the preoptic area (Gorski *et al.*, 1978), the cell number and size of the spinal nucleus of the bulbocavernosus (Breedlove, 1992), the cell number of the major pelvic ganglia (Greenwood *et al.*, 1985) and many more in rats (Fitch & Denenberg, 1998).

The downstream molecular processes of steroid action in sexual differentiation have not been delineated. Steroids may influence neurotransmitter metabolism, neuronal conductivity and synaptic connectivity, of developing neurons, which may lead to permanent changes in synaptic transmission and overall neuronal activity (Becú-Villalobos *et al.*, 1997). Interactions between steroids and neurotransmitters have been widely investigated in adult animals, and neurotransmitters may also serve active roles during the process of sexual differentiation (Dohler, 1991).

So far the research on sexual differentiation of copulatory behavior has been focused on systemic injections of steroids, steroid agonists and antagonists, receptor blockers, or enzyme blockers during specific critical periods. However, the results of these studies are sometimes difficult to interpret because steroids have widespread effect on many cross-reacting pathways in the brain. The use of PCBs on the study of sexual development of copulatory behavior has some advantages over steroids. PCBs act upon specific neural substrates, e.g., dopaminergic neurons, and the interaction between estrogenic and nonestrogenic components of sexual differentiation can be distinguished if

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estrogenic and nonestrogenic PCBs are used in comparison. Another drawback of early studies of sexual differentiation of female sexual behavior is that only one behavioral parameter was used, the lordosis quotient. A new way of measuring female copulatory behavior, the female-pacing test, was used in our research to provide insights into the process of sexual differentiation. Since we were interested in whether PCBs can disrupt sexual differentiation, we examined sexual behavior in PCB-treated and control female rats and also investigated changes in related dopaminergic brain systems to answer these questions:

1. Do PCBs affect sexual differentiation as measured by behavioral parameters?
2. Do PCBs affect the incertohypothalamic dopaminergic system?
3. Is there a critical period for PCB treatment to affect sexual differentiation and sexual behavior?
4. Is there any relation between changes in behavior and changes in the brain in PCB-treated rats?

CHAPTER 2: GENERAL METHODS

Animals

Sexually mature Long-Evans rats (*Rattus norvegicus*) were purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN. Upon arrival, females rats (60-day-old; 3 rats/cage) and male rats (90-day-old; 2 rats/cage) were housed separately in standard plastic cages (57x25x20 cm) in a vivarium with 48% humidity and temperature at 22°C (lights off 1100-1900). Standard rat feed (Harlan Teklad 22/5 rodent diet 8640, Madison, WI) and tap water were available *ad libitum*. All experiments were conducted in our laboratory in accordance with the *Guidelines for the Use of Animals in Research* (National Research Council, 1997) and the policies of the University Committee on Animal Use and Care at Michigan State University.

Prior to behavioral testing, female rats were ovariectomized. Seven days after surgery, the ovariectomized rats were injected subcutaneously (sc) with estradiol benzoate (Sigma Chemical Co., St. Louis, MO) 0.5 μg/0.1 ml/rat (dissolved in sesame oil) for 3 consecutive days then given progesterone on the fourth day (Sigma Chemical Co., St. Louis, MO), 0.5 mg/0.1 ml/rat sc (dissolved in sesame oil) four hours prior to behavioral testing (All the injections were administered at 0900 hr). Females were placed in the behavior test arena, after each injection, for 10 min to familiarize them with the testing apparatus. They were tested for sexual behavior once per week at 1300 hr. The purpose of ovariectomy and hormone injections was to standardize the hormone milieu in each subject for the behavior test.

Female Behavior Tests

Female sexual behavior was evaluated with two separate test paradigms: a traditional lordosis test and a pacing test. In the traditional test, the female has no opportunity to escape from the male; therefore, she has minimal control of the timing of copulatory behavior. In the pacing test where the female can escape from the male, she has the opportunity to control the pacing of the sexual contacts.

Traditional Lordosis Tests

Sexually receptive females respond to mounts by a male by exhibiting a pronounced arching of the back, lordosis. Following treatment with estradiol benzoate and progesterone, sexual receptivity was measured by counting the number of times the female responded to the male with lordosis in ten mounts (lordosis quotient, LQ). The male rat was placed in the testing arena, a plexiglas cage (56x44x49 cm) 5 min before the female was placed in the test chamber where she remained until mounted ten times by the male.

Pacing Tests

To determine whether females were actively motivated to copulate, they were also tested with a male in a two-compartment cage. For this test, the testing chamber (56x44x49 cm) was divided into a main chamber (34x44x49 cm) and an escape chamber (22x44x49 cm). During copulatory tests, females could escape from the male through holes in the dividing partition through which the larger male was unable to pass. The female was introduced into the escape chamber 5 min after the male was placed in the

main chamber. The time course of the behavior was recorded with a computer using an event-recording program designed by Dr. Kalle Karu (Department of Mathematics, Harvard University, Cambridge, MA).

In the pacing paradigm, several latency measurements were used to assess the temporal pattern of female sexual behavior. Approach latency was the time from the introduction of female into the escape chamber to her crossing the partition to approach the male. Mount return latency, intromission return latency and postejeculatory refractory period were the measures of time that the female spent in the escape chamber after each male copulatory event (mount, intromission or ejaculation, respectively). We also used the percentage of times that the female escaped from the male after different copulatory events, i.e., percentages of mount leave (frequency of mount leave divided by frequency of mount) and intromission leave (frequency of intromission leave divided by frequency of intromission) to assess female sexual behavior. The percentage of ejaculation leave was excluded because females always escaped from the males' chamber after an ejaculation.

Immunohistochemistry

Tyrosine hydroxylase immunoreactivity (TH-IR) was used to identify dopaminergic neurons. Some brain sections were also double labelled with FOS immunohistochemistry. FOS, the protein product of an immediate early gene c-fos, is rapidly and transiently expressed in brain tissues in response to various stimulation (Wu *et al.*, 1992; Pfaus *et al.*, 1994, 1996; Coolen *et al.*, 1996; McCarthy *et al.*, 1997; Pfaus & Heeb, 1997). FOS immunoreactivity (FOS-IR) has been used as a metabolic marker in neuronal pathway

tracing (Dragunow and Faull, 1989) and as a marker of neuronal activity (Hoffman *et al.*, 1994a). It has been successfully applied in studies relating sexual behavior to activity of specific brain areas (Robertson *et al.*, 1991; Baum and Everitt, 1992; Erskine, 1993; Flanagan *et al.*, 1993; Pfaus *et al.*, 1993; Rowe and Erskine, 1993; Tetel *et al.*, 1993; Wersinger *et al.*, 1993; Flanagan-Cato and McEwen, 1995; Gréco *et al.*, 1996). The purpose of double labelling TH and FOS was to identify mating-activated dopaminergic neurons.

Prior to sacrifice, female rats were anesthetized with sodium pentobarbital (75 mg/Kg), then perfused with 0.87% NaCl then 4% paraformaldehyde (in 0.1 M phosphate buffer, pH 7.4). Brains were post-fixed in 4% paraformaldehyde at 4°C over night then transferred to 20% sucrose (in 0.1 M phosphate buffer, pH 7.4) for cryoprotection. Once the brains had sunk, they were sectioned coronally with a cryostat. Frozen sections were collected and processed for FOS and/or TH immunohistochemistry. Thionine staining was performed on the adjacent sections of those processed for FOS/TH immunohistochemistry. For TH immunohistochemistry, sections were rinsed three times in 0.1 M phosphate buffer saline (PBS, pH 7.4) and incubated with 0.5% hydrogen peroxide (diluted with TBS) for 30 min. They were rinsed three times afterwards with PBS and then incubated with the primary antibody for TH (monoclonal mouse-anti-TH, 1:10,000, Sigma Chemical Co., St. Louis, MO) and normal horse serum (Vector Laboratories, diluted with PBS/0.3% Triton X-100 according to the manufacturer's directions) for 2 days at 4°C. After three rinses with PBS, sections were incubated with biotinylated secondary antibody (horse-anti-mouse IgG, Vector Laboratories, diluted with PBS/0.3% Triton X-100 according to the manufacturer's directions) for 2 hr at room temperature. After three rinses in PBS, sections were incubated with ABC solution (Vector Laboratories, diluted

with PBS/0.3% Triton X-100 according to the manufacturer's directions) for 1.5 hr at room temperature. They were rinsed three times with PBS and then reacted with VIP (VIP Substrate Kit, Vector Laboratories) for 10 min. After staining, the sections were rinsed three times with PBS and mounted on gelatin-coated slides, dehydrated in an ethanol series (70%, 95%, 100%, 2 min each) and xylene (15 min), then coverslipped using DPX mounting media (Fluka Chemical Corp., St. Louis, MO).

For FOS/TH immunohistochemistry, sections were rinsed three times in 50 mM Tris buffer saline (TBS, pH 7.2) and incubated with 0.5% hydrogen peroxide (diluted with TBS) for 30 min. They were rinsed three times with TBS and then incubated with the primary antibody (details described in each chapter) and normal serum (details described in each chapter; Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions) for 2 days at 4°C. After three rinses with TBS, sections were incubated with biotinylated secondary antibody (details described in each chapter; Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions) for 2 hr at room temperature. After three rinses in TBS, sections were incubated with ABC solution (Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions) for 1.5 hr at room temperature. They were rinsed three times with TBS and then reacted with DAB/Ni (DAB Substrate Kit, Vector Laboratories) for 10 min. After the first set of immunohistochemistry, sections were incubated in 5% dimethyl sulfoxide for 30 min and rinsed three times with TBS, the second set of the immunohistochemistry was performed with the primary antibody for TH (monoclonal mouse-anti-TH, 1:10,000, Sigma Chemical Co., St. Louis, MO) and with normal horse serum as the same steps described above except using different secondary antibody (horse-

anti-mouse IgG, Vector Laboratories) and DAB (Vector Laboratories) as the chromagen. After staining sections were rinsed three times with TBS and mounted onto gelatin-coated slides, dehydrated in an ethanol series (70%, 95%, 100%, 2 min each) and xylene (15 min), then coverslipped using DPX mounting media (Fluka Chemical Corp., St. Louis, MO). TH- and/or FOS-IR perikarya from both sides of the sections were quantified using camera lucida. (Note: TH-IR is cytosolic while FOS-IR is nuclear.)

A systematic section method was used for cell counting, and the estimated neuronal population was calculated by the product of sample count and the period of the systematic sample (Konigsmark, 1970).

CHAPTER 3: EFFECTS OF PERINATAL EXPOSURE TO PCBS ON THE DEVELOPMENT OF FEMALE SEXUAL BEHAVIOR AND THE INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS

Introduction

Estrogen plays a key role in the sexual differentiation of rats as described in Chapter 1. It is capable of defeminizing copulatory behavior of female rats as indicated by low lordosis quotients (LQ), during critical periods, which extends from gestation day 18 to about one week after parturition in the laboratory rats (McEwen *et al.*, 1977; MacLusky and Naftolin, 1981; Elkind-Hirsch *et al.*, 1984). Consequently, estrogenic PCBs have the potential to disrupt sexual differentiation and sexual behavior in the adult. A dramatic example has been found in turtles where the estrogenic effect of hydroxy-PCBs reverses temperature-dependent sex determination (Bergeron *et al.*, 1994; Crews *et al.*, 1995). Reports of abnormal sexual development in reptiles (Guillette *et al.*, 1994, 1995) and feminized responses in male fish (Jobling & Sumpter, 1993; White *et al.*, 1994) have also suggested an association with environmentally active chemicals functioning as estrogens. Therefore, the disruption of steroid hormone homeostasis and activity by environmental contaminants has become a cause for concern (Wolff *et al.*, 1993; Schmidt, 1994; Dibb, 1995).

As noted earlier, some PCBs and their metabolites exhibit estrogenic activity (Bitman and Cecil, 1970; Örberg and Kihlström, 1973; Gellert, 1978; Korach *et al.*, 1988). For example, Aroclor 1221 (A1221), one commercial PCB mixture, has been reported to exhibit estrogenic activity (Bitman and Cecil, 1970) and cause precocious puberty, persistent vaginal estrus, and anovulation in female rats (Gellert, 1978). Other PCBs, on the contrary,

exhibit minimal binding to estrogen receptors (Moore *et al.*, 1997). Aroclor 1254 (A1254), for instance, did not show estrogenic effects (Bitman and Cecil, 1970). Based on these considerations, we would predict that estrogenic PCBs (A1221) would defeminize female rats (show low LQ) whereas nonestrogenic PCBs (A1254) would not.

In addition to the estrogenic effects, PCBs also exhibit neurotoxic effects on dopaminergic neurons (Safe *et al.*, 1985; Seegal *et al.*, 1990, 1991; Shain *et al.*, 1991; Brouwer *et al.*, 1995). Dopamine is an important neurotransmitter involved in female sexual behavior (Everitt *et al.*, 1974; Carter & Davis, 1977; Foreman & Moss, 1979; Foreman & Hall, 1987; Grierson *et al.*, 1988; Mani *et al.*, 1994), and it is possible that PCBs could disrupt sexual behavior by their actions on the development of dopaminergic neurons.

Dopaminergic neurons of the incertohypothalamic system are the main dopamine input to the medial preoptic area of the hypothalamus (MPOA) (Björklund *et al.*, 1975; Lindvall and Björklund, 1983; Simerly and Swanson, 1986), an area of major importance for sexual behavior (Powers & Valenstein, 1972; Foreman & Moss, 1979; Clark *et al.*, 1986; Warner, 1991; Hull *et al.*, 1995). On the basis of the fiber distribution, a caudal (A11 and A13 cell groups) and a rostral part (A14 cell group) of the incertohypothalamic system can be discriminated. The projection areas of these neurons signify an involvement of this system in the control of secretion of pituitary hormones and sexual behavior (Björklund *et al.*, 1975). Chemical lesions of the A13 region have been shown to decrease lordosis behavior in rats (Dornan *et al.*, 1991). MPOA lesions have also been shown to disrupt the temporal pattern of sexual behavior in female rats (Whitney, 1986; Yang and Clemens, 1996). It is possible that PCBs might disrupt sexual behavior by their actions on incertohypothalamic dopaminergic neurons. An estrogenic effect of PCBs on the

development of dopaminergic systems cannot be overlooked. Since estrogen is concentrated in hypothalamic dopaminergic neurons (Sar, 1983), estrogen may well affect the development of these neurons. Indeed, the development of a sexually dimorphic population of dopaminergic neurons in the hypothalamus has been shown to depend upon gonadal hormones (Simerly, 1989; Davis *et al.*, 1996). The interactions between estrogen and dopamine are also well documented (Euvrard *et al.*, 1980; Stumpf *et al.*, 1983; Mermelstein and Becker, 1995; Xiao & Becker, 1997).

From this brief summary it is clear that PCBs have the potential to affect behavioral sexual differentiation by at least two modes of action — estrogenic effects or neurotoxic effects on brain dopaminergic systems. Since these outcomes of PCB toxicity are not mutually exclusive, our strategy will be to measure changes in behavior as a result of PCB treatments, and subsequently determine whether these behavioral changes are related to effects on brain dopaminergic systems.

Materials and Methods

To determine whether exposure to PCBs during early development would affect adult sexual behavior, perinatal PCB exposure was delivered through the dam. Three PCB intraperitoneal injections were administered to the dam: one was given on gestation day 14, the second at parturition (day 1) and the third on day 10 after birth to cover the entire period of sexual differentiation. Since PCBs accumulate in animals' body fat storage owing to their high lipophilic nature and low biodegradation rate, the actual exposure time continues long after the last injection.

Animals

Long-Evans rats, described in Chapter 2, were randomly paired and mated over night. Pregnant female rats were then housed alone. Each pregnant rat received an intraperitoneal injection of 0.5 ml sesame oil (Sigma Chemical Co., St. Louis, MO) or PCB solution (Fisher Scientific, Pittsburgh, PA) on gestation day 14, parturition day (day 1) and day 10 after birth. The PCB solution for each injection was prepared by dissolving 5 mg or 15 mg A1221 (or A1254) in 1.5 ml sesame oil for each animal (3.33 mg/ml and 10 mg/ml, respectively). All the injections were administered at 0900 hr (2 hr before lights off) thus providing offspring with perinatal exposure to the PCBs of total maternal dosage of 0 mg (sesame oil group, control), 5 mg (about 14 mg/Kg) or 15 mg (about 42 mg/Kg) A1254 (or A1221). [Note: The actual dosage transferred to the pups is estimated to be about 10 μg (1.7 mg/Kg) and 30 μg (5 mg/Kg), respectively (according to Takagi *et al.*, 1986).] Pups were weaned from their mothers at day 28. Males and females were housed in separate cages after weaning. At 60 days of age, female offspring were ovariectomized and rehoused (3 rats/cage). Seven days (day 67 after parturition) after surgery, the ovariectomized rats were treated with hormones and tested for sexual behavior as described in Chapter 2.

Immunohistochemistry for Caudal Incertohypothalamic Dopaminergic Neurons

Tyrosine hydroxylase immunoreactivity (TH-IR) was used to identify dopaminergic neurons in the caudal incertohypothalamic region of the brain. Female rats were randomly selected (at least one from each litter in each treatment group) for immunohistochemical processing and sacrificed right after the last behavior test. The

brain samples for the 5 mg A1254 treatment group were accidentally lost and TH immunohistochemistry could not be performed. Brains were sectioned coronally at 30 μm with a cryostat. Frozen sections were collected and every third section was processed for TH immunohistochemistry and TH-IR perikarya were quantified (see Chapter 2).

Statistics

The mean of each behavioral parameter in each test was automatically calculated by the computer program. The mean of four behavior tests were then used to calculate the mean for each litter and for further statistical analyses using StatView (Abacus Concepts, Inc., Berkeley, CA). Approach latency, mount return latency, intromission return latency and postejaculatory refractory period were analyzed by one-way ANOVA. Fisher's LSD post hoc test was performed when there was a significant difference among treatment groups ($p<0.05$). Percentages of mount leave and intromission leave were analyzed by Kruskal-Wallis Test. LQ, the percentage of times that females showed a lordosis response in 10 mounts, was also analyzed by Kruskal-Wallis Test. The post hoc tests were performed between the control and PCB-treated group when there was a significant difference among treatments ($p<0.05$). The number of TH-IR neurons in A11 or A13 region was analyzed by both one-way ANOVA and Kruskal-Wallis Test. To compare the measurements of two sides of A11 or A13, two-way ANOVA (two sides as repeated measurements) was used.

Results

In both the 5 mg and 15 mg groups, perinatal A1221 exposure lowered the

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lordosis quotient of female rats during copulation (Figure 6). Perinatal 5 mg A1221 exposure increased the latencies for female rats to approach males and return to them after intromissions (Figure 7). These females also had a higher percent leave after receiving an intromission (Figure 8).

Perinatal A1254 treatment increased the percent leave following mounts or intromissions (Figure 9) but did not change other measurements of sexual behavior.

Perinatal A1221 or A1254 treatment did not change the number of TH-IR neurons in the A11 or A13 regions. However, we found an asymmetry in A11 (Figure 10) and A13 (Figure 11) TH-IR cell number on two sides of the brain (pooled data from all groups, $p<0.001$ and $p = 0.0001$, respectively), with one side having more neurons (major) than the other (minor).

Discussion

Perinatal A1221 treatment affects female sexual behavior.

Perinatal A1221 treatment lowered the lordosis quotient of female rats while perinatal A1254 did not, which is consistent with an estrogen-induced defeminizing effect of A1221. A1221 has been reported to have estrogenic activity when the dose exceeds 1 mg using 18-hr glycogen response as a measure of estrogenicity (Bitman and Cecil, 1970). Testosterone is able to defeminize female rats during sexual differentiation, which extends from gestation day 18 to about one week after parturition in rats (McEwen *et al.*, 1977; Maclusky and Naftolin, 1981; Elkind-Hirsch *et al.*, 1984). Testosterone and estrogen treatment during gestation or shortly after birth results in a pattern of anovulatory sterility in adulthood (Gorski & Wagner, 1965; Gorski, 1971). Gellert (1978) treated two or three-day-

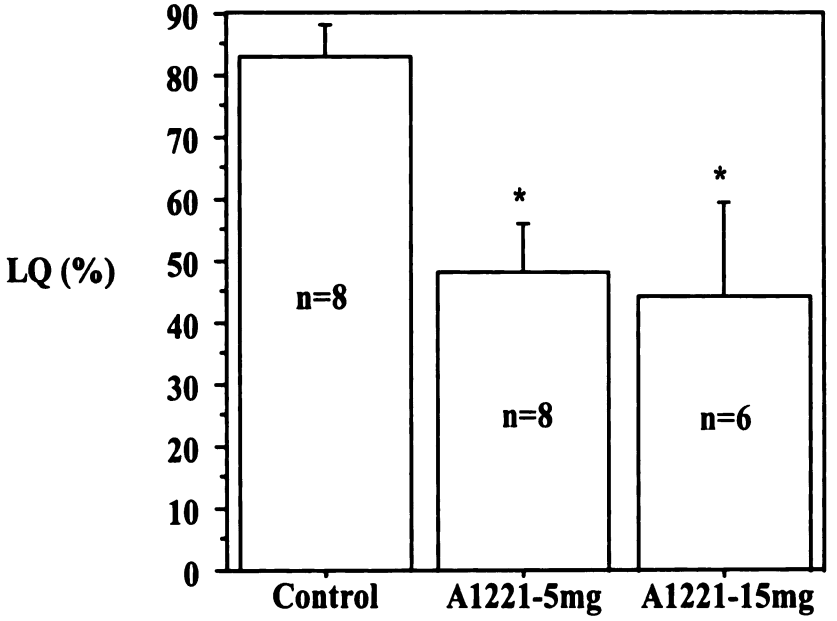
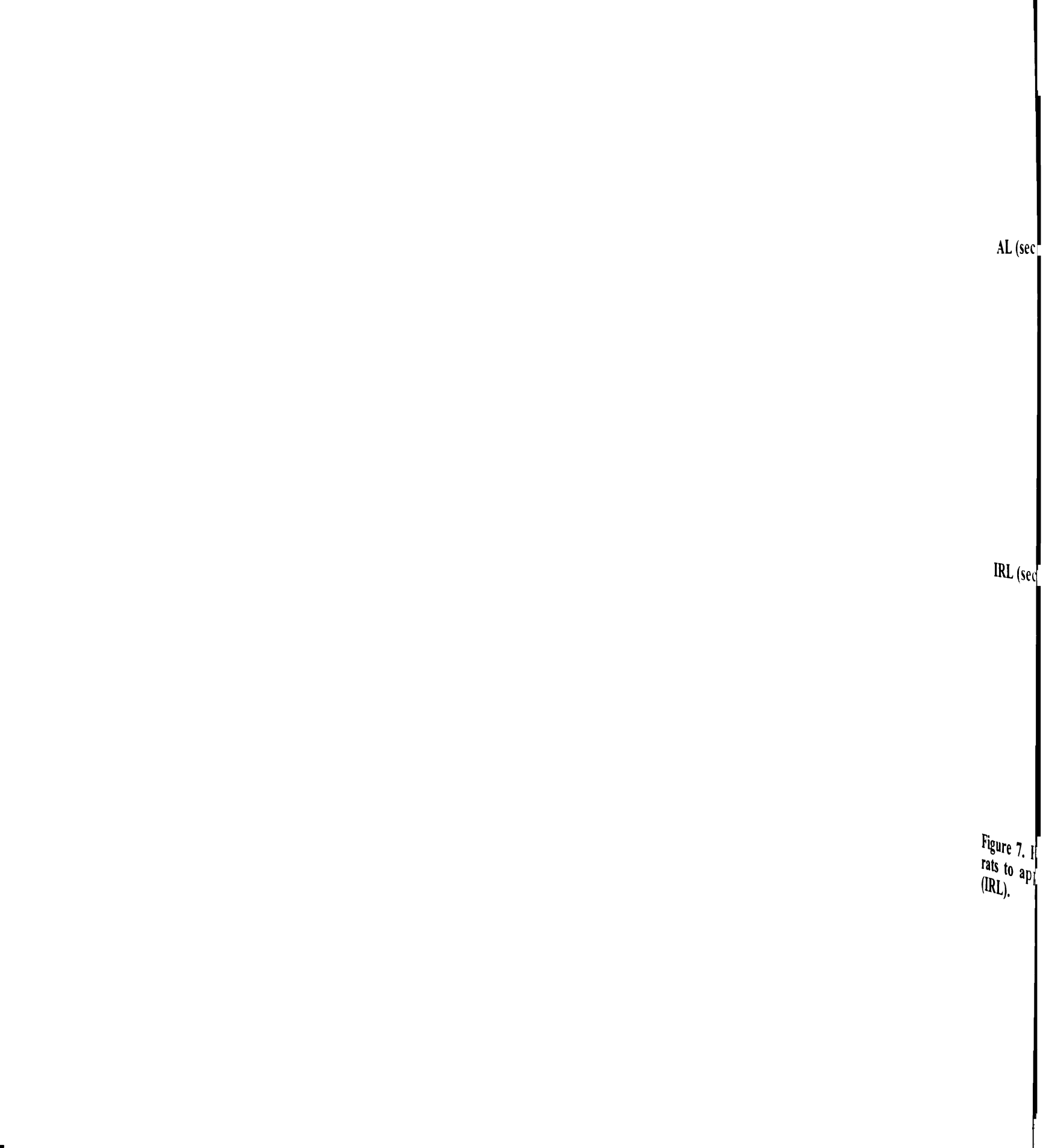


Figure 6. Perinatal A1221 treatment decreased the lordosis quotient (LQ=frequency of lordosis/frequency of 10 mount) of female rats.



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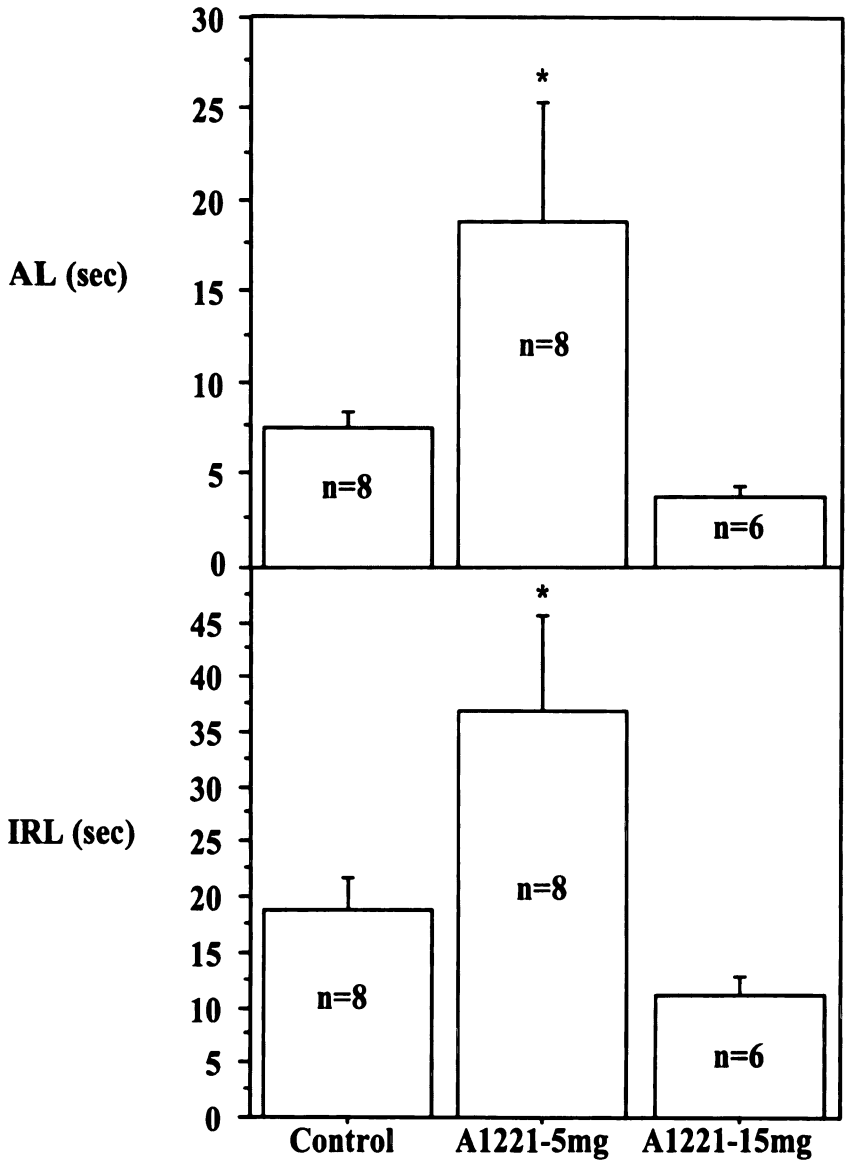

Figure 7. Perinatal A1221 treatment increased the latencies for female rats to approach (AL) males and return to them after intromissions (IRL).

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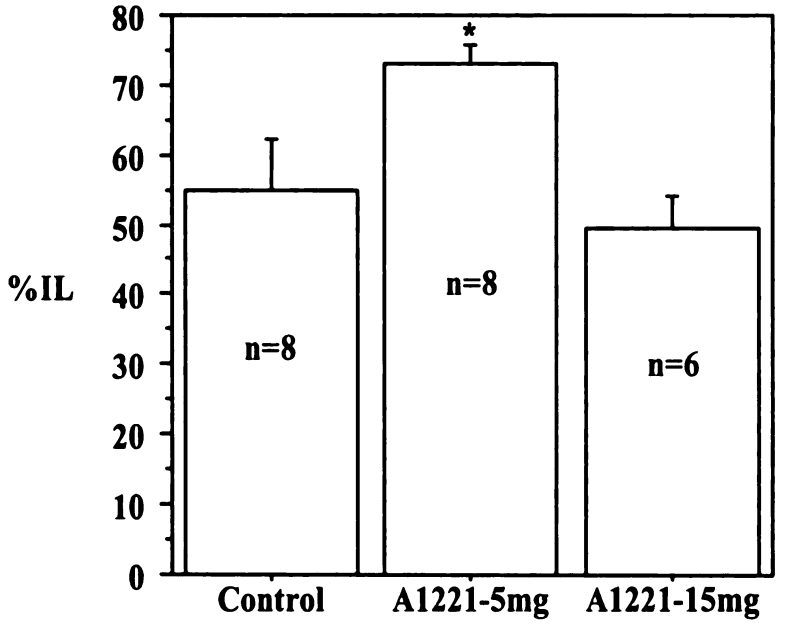
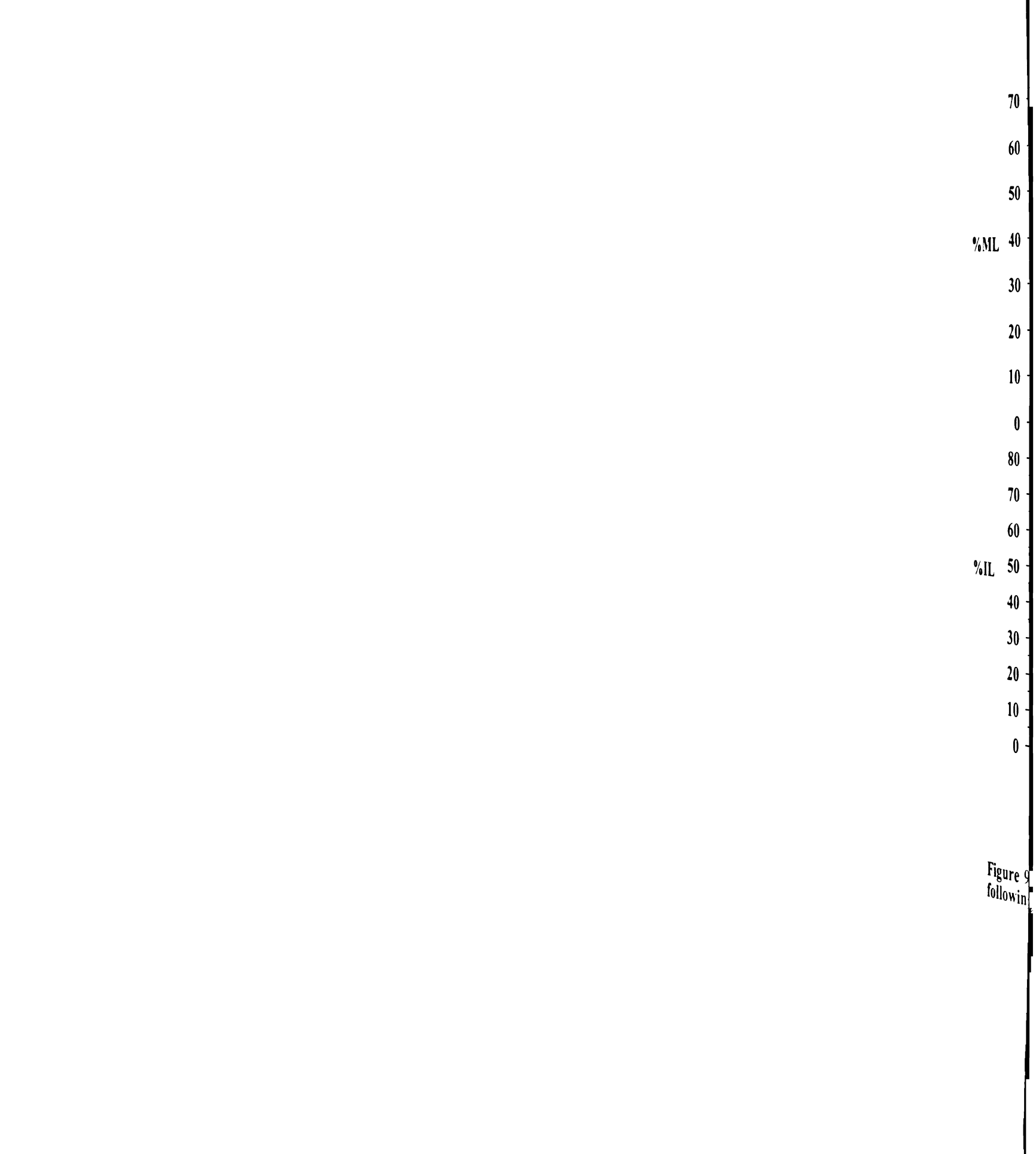


Figure 8. Perinatal A1221 treatment increased the percentage of times that females leave the male after receiving intromissions (%IL=frequency of intromission leave/frequency of intromission).



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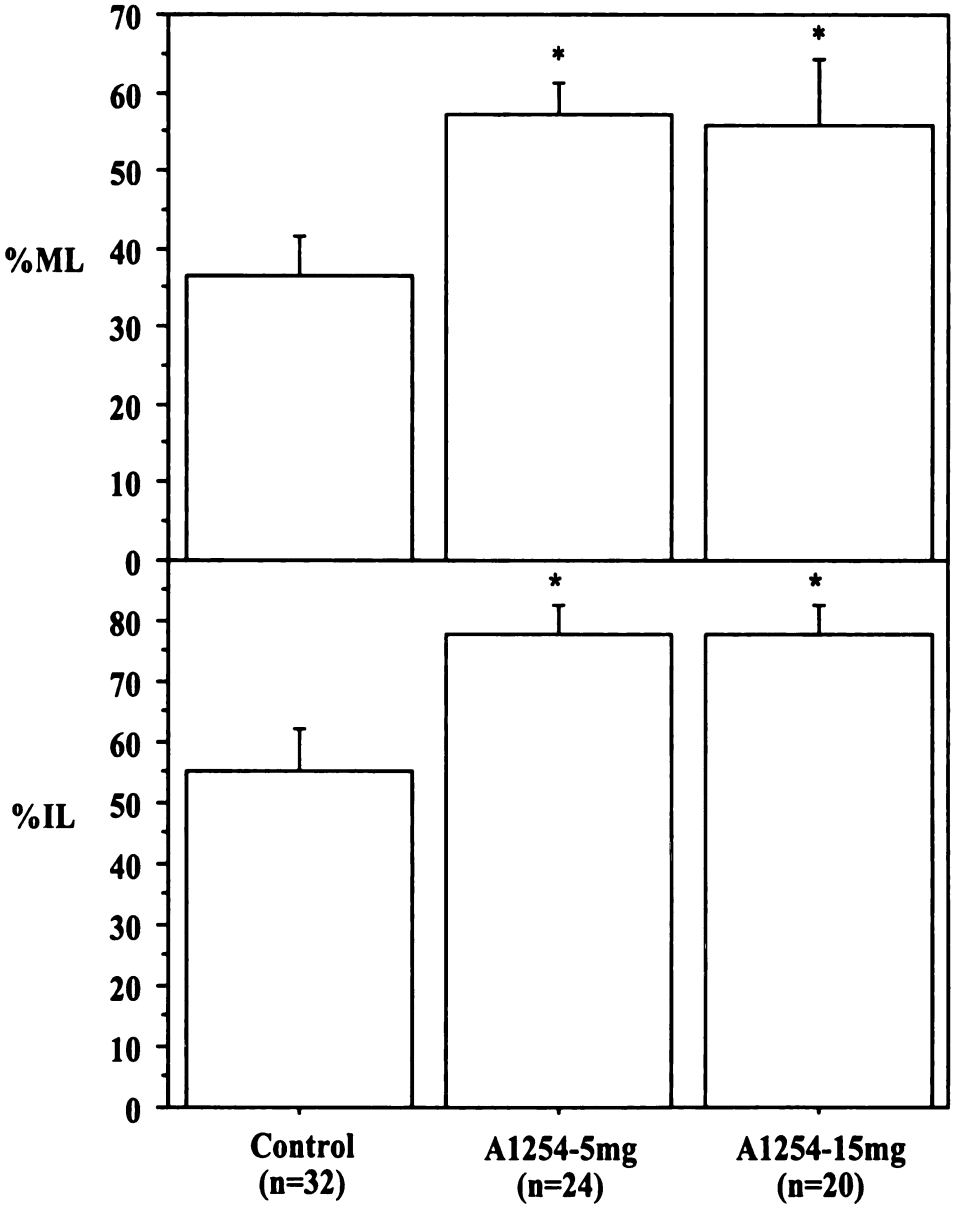

Figure 9. Perinatal A1254 treatment increased the percent leave following mounts (%ML) or intromissions (%IL).

Figure 10.

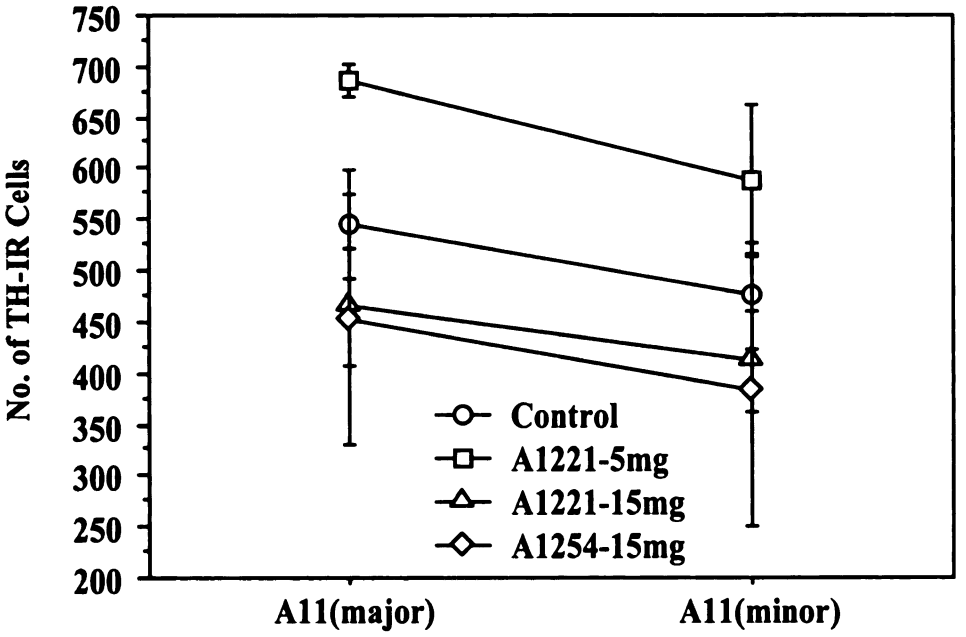

Figure 10. The main effect of asymmetry in A11 dopaminergic neurons.

Figure 11.

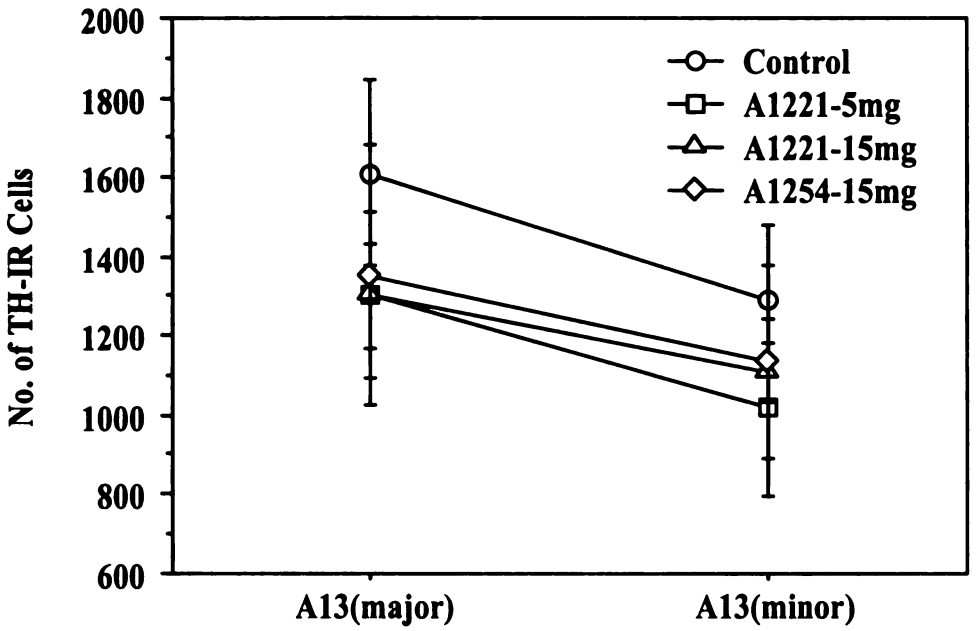

Figure 11. The main effect of asymmetry in A13 dopaminergic neurons.

old female rats with A1221 (20 mg/rat) and reported similar precocious puberty, persistent vaginal estrus, and anovulation. However, only testosterone or antiestrogen decreased the lordosis behavior. It is believed that testosterone has to be aromatized in the brain tissue into estrogen to affect the brain circuits involved in lordosis behavior (McEwen *et al.*, 1977).

We did not find any correlation between the lordosis quotient change and the A11 or A13 anatomic parameters. Therefore, A1221 may have acted on other systems related to lordosis behavior. Several dopaminergic systems in the hypothalamus and midbrain have been associated with lordosis behavior in female rats, including the substantia nigra (A9), central tegmental area (A10) (Herndon, 1976) and the arcuate nucleus (A12) (Ahren *et al.*, 1971; Lofstrom, 1977). Indeed, PCBs have been shown to decrease dopamine content in the substantia nigra and other hypothalamic nuclei (Seegal *et al.*, 1990, 1991), and it is possible that changes in these dopaminergic systems may be related to the low lordosis quotient in our study. Alternatively, although the whole population of A11 or A13 dopaminergic neurons did not show correlations with lordosis behavior, some subpopulation of these neurons may be related to this behavior, e.g., the A11 or A13 dopaminergic neurons that were activated during copulation. Other methods of measuring neuronal activities may be a better index than TH-IR cell counts.

In the pacing tests, perinatal A1221-treated (5 mg) females took a longer time to approach the male, and returned to the male with longer latencies after intromission than control females. The percentage of times these females left the male following an intromission was also increased. These behavioral alterations may reflect changes in sexual motivation of the female rats and could be the result of increased dopamine level in the nucleus accumbens and the striatum due to the estrogenic effect of A1221 in the critical

period of development. Xiao and Becker (1997) found that estrogen implantation in the nucleus accumbens increased dopamine level in this brain region and prolonged the return latencies of female rats receiving intromissions and the implantation into the striatum facilitated the percentage of females leaving males after copulatory events. This pathway appears to be involved in the maintenance but not in the initiation of copulation (the approach latency). On the other hand, since the temporal pattern of female copulation depends heavily on peripheral sensory factors (Erskine, 1989), PCB treatments may have influenced the female's sensitivity to vaginal and cervical stimulation during copulation.

In addition, lesions of the medial preoptic area (MPOA) have been shown to disrupt the temporal pattern of sexual behavior in female rats (Whitney, 1986; Yang & Clemens, 1996). It is plausible that PCBs disrupted development of the MPOA which in some way produced deficits in the temporal pattern of female sexual behavior.

Differential Effect of A1221 and A1254

A possible explanation for different effects of Aroclor 1254 and Aroclor 1221 lies in the components of these two PCB mixtures. Aroclor 1221 consists of 40% coplanar and 60% ortho-substituted PCBs (Webb and McCall, 1972; Willis and Addison, 1972). Aroclor 1254, on the other hand, is mainly composed of ortho-substituted PCBs (about 99%; Sissons and Welti, 1971; Webb and McCall, 1972). Comparing the results from A1221 and A1254 treatments, it appears that the lordosis reflex may have been altered by the estrogenic effects of coplanar PCBs since only A1221 decreased the lordosis quotient, while the increase in leaving after a copulatory event may be due to the effect of the ortho-substituted PCBs because both A1221 and A1254 showed changes in these parameters.

A1221, 5 mg, had effects on the female's response latencies (Figure 7) but the dose of 15 mg A1221 did not. Since estrogen interacts with the dopaminergic system (Mani *et al.* 1994), and since coplanar and ortho-substituted PCBs have opposite effects on dopamine levels (Seegal *et al.* 1990, 1997), the differential effect in the A1221 5 mg and 15 mg groups may be attributed to the antagonism between two types of PCBs. Interestingly, the dose response curve of commercial PCBs is sometimes biphasic (Chishti *et al.*, 1996). The biphasic dose response may also result from the antagonism between the coplanar and ortho-substituted PCBs.

Aroclor and the Ah and Cholinergic Receptors

It is well known that coplanar PCBs can mimic the structure of TCDD as described in Chapter 1, and therefore bind to the Ah receptor. However, there are two structural requirements for induction of Ah receptor activity: the presence of at least two adjacent halogen atoms in the lateral positions of each benzene ring (positions 3,4,3',4') and the absence of halogen atoms adjacent to the biphenyl bridge (position 2,6,2',6') (Poland & Glover, 1977). None of the coplanar components in A1221 fulfill these requirements.

In addition to changes in brain dopamine function, postnatal day 10 exposure to individual congeners (including ortho-substituted congeners and 3,4,3',4'-TCB) resulted in long-term changes in behavior and regional brain muscarinic and nicotinic receptor densities (Eriksson *et al.*, 1991; Eriksson & Fredriksson, 1996). The persistent changes in cholinergic receptor function may alter the normal dopaminergic/cholinergic balance, resulting in altered brain function (Consolo *et al.*, 1992; Marchi *et al.*, 1992; Di Chiara &

Morelli, 1993).

In summary, permanent changes were seen in feminine sexual behavior after perinatal treatment with the PCB mixture, A1221 or A1254. The lordosis reflex may be altered by the estrogenic effects of the coplanar PCBs, while the increase in leaving after a copulatory event may be due to the effect of the ortho-substituted PCBs.

CHAPTER 4: NEONATAL AND ADULT TOXICITY OF PCB EXPOSURE ON FEMALE SEXUAL BEHAVIOR AND INCERTOHYPOTHALAMIC DOPAMINERGIC NEURONS IN RATS

Introduction

In the previous study we found that perinatal PCB treatments produced deficits in female sexual behavior. However, the timing of PCB treatment seems to be a crucial factor in PCB toxicity (Seegal, 1992; Brouwer *et al.*, 1995). In contrast to the decreases in dopamine concentrations observed following adult PCB exposure, perinatal exposure to Aroclor 1016 significantly increased brain dopamine concentrations (Seegal, 1992). In addition, coplanar PCBs only showed toxic effects during development. Adult coplanar PCB treatment did not produce deficits in treated animals. On the contrary, ortho-substituted PCBs are active during both development and adulthood (Brouwer *et al.*, 1995). Furthermore, the process of sexual differentiation and the physiological environment are different at various developmental stages (Maclusky & Naftolin, 1981). Hypothalamic dopaminergic neurons start to display sex differences in dopamine metabolism at birth (Reisert & Pilgrim, 1991) and around puberty (Davis *et al.*, 1996). Therefore, it is important that we also examine the neonatal and adult effects of PCBs. In this chapter, we compare the effects of neonatal treatment with two commercial PCB mixtures, A1221 (compounds with estrogenic activity) and A1254 (non-estrogenic compounds), on the development of female sexual behavior and incertohypothalmic dopaminergic neurons as well as the effect of these compounds when administered to adult female rats. The dose was chosen as 1/3 of the perinatal treatment to mimic the day 1 injection used in the perinatal study. To ensure the delivery of PCBs, I used daily

injection for one week. In this experiment I also used FOS immunoreactivity to further refine our assessment of the function of incertohypothalamic dopaminergic neurons during copulation.

Materials and Methods

Neonatal PCB exposure from day 1 to day 7 was used to determine whether PCB exposure would affect adult sexual behavior. To ascertain whether the effects were specific to development, I also exposed adult rats from day 67 to day 72. Since PCBs accumulate in body fat, owing to their high lipophilicity and low biodegradation rate, the actual exposure can extend beyond the time of injection.

Animals

Neonatal Treatment

Long-Evans rats were randomly paired and placed with the male over night. Pregnant female rats were then housed alone. After parturition, lactating rats were treated daily with sesame oil or a PCB solution (Fisher Scientific, Pittsburgh, PA) from day 1 (parturition day) to day 7. In the A1254 experiment, 10 mg or 20 mg A1254 were dissolved in 14 ml sesame oil (Sigma Chemical Co., St. Louis, MO) as the working solution (0.71 mg/ml and 1.43 mg/ml, respectively). In the A1221 experiment, 12.5 mg or 25 mg A1221 were dissolved in 17.5 ml sesame oil as the working solution (about 0.71 mg/ml and 1.43 mg/ml, respectively). Lactating rats were given daily intraperitoneal (ip) injections of sesame oil or the PCB working solution (0.5 ml/rat; daily dosage about 0.357 mg and 0.714 mg, respectively) at 0900 hr (2 hr before lights off) thus providing

offspring with lactational exposure to the PCBs at a total dosage of 0 mg (sesame oil group, control), 2.5 mg (about 7 mg/Kg) or 5 mg (about 14 mg/Kg) A1254 (or A1221). [Note: The actual dosage transferred to the pups was estimated to be 7 μg (1.2 mg/Kg) and 14 μg (2.3 mg/Kg), respectively (Takagi *et al.*, 1986).] Pups born on the same day were randomly assigned to the dam and the number of pups per dam was reduced to eight at parturition. On the same day, at least one dam was randomly assigned to each treatment (control, 2.5 mg PCBs and 5 mg PCBs). Pups were weaned on day 28. Males and females were housed in separate cages after weaning. At 60 days of age, female offspring were ovariectomized and treated with hormones for behavior tests as described in Chapter 2.

Adult Treatment

In order to separate developmental PCB effects from concurrent residual effects of stored PCBs, I examined the behavior of females treated with PCBs as adults. Sixty sexually mature (60-day-old) female rats were ovariectomized and treated with hormones as described in Chapter 2. After four behavior tests, they were randomly divided into five groups [control, 2.5 mg (8 mg/Kg) A1221, 5mg (16 mg/Kg) A1221, 2.5 mg (8 mg/Kg) A1254, 5 mg (16 mg/Kg) A1254]. To prepare the PCB working solution, 30 mg or 60 mg A1254 (or A1221) was dissolved in 8.4 ml sesame oil (3.57 mg/ml and 7.14 mg/ml, respectively). The control group received 0.1 ml sesame oil (ip injection at 0900 hr) per day for a week. The other four groups received 0.1 ml of the PCB working solution for a week [total dosage 2.5 mg (daily dosage 0.357 mg, about 1 mg/Kg) or 5 mg (daily dosage 0.714 mg, about 2.4 mg/Kg) A1254 (or A1221), ip injection at 0900 hr]. Four hours after the last PCB treatment, rats were tested for female sexual behavior (1300 hr, 2

hr after lights off). Behavioral analyses were based on data from the last two tests for the pre- and post-treatment comparisons.

Immunohistochemistry for Caudal Incertohypothalamic Dopaminergic Neurons

Female rats were randomly selected (at least one from each assigned litter in the neonatal treatment groups) for immunohistochemical processing and sacrificed one hour after the introduction of the female into the testing arena in the last behavior test. Since there was no difference in the behavior of adult treatment groups, immunohistochemistry was not performed in the adult experiment. In the other groups, brains were sectioned coronally at 25 μm with a cryostat. Frozen sections were collected and every fourth section was processed for FOS and TH immunohistochemistry as described in Chapter 2. The primary antibody (c-FOS oncoprotein mouse monoclonal antibody, 1:40, Novocastra Laboratories Ltd., UK/Vector Laboratories, CA) and normal goat serum (Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions) were used. The biotinylated secondary antibody was goat-anti-mouse IgM (Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions). The primary antibody for TH was monoclonal mouse-anti-TH (1:10,000, Sigma Chemical Co., St. Louis, MO) with normal horse serum and the secondary antibody was horse-anti-mouse IgG (Vector Laboratories).

Statistics

Neonatal Treatments

The mean of each behavioral parameter in each test was automatically calculated

by the computer program. The average data of four behavior tests were then used for statistical analyses. Since there was no difference between assigned litters in each treatment group, pooled data for treatment groups were used for further statistical analyses. Approach latency, mount return latency, intromission return latency and postejaculatory refractory period were analyzed by one-way ANOVA. Fisher's LSD post hoc test was performed between the control and PCB-treated group when there was a significant difference among treatments ($p<0.05$). Percentages of mount leave, intromission leave, mount stay and intromission stay were analyzed by Kruskal-Wallis Test. LQ, the percentage of times that females showed a lordosis response in 10 mounts (standard test) or more (pacing test), was also analyzed by Kruskal-Wallis Test. The post hoc tests were performed between the control and PCB-treated group when there was a significant difference between treatments ($p<0.05$). The number of TH-IR neurons in the A11 or A13 region was analyzed by both one-way ANOVA and Kruskal-Wallis Test. To compare the measurements of two sides of A11 or A13, two-way ANOVA (two sides as repeated measurements) was used. I used the number of TH-IR neurons (or FOS/TH-IR neurons) on the major side divided by the number of TH-IR neurons (or FOS/TH-IR neurons) on the minor side as an index of asymmetry. The asymmetric indices were analyzed by Kruskal-Wallis Test.

Adult Treatment

Data from the fourth and the fifth behavior tests were treated as repeated measurements (pre- and post- treatments). Two-way ANOVA was used to analyze female approach latency, mount return latency, intromission return latency and postejaculatory refractory period. Percentages of mount leave, intromission leave, mount

stay, intromission stay and LQ were analyzed by Friedman's test.

Results

There was no adverse effect on the general health as measured by body weight (data not shown) of the exposed animals in the neonatal or adult PCB treatment groups. At adulthood, experimental females showed regular estrous cycles as indicated by daily vaginal smears for ten days before ovariectomy.

Neonatal treatment with A1254 significantly reduced the lordosis quotient (Figure 12), and shortened the latency for female rats to return to the male after an intromission (Figure 13). Other measurements of female sexual behavior were not affected. Neither neonatal treatment with A1221 nor adult treatment with either PCB compounds affected female sexual behavior.

Relation of Neonatal A1221 Treatment to A11 and A13 Dopaminergic Neurons

Neonatal A1221 treatment had no effect on the number of A11 or A13 TH-IR neurons across treatment groups. Since groups did not differ the data were pooled. Analysis revealed an asymmetry in both the A11 and A13 TH-IR cell groups with one side of brain (major side) containing more TH-IR cells than the other (minor) ($p<0.001$ and $p<0.005$, respectively). The FOS/TH-IR A13 neurons also showed significant asymmetry ($p=0.005$) while the FOS/TH-IR A11 neurons did not.

The Effect of Neonatal A1254 Treatment on A11 and A13 Dopaminergic Neurons

There was no difference in the number of A11 or A13 TH-IR neurons between

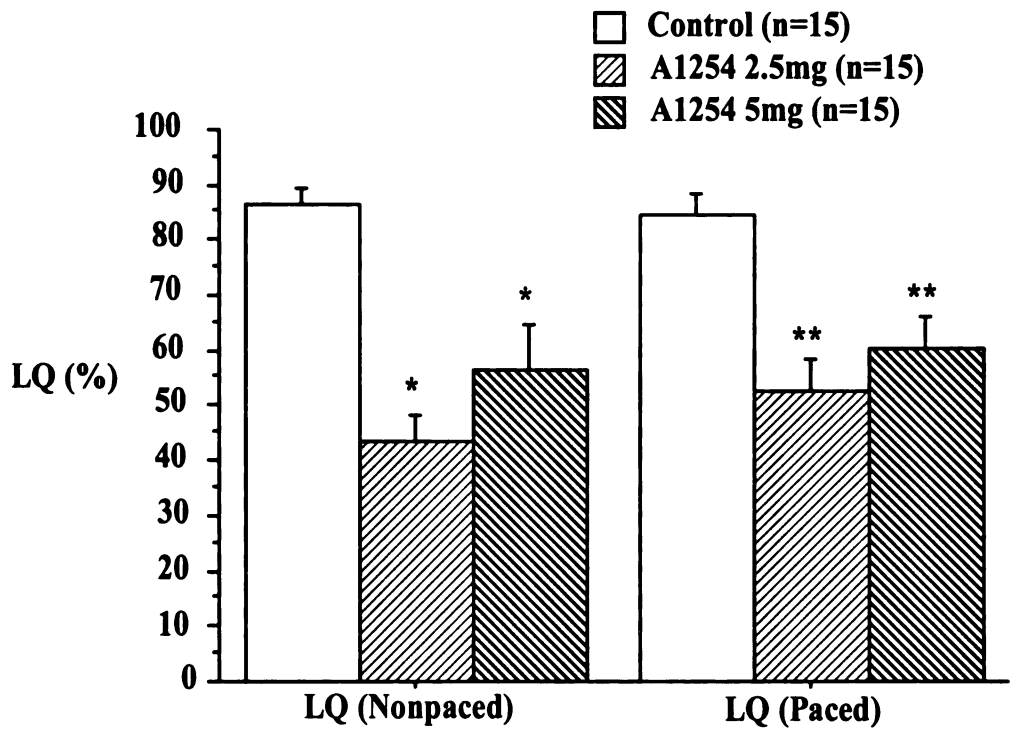

Figure 12. Neonatal A1254 treatment reduced female lordosis quotient (LQ).

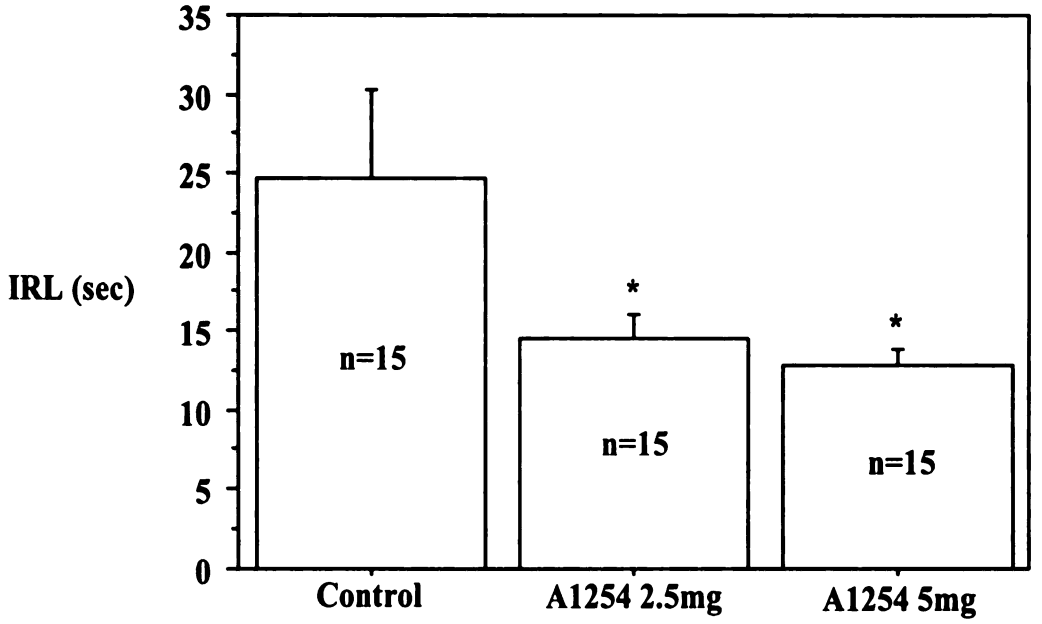

Figure 13. Neonatal A1254 treatment shortened the latency for female rats to return to the male after an intromission (IRL).

neonatal A1254 treatment groups. However, there was a significant interaction between neonatal A1254 treatments and the number of A11 or A13 TH-IR neurons on the two sides of the brain ($p<0.05$ and $p<0.005$, respectively). In control females, one side of the A11 and A13 region had more TH-IR neurons (major) than the other (minor) ($p<0.0001$ and $p<0.05$, respectively). A1254 treatment decreased the asymmetry of A13 TH-IR neurons in A1254-treated females (Figure 14, showing different slopes). The FOS/TH-IR A13 neurons also showed the pattern of asymmetry ($p<0.05$), and the interaction between A1254 treatment and the number of A13 FOS/TH-IR neurons was also significant ($p<0.005$). The FOS/TH-IR A11 neurons also showed the pattern of asymmetry ($p<0.001$), but no interaction was found between A1254 treatment and the number of A11 FOS/TH-IR neurons.

Since there was a significant interaction between neonatal A1254 treatment and the asymmetry of FOS/TH-IR A13 neurons, we examined the possible correlation between the asymmetry of FOS/TH-IR A13 neurons and the behavioral changes in the intromission return latency and the lordosis quotient. To represent the asymmetry of FOS/TH-IR A13 cells, we used the number of cells in the major side divided by the number of cells in the minor side as the asymmetric index. Correlation analyses revealed that the asymmetric index of FOS/TH-IR A13 cells was significantly ($r=0.577$, $p<0.05$) correlated with the intromission return latency but not the lordosis quotient. A linear regression analysis showed a significant linear relation between intromission return latency and the asymmetric index of FOS/TH-IR A13 neurons ($r=0.577$, $p<0.05$, Figure 15).

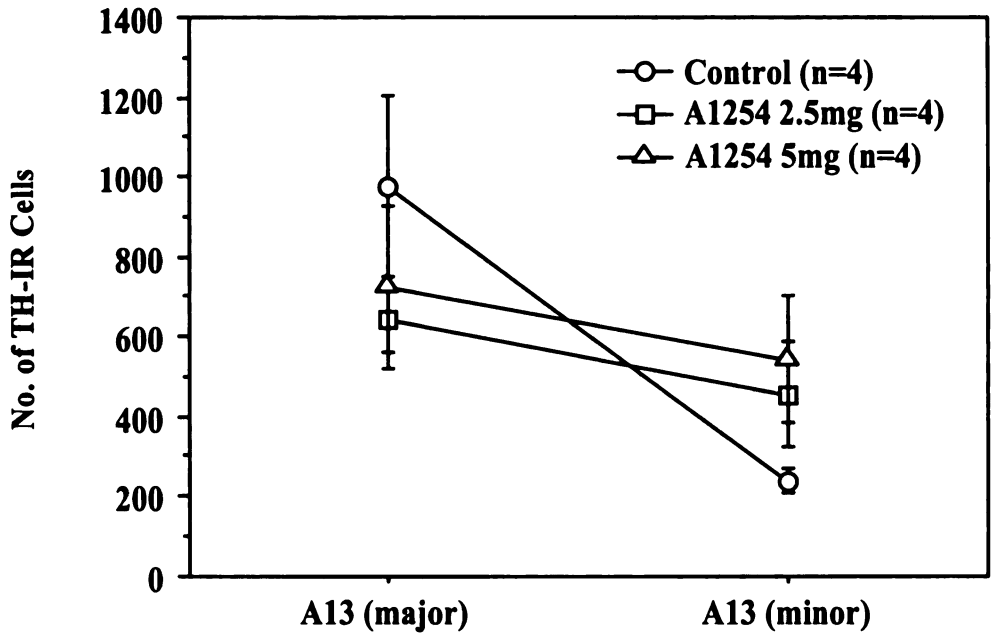

Figure 14. Interaction between neonatal A1254 treatment and the number of TH-IR cells in the A13 region. A1254 decreased the asymmetry as shown by different slopes.

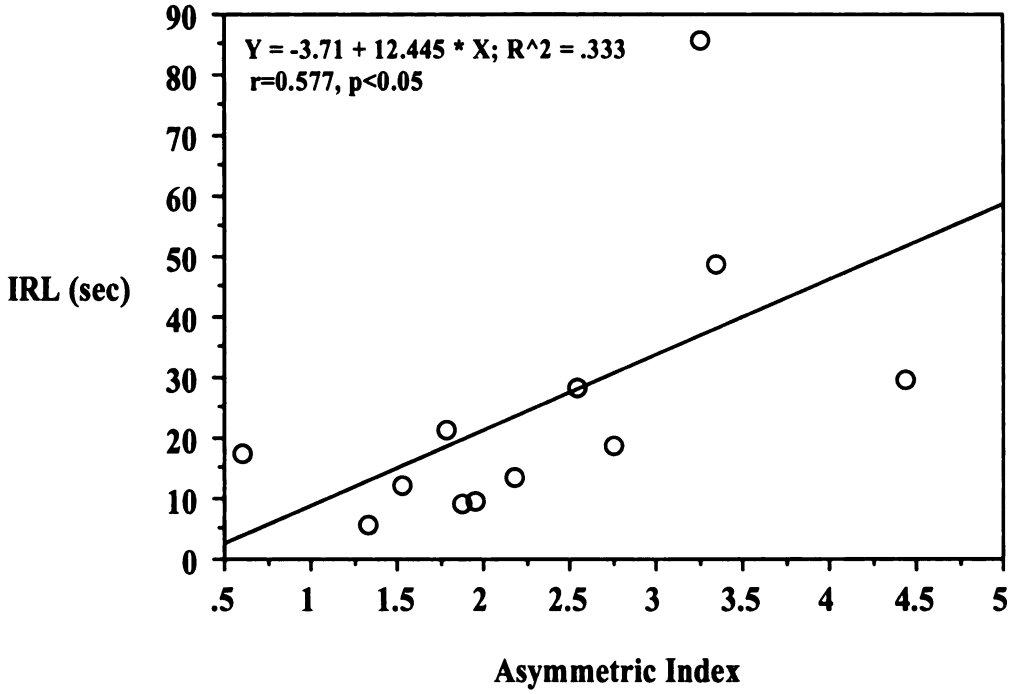

Figure 15. There is a significant correlation between the intromission return latency and the asymmetric index of FOS/TH-IR cells in the zona incerta. Asymmetric index = No. of FOS/TH-IR cells (major) / No. of FOS/TH-IR cells (minor).

Discussion

Neonatal treatment with the nonestrogenic PCB mixture, A1254, significantly altered female sexual behavior and the organization of dopaminergic neurons in the A13 region of the brain. However, neonatal treatment with the estrogenic PCBs, A1221, had no detectable effect either on female sexual behavior or on dopaminergic neurons in A11 and A13 regions. Comparing these results with those from the previous chapter, it is clear that the estrogenic component of PCBs has the potential to alter development of lordosis and pacing behaviors but such effects appear restricted to prenatal development. On the other hand, the nonestrogenic component of PCBs may disrupt sexual differentiation in a manner quite different from the estrogenic PCBs and the critical period for this action occurs during neonatal development. Since both estrogenic and nonestrogenic PCBs reduced lordosis behavior but had opposite effects on pacing behavior, it appears that these two behaviors are controlled by different developmental mechanisms. In addition, there may be at least two phases for the differentiation of brain systems involved in lordosis behavior: the initial prenatal phase may be influenced by estrogen whereas the second phase may be related to the development of dopaminergic systems. Furthermore, PCB toxicity is region-specific with A13 being more susceptible to PCB treatment than A11.

Although the reduction of LQ in A1254-treated animals was not related to the brain regions examined in this study, the short intromission return latency did appear to be associated with a reduction in putative dopamine asymmetry in the A13 region. This brain area sends fibers rostrally to several nuclei in the hypothalamus involved in the control of sexual behavior (Lindvall & Björklund, 1983), e.g., the medial preoptic area

(MPOA). Incertohypothalamic dopaminergic neurons (including A11, A13 and A14) provide the major dopaminergic inputs to the MPOA region (Lindvall & Björklund, 1983; Simerly & Swanson, 1986), and lesions of the MPOA have been shown to disrupt the temporal pattern of sexual behavior in female rats (Whitney, 1986; Yang & Clemens, 1996). The A13 region also receives return projections from the MPOA (Swanson *et al.*, 1987).

Asymmetry of dopaminergic systems in the brain is well documented (Glick *et al.*, 1982; Caligiuri *et al.*, 1989; Afonso *et al.*, 1993). In the striatum dopamine laterality has been correlated with turning behavior in females that showed a strong turning preference towards one side while a similar correlation was not seen in males (Dark *et al.*, 1984). Asymmetry in neostriatal and accumbens dopamine metabolism has been associated with variations in locomotion (Speciale *et al.*, 1986) and hippocampal dopamine concentration has been related to lateralization of T-maze choice in rats (Diaz-Palarea *et al.*, 1987). The laterality of the A13 shown in the present study provides an example of how asymmetrical brain organization may be related to behavior involving approach and avoidance components (e.g. female-paced sexual behavior).

The zona incerta (including A13 dopaminergic neurons) is involved in the initiation of locomotor (Milner & Mogenson, 1988) and orienting movements (Kim *et al.*, 1992). Electrical stimulation of the zona incerta in cats may lead to escape responses (Kaelber, 1979). The initiation of female-paced sexual behavior falls into the category of orienting movement since it has a desired direction (towards the male). The escape of the female from the male after a mount may result from the nociception provoked by body contact, and may parallel the escape response in cats. Thus, the two sides of the A13 may

play separate roles in approaching or leaving the male. Perhaps the activation of one side of the A13 is related to approach behavior while the activity of the other side favors the avoidance component of the same behavior. It is intriguing to consider the possibility that when both sides of the A13 region are active during copulation, the asymmetry of neuronal activities between the two sides may be associated with the interval between the approach and the avoidance components (return latency).

In subsequent work, we have found that this asymmetry in FOS/TH-IR is a dynamic phenomenon associated with recent experience (next chapter). In view of this, caution is warranted concerning interpretation since it is not clear whether the asymmetry is a functional aspect of the system that mediates sexual behavior, or whether the behavior results in the emergence of the asymmetrical FOS/TH immunoreactivity.

While the role of A11 dopaminergic neurons in female sexual behavior is unclear, anatomical distribution of the terminal fields for these cells suggest that they may have an inhibitory effect on mating-induced noxious input to the spinal cord and mediate an analgesic effect during copulation (Jensen & Smith, 1982; Jensen & Yaksh, 1984). The A11 cell group provides spinal dopaminergic projections (Lindvall & Björklund, 1983), which are involved in autonomic regulatory processes (Smith & Devito, 1984; Skagerberg & Lindvall, 1985) and the inhibition of noxious input to the spinal cord (Fields, 1978; Jensen & Smith, 1982; Jensen & Yaksh, 1984). Mechanical or copulatory stimulation of the vagina and cervix of the rat produces a variety of behavioral effects (Komisaruk, 1978) including facilitation of lordosis (Rodriguez-Sierra *et al.*, 1975) and selective inhibition of responses to noxious stimulation (Crowley *et al.*, 1976; Komisaruk *et al.*, 1976; Komisaruk & Wallman, 1977; Gómora *et al.*, 1994). Both the pelvic and

hypogastric nerves play a critical role in conveying genital sensory activity relevant to vaginocervical stimulation-evoked responses during mating (Cunningham *et al.*, 1991; Cueva-Rolón *et al.*, 1996). Bilateral transection of both the pelvic and hypogastric nerves not only eliminates the analgesic effects of vaginocervical stimulation (VCS) (Gómora *et al.*, 1994) but also reduces the intromission return latency (Erskine, 1992). Coincidentally, the A11 dopaminergic fibers in the spinal cord are located at sites where the pelvic and hypogastric nerves enter the spinal cord (Lindvall & Björklund, 1983) and where the neural circuitry mediating VCS-induced analgesia is found (Watkins *et al.*, 1984). Therefore, A11 dopaminergic neurons could play an indirect role in regulating the time females stay with the male and the lordosis intensity through some form of A11 analgesic effect. On the other hand, other brain areas, e.g. A13, might dictate the motor components directly.

In addition to possible central effects of A1254, there is a possibility of the peripheral effects. PCBs have been reported to alter peripheral nerve conduction velocity (Murai & Kuroiwa, 1971). Such effects could result in altered sensitivity to sexual stimulation. Severing the pelvic nerve decreased female return latencies in female pacing tests (Erskine, 1992), a result similar to the effect of neonatal A1254 treatment. It is possible that PCBs altered the pelvic nerve processes and/or decreased pelvic sensitivity of the female resulting in the shortening of the return latencies in our neonatal experiments.

Adult PCB treatment had no effect on female sexual behavior, suggesting that effects seen as a result of neonatal exposure represent developmental influences of PCBs, not concurrent effects. Seegal suggested that the prolonged suppression of brain

dopamine concentrations following PCB exposure was due to biochemical alterations induced by the initial exposure to PCBs, rather than to continual, low-level exposure via fat stores (Seegal *et al.*, 1992, 1994). Our adult study further supported the idea that changes in sexual behavior were developmental effects, not the result of residual PCBs. Moreover, the dose needed to disrupt sexual behavior was much lower in neonates than in adults.

In summary, permanent changes were seen in feminine sexual behavior after neonatal treatment with the PCB mixture, A1254, but not after treatment with the estrogenic PCBs, A1221. These behavioral changes were associated with the disruption of developing A13 dopaminergic system. The asymmetry of A13 may provide a good model for studying motor control of female-paced sexual behavior. The A11 dopaminergic system may indirectly modulate this behavior. These studies suggest that PCBs could disrupt the development of A13 dopaminergic systems and thereby reduce the reproductive success of exposed animals.

CHAPTER 5: FEMALE-PACED SEXUAL BEHAVIOR AND ITS LINK TO REPRODUCTION: A TIME COURSE STUDY

Introduction

When placed in a two-compartment chamber which allows free entrance and exit from a compartment containing a sexually active male, estrous female rats spontaneously regulate or pace the timing of the copulatory stimulation received from males (Bermant, 1961; Peirce & Nuttall, 1961; Krieger *et al.*, 1976; Erskine, 1985). Female-paced sexual behavior appears to regulate the timing of vaginocervical stimulation and plays an important role in ensuring the activation of the neuroendocrine reflex responsible for prolongation of ovarian corpora luteal function for pregnancy (Erskine, 1989).

My previous studies showed that the distribution of FOS immunoreactive A13 dopaminergic neurons on two sides of the brain might be important for female-paced sexual behavior (Figure 14). However, the correlation did not clarify whether the change in the asymmetry of A13 dopaminergic activity was the cause of behavioral disruption or *vice versa*. Further, since these data were collected from animals that had been treated neonatally, I decided to investigate more closely the relationship of copulatory behavior and changes in brain activity using normal females.

Since female-paced sexual behavior is dynamic in its nature, I predict that the neuronal activity in the A13 region would also be dynamic during copulation if A13 dopaminergic neurons are involved in female-paced sexual behavior. Thus, FOS immunoreactivity (an index for neuronal activity as described in Chapter 2) in the A13 region should show differences among females that have copulated and those that have not.

A time-course study of FOS immunoreactivity in the A13 region should also show dynamic changes during the course of female-paced copulation. In addition, a comparison of female-paced and male-paced females should reveal differences in this brain area as well as in efferent A13 target sites.

Since neurons of the incertohypothalamic system (including A11, A13, and A14) provide dopaminergic input to the medial preoptic area of the hypothalamus (MPOA) (Lindvall & Björklund, 1983; Simerly & Swanson, 1986), and since MPOA lesions have been shown to disrupt the temporal pattern of female-paced sexual behavior in rats (Whitney, 1986; Yang & Clemens, 1996), we propose that the MPOA is a potential efferent of the A13 region that is related to female-paced sexual behavior. We would then predict that FOS immunoreactivity would differ between female-paced and male-paced animals and also vary over time in the A13 and MPOA regions.

In this chapter, we compared the A11, A13 and the medial preoptic nucleus of the MPOA (MPON) from normal female rats tested in female-paced and male-paced paradigms for different time periods (10 min, 30 min, 1 hr) as well as with different amounts of vaginocervical stimulation (1E, 2E, 3E and 4E). A group of noncopulated females served as the baseline control.

Materials and Methods

Animals

Sixty-day-old Long-Evans female rats were ovariectomized and given hormone treatments for behavior tests as described in Chapter 2.

Female Behavior Tests

We divided 42 rats into seven time-course groups (6 rats/group). The control group received no sexual stimulation and stayed in the home cage. FP-10, FP-30 and FP-1h groups were placed with a male for 10 min, 30 min and 1 hr respectively under female-paced condition. MP-10, MP-30 and MP-1h groups remained with a male for 10 min, 30 min and 1 hr under male-paced condition. Females were returned to their home cage after the behavior test except the FP-1h and MP-1h groups which were immediately sacrificed. One hour after the first sexual encounter, all the other females were also deeply anesthetized with sodium pentobarbital (75 mg/Kg) and sacrificed for brain tissue processing. Thus, the time from the beginning of sexual stimulation was constant.

Four latencies were measured in the male-pacing test: mount latency, intromission latency, ejaculation latency, and postejaculatory interval. Mount latency was the time interval measured from the introduction of the female to the first mount or intromission performed by the male. Similarly, intromission latency was the time interval measured from the introduction of the female to the first intromission performed by the male. Ejaculation latency, however, was the time interval from the first intromission to the following ejaculation performed by the male. Postejaculatory interval was the time interval measured from the first ejaculation to the following intromission. The frequencies of different copulatory events (mount frequency and intromission frequency) were also recorded. Inter-intromission interval, the average interval between intromissions, and intromission rate, the rate of intromissions per minute, were analyzed as well.

In the female-paced paradigm, other parameters were used together with the above

measures as described in Chapter 2.

Immunohistochemistry for FOS and TH

Tyrosine hydroxylase immunoreactivity (TH-IR) was used to identify dopaminergic neurons in the A11 and A13 regions of the brain. We also double labelled the brain sections with FOS immunohistochemistry to identify mating-activated dopaminergic neurons. FOS-IR of the medial preoptic nucleus of the hypothalamus (MPON) was also analyzed.

Female rats were randomly selected for immunohistochemical processing and sacrificed 1 hr after the first sexual encounter (the introduction of female into the testing arena). The procedure for FOS/TH immunohistochemistry was described in Chapter 2. Brains were sectioned coronally at 25 μm with a cryostat. Frozen sections were collected and every fourth section was processed for FOS and TH immunohistochemistry. At least one brain from each group was processed simultaneously. (Note: All the sections for MPON were double stained with FOS/TH except the ones for the thionine staining.) The primary antibody (Ab-5 c-FOS rabbit polyclonal antisera, 1:10,000, Oncogene Research Products, MA) and normal goat serum (Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions) were used. The biotinylated secondary antibody was goat-anti-rabbit IgG (Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's directions). After the FOS immunohistochemistry, sections were incubated in 5% dimethyl sulfoxide for 30 min and rinsed three times with TBS. They then underwent the immunohistochemistry with the primary antibody for TH (monoclonal mouse-anti-TH, 1:5,000, Sigma Chemical Co., St. Louis, MO) with normal horse serum (Vector Laboratories, diluted with TBS/0.3% Triton X-100 according to the manufacturer's

directions) and the same steps as described in Chapter 2.

TH-IR and FOS/TH-IR perikarya from both sides of the sections were quantified in the A11 and A13 regions using camera lucida. Systematic section method was used for cell counting, and the estimated neuronal population was calculated by the product of sample count and the period of the systematic sample (Konigsmark, 1970). FOS-IR in the MPON was quantified on a Macintosh computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/). Brain sections from each animal were selected to match 5 plates from the Atlas of the rat brain (Paxinos & Watson, 1986) at the level from plate 18 (Bregma: -0.26 mm, Interaural: 8.74 mm) to plate 22 (B: -0.92 mm, I: 8.08 mm). The images were taken at 100x maginification. The plates were magnified to the appropriate size to match the computer printouts of the image. The plate was then superimposed on the computer printouts and FOS-IR nuclei were counted inside the MPON region. All the slides were number-coded and the person responsible for counting was not aware of the experimental design.

Statistics

The mean of each behavioral parameter in each test was automatically calculated by the computer program. The average data of four behavior tests were then used for statistical analyses. Mount latency, intromission latency, ejaculation latency and postejaculatory interval were analyzed by one-way ANOVA. Fisher's LSD post hoc test was performed when there was a significant difference among groups ($p<0.05$). Mount frequency, intromission frequency, inter-intromission interval and intromission rate were analyzed

by the Kruskal-Wallis Test. A post hoc test was performed when there was a significant difference among groups ($p<0.05$). Approach latency, mount return latency, intromission return latency and postejaculatory refractory period were analyzed by one-way ANOVA. Fisher's LSD post hoc test was performed between different time course groups when there was a significant difference among treatments ($p<0.05$). Percentages of mount leave, intromission leave, mount stay and intromission stay were analyzed by the Kruskal-Wallis Test. LQ, the percentage of times that females showed a lordosis response in 10 mounts (standard test) or more (pacing test), was also analyzed by the Kruskal-Wallis Test. The post hoc tests were performed between different groups when there was a significant difference among treatments ($p<0.05$). The number of TH-IR neurons in the A11 or A13 region was analyzed by both one-way ANOVA and Kruskal-Wallis Test. The number of FOS-IR cells in the MPON region was analyzed by both one-way ANOVA and Kruskal-Wallis Test. To compare the measurements of two sides of A11, A13 or MPON, two-way ANOVA (two sides as repeated measurements) was used.

Results

Time Effect

Copulation increased the total number of TH-IR ($p<0.005$, Figure 16) and FOS/TH-IR ($p<0.0005$, Figure17) cells in the A13 region. The induction of A13 TH-IR or FOS/TH-IR cells was asymmetric ($p<0.0001$, $p<0.001$, respectively) on two sides of the brain.

Copulation for 10 min increased the number of A13 TH-IR cells under the female-paced condition, but not in the male-paced paradigm. An increase in TH-IR cell number was only seen in the male-paced females that copulated for 30 min and 1 hr. The number of TH-

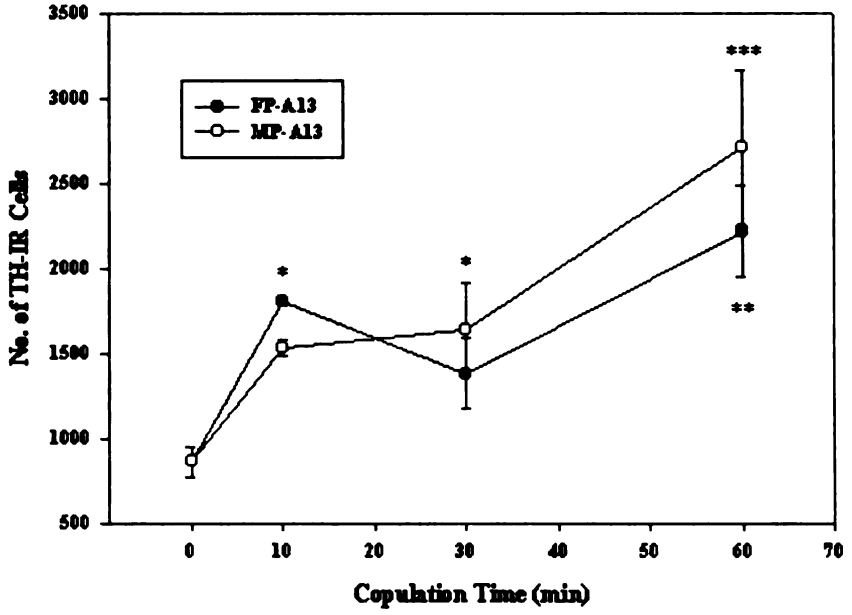

Figure 16. Time course of tyrosine hydroxylase immunoreactivity (TH-IR) in the A13 region. FP: female-paced; MP: male-paced; *: significantly different from the noncopulated group (0 min), $p<0.05$; **: significantly different from the noncopulated group, $p<0.005$; *: significantly different from the noncopulated group, $p<0.0001$).**

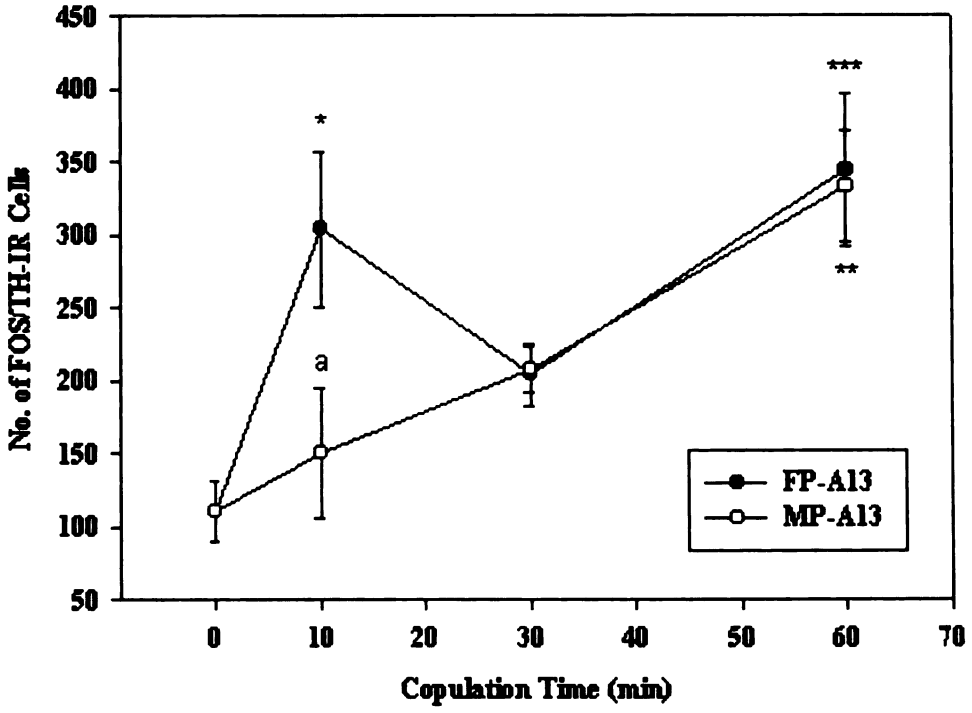

Figure 17. Time course of FOS/TH immunoreactivity (IR) in the A13 region. FP: female-paced; MP: male-paced; a: significantly different from the FP-10 group, $p<0.05$; *: significantly different from the noncopulated group (0 min), $p<0.005$; **: significantly different from the noncopulated group, $p<0.0005$; *: significantly different from the noncopulated group, $p<0.0001$.**

IR cells in the female-paced 30 min group was low, but an increase in TH-IR cells was seen in the female-paced 1 hr group (Figure 16).

Copulation for 10 min increased the number of A13 FOS/TH-IR cells in female-paced rats, but not in the male-paced paradigm. The induction of FOS/TH-IR cell number was not seen in either condition at 30 min. The increase in FOS/TH-IR cells occurred in both female-paced and male-paced 1 hr groups (Figure 17).

Copulation did not increase the total number of TH-IR cells in the A11 region. However, the increase in FOS/TH-IR cells was seen in the male-paced females copulated after 1 hr ($p<0.001$, Figure 18) and the induction was also asymmetric ($p<0.001$).

Copulation increased the number of FOS-IR cells in the MPON as measured by the sum of five plates ($p<0.01$, Figure 19). The increase in FOS-IR cells was restricted in plate 20 ($p<0.005$) and plate 21 ($p<0.05$) and the induction was significant in both female-paced and male-paced females that copulated for 1 hr (Figure 19).

Effect of the Amount of Sexual Stimulation

To control for the amount of sexual stimulation that the female received within the same time period under a different testing paradigm, I also analyzed data using ejaculation as a unit for sexual stimulation. Copulation increased the number of TH-IR cells at the major side of the A13 region ($p<0.05$). The induction of A13 TH-IR cells was asymmetric ($p<0.0001$) with respect to the two sides of the brain. More sexual stimulation did not induce more TH immunoreactivity after one ejaculatory series since there was no difference among different ejaculatory series.

Copulation increased the number of FOS-IR cells in the MPON as measured by the

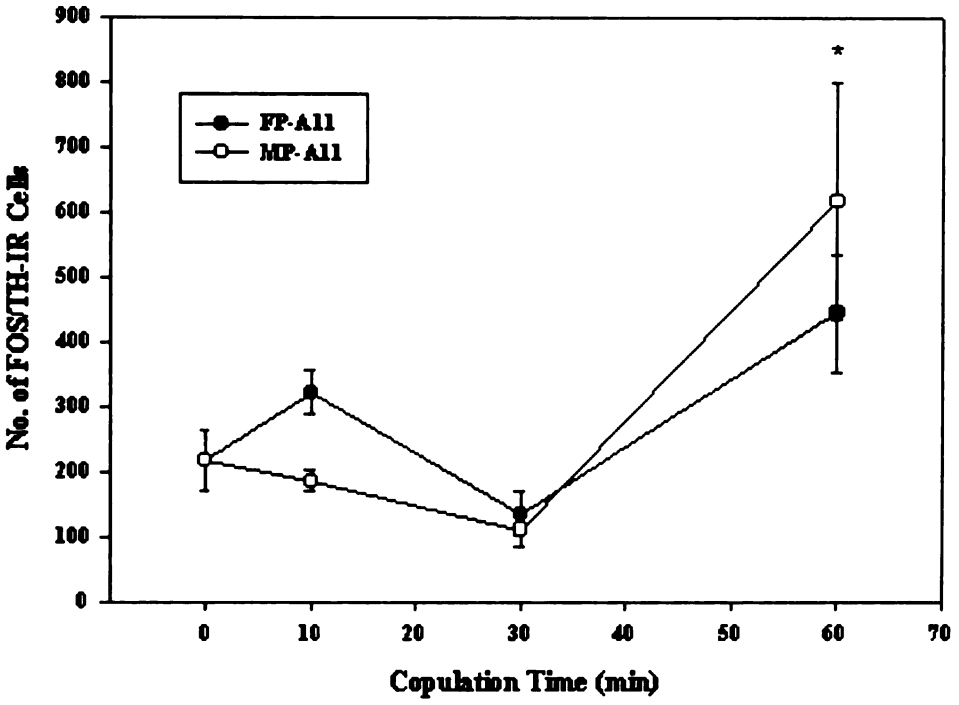

Figure 18. Time course of FOS/TH immunoreactivity in the A11 region. FP: female-paced; MP: male-paced; *: significantly different from the noncopulated group (0 min), $p<0.005$).

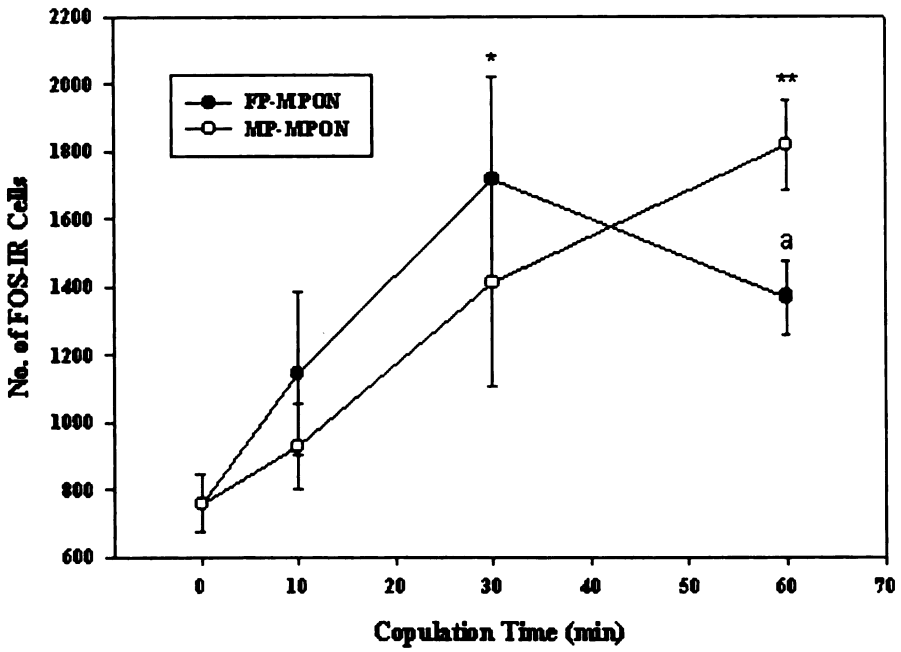

Figure 19. Time course of FOS immunoreactivity in the medial preoptic nucleus of the hypothalamus (MPON). FP: female-paced; MP: male-paced. *: significantly different from noncopulated group (0 min), $p<0.05$; **: significantly different from noncopulated group (0 min), $p<0.005$; a: significantly different from MP-1h group, $p<0.05$.

sum of five plates ($p<0.01$, Figure 20). The increase in FOS-IR cells was seen only in plate 20 ($p<0.05$), 21 ($p<0.05$) and 22 ($p<0.005$) of both 1-hr groups (Figure 20). There was no FOS-IR asymmetry between two sides of the brain in the MPON.

A striking finding showed that a dense core of FOS-IR cells in the sexually dimorphic nucleus of the preoptic area (SDN-POA) occurred in the FP-1E group but not in the MP-1E group (Figure 21). After copulation for 2 ejaculatory series, both female-paced and male-paced females showed this dense core of FOS-IR cells in the SDN-POA (Figure 21).

There was no difference between the male behavioral parameters in different testing situations (data not shown).

Discussion

I was able to replicate our previous finding of A11 and A13 TH-IR asymmetry following copulation in normal female rats. The asymmetry may be a dynamic phenomenon associated with recent sexual experience since copulation for various lengths of time induced differential changes in TH immunoreactivity. In addition, the TH-IR asymmetry in these two brain regions may be fundamentally different since copulation for various time periods changed the total number of detectable TH-IR cells in the A13 but not in the A11 region. Moreover, different testing situations also affected the timing of TH-IR induction.

Copulation also induced FOS/TH-IR in both A11 and A13 regions. However, the timing of induction seemed to be different for the two brain areas. In the A11 region, copulation induced more detectable FOS/TH-IR cells only in the 1 hr male-paced group but not in the other groups. As for the A13 region, the number of detectable FOS/TH-IR cells

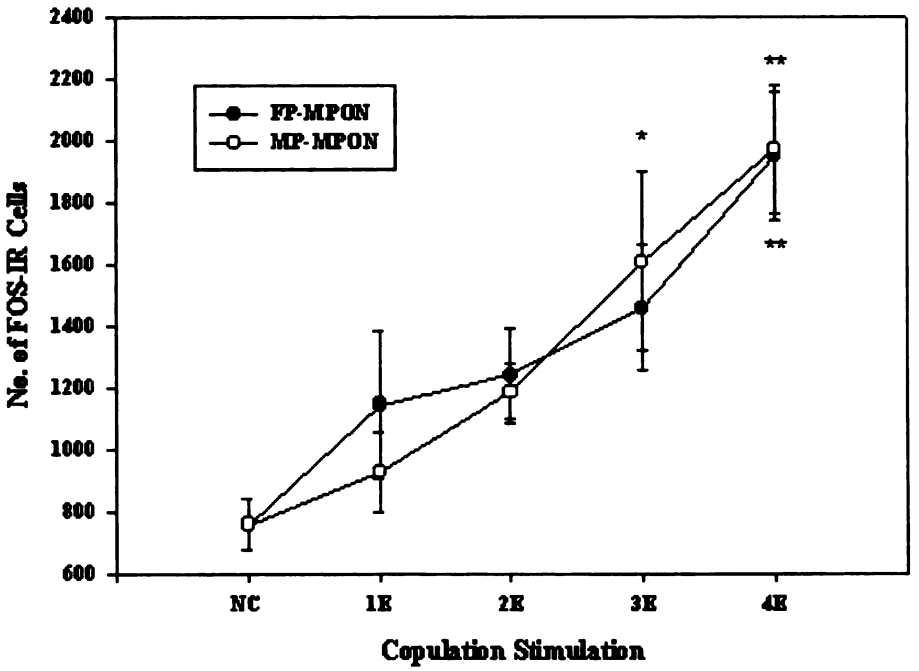

Figure 20. FOS immunoreactivity in the MPON region. FP: female-paced; MP: male-paced; E: ejaculatory series; *: significantly different from the noncopulated group (0 min), $p<0.05$; **: significantly different from the noncopulated group, $p<0.005$).

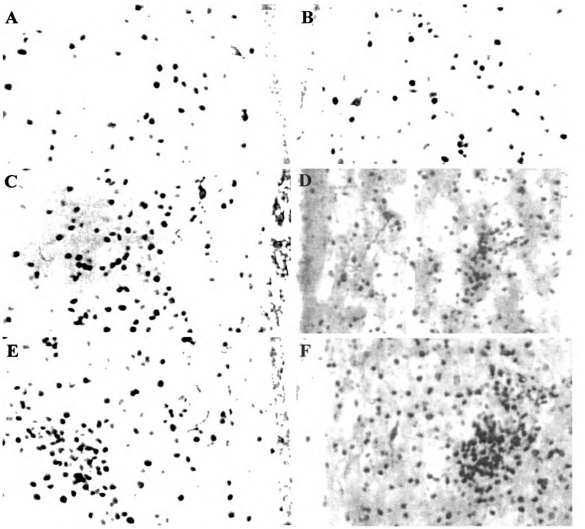

Figure 21. FOS immunoreactivity in the sexual dimorphic nucleus of the preoptic area of the hypothalamus under various sexual stimulation. A, B: noncopulated; C: male-paced 1 ejaculatory series; D: female-paced 1 ejaculatory series; E: male-paced 2 ejaculatory series; F: female-paced 2 ejaculatory series.

dramatically increased in the FP-10 females. While a similar increase was not seen in the FP-30 females, the number did increase in FP-1h females. In the male-paced females, the induction of FOS/TH-IR did not show significant difference until the 1-hr test period. Female-paced mating seemed to maximize the induction of FOS/TH-IR cells in the A13 region in a short period of time (10 min) while male-paced mating required a longer period (1 hr). It is unclear from these data whether there was a decline of FOS/TH-IR cells in the FP-30 females or whether the increase seen at 10 min was followed by a decline.

The induction of FOS-IR cells in the MPON seemed to follow the same pattern of the induction of FOS/TH-IR cells as seen in the A13 region with a little delay in time. Considering the reciprocal connections between MPON and A13, it is quite reasonable that these two regions should share the same pattern of neuronal activity during copulation. A13 activity may be the trigger of the induction of FOS-IR in the MPON.

Pfaus *et al.* (1996) found that artificial vaginocervical stimulation (VCS) produced a threshold pattern of FOS activation in the MPOA. The artificial VCS was designed to stimulate an intromission. In the rostral MPOA, generally moderate and steady numbers of FOS-IR cells were induced between 1 and 20 VCSs, but a sharp rise in FOS-IR cells occurred following 30-50 VCSs. In the caudal MPOA, VCS appeared to induce three different thresholds of FOS expression, the first increased from 1 to 10 VCSs, a second increase from 10 to 40 VCSs, and a third increase at 50 VCSs. To consider the factor of VCS through the time course, we also examined the FOS induction using ejaculation as a unit since the amount of VCSs female received in 1 ejaculatory series (E) in our behavior test is close to 10 VCSs in the artificial treatment in Pfaus's study (1996).

Perhaps the most striking finding in this Chapter is that the SDN-POA is activated at

1E in the female-paced but not in the male-paced females. Although the SDN-POA was characterized over 20 years ago (Gorski *et al.*, 1978), no true function has been assigned to this sexually dimorphic nucleus. Considering the timing of SDN-POA activation in the female-paced and male-paced females and its anatomical connections (Simerly & Swanson, 1986, 1988), I propose that the function of SDN-POA in female rats may be related to the termination of estrus, stimulation of the prolactin release for pregnancy and the inhibition of LHRH release for normal cycling (Figure 22).

In rats, one of the behavioral consequences of mating is an abbreviation of the period of estrus (Hardy & DeBold, 1972; Erskine, 1989). Although large numbers of temporally uncontrolled intromissions can induce estrus abbreviation (Erskine & Marcus, 1981), many fewer are sufficient if the female is allowed to pace coital contacts with males (Erskine & Baum, 1982; Erskine, 1985). Estrus abbreviation may occur after 10 intromissions in the female-paced situation (Erskine,1985), but has been demonstrated under male-paced condition only after 25 intromissions (Erskine & Marcus, 1981) or after *ad lib* mating has occurred for 40 min (Lodder & Zeilmaker, 1976). The stimulation needed for the induction of SDN-POA FOS-IR cells in our female-pacing test (1E) and male-pacing test (2E) is quite compatible with 10 intromissions mentioned in Erskine's female-paced (1985) and male-paced (25 intromissions, 1981) paradigms. Therefore, considering the timing and the stimulation for the induction of SDN-POA FOS-IR, it is likely that this area might be related to the termination of estrus in female rats. Indeed, a significant increase in dopamine concentration in the preoptic-anterior hypothalamic area of the sexually refractory females was observed (Clark *et al.*, 1986). The termination of estrus (sexually refractory) may be due to the increase dopamine transmission from the incertohypothalamic system (including A13)

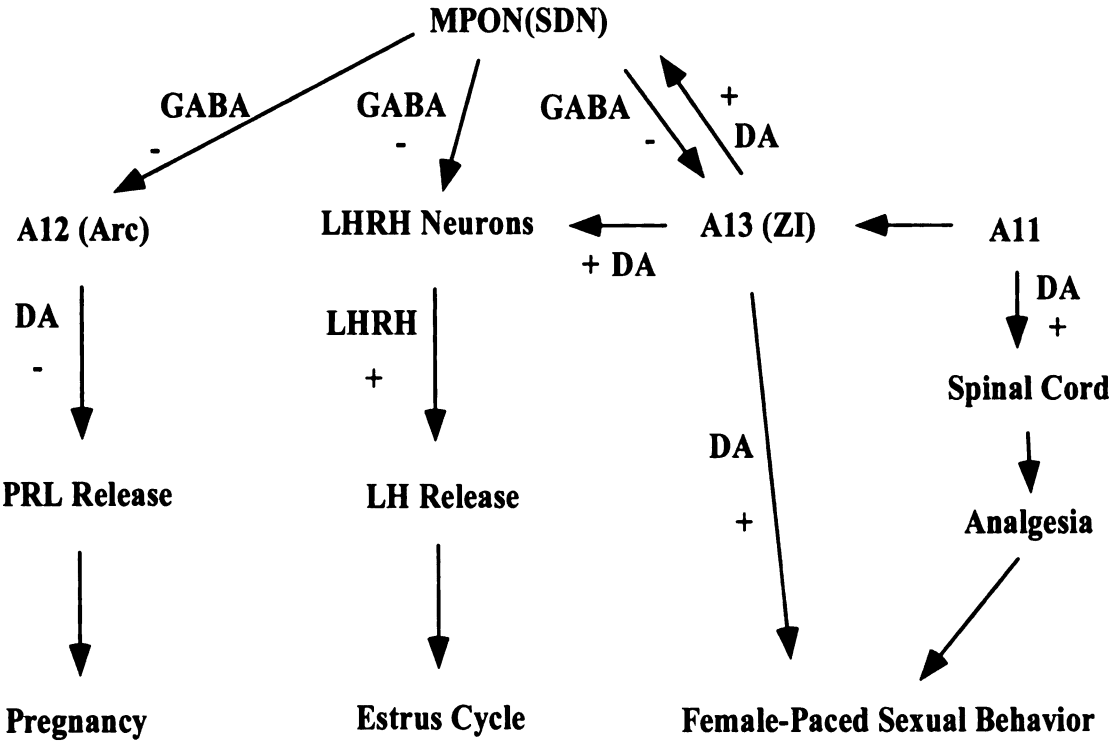

Figure 22. Hypothetical diagram for the possible functions of the sexually dimorphic nucleus of the preoptic area (SDN). DA: dopamine; GABA: gama-aminobutyric acid; LH: luteinizing hormone; LHRH: luteinizing hormone-releasing hormone; MPON: medial preoptic area; PRL: prolactin; ZI: zona incerta.

to the medial preoptic area (including SDN-POA).

Female-paced sexual behavior can regulate the vaginocervical stimulation (VCS) and play an important role in ensuring the activation of the neuroendocrine reflex responsible for prolongation of ovarian corpora luteal function (Erskine, 1989). In a variety of species, copulation with intromission or manual VCS results in the release of prolactin to sustain luteal function (Terkel & Sawyer, 1978) which prepares the uterus for implantation of the embryos. The induction of this progestational state is critical for the establishment and maintenance of pregnancy, and is positively correlated with the number of female-paced intromissions received by the female (Adler *et al.*, 1970; Komisaruk & Steinman, 1986). It has been shown that the frequency of intromissions received by the female rat during estrus is a critical determinant of whether the ovarian corpora lutea secrete the quantities of progesterone necessary to maintain pregnancy (Adler, 1969, 1983; Chester & Zucker, 1970). VCS activates a neuroendocrine reflex arc which initiates a pattern of twice-daily surges of the prolactin secretion; these nocturnal and diurnal prolactin surges are necessary for the prolonged luteal progesterone secretion of pregnancy and pseudopregnancy (Gunnet & Freeman, 1983). Intromissions received by females during female-paced mating induce pregnancy/pseudopregnancy more readily than do male-paced intromissions (Erskine *et al.*, 1989). Female-paced as opposed to male-paced mating appears to enhance the effectiveness of each intromission in inducing these neuroendocrine changes (Erskine, 1989). The differences between female-paced and male-paced females in the behavioral and neuroendocrine responses to coital stimulation may result from differences in intromission duration (Erskine *et al.*, 1989) or ejaculation duration (Fang *et al.*, 1998) by males (longer in the female-paced situation).

The effect of the SDN-POA in stimulating the prolactin release might be through the inhibition of the other POA region (Clemens *et al.*, 1971, 1976; Wiegand *et al.*, 1980; Gunnet & Freeman, 1983, 1985) or the dopaminergic neurons in the arcuate nucleus (Moore, 1987) by its GABAergic neurotransmission or due to the release of a prolactin releasing factor, thyrotropin-releasing hormone (Tashjian *et al.*, 1971). The SDN contains thyrotropin-releasing hormone neurons (Simerly *et al.*, 1986) and GABAergic neurons (Sagrillo & Selmanoff, 1997). The POA is known to tonically inhibit the expression of the nightly surge of prolactin during pseudopregnancy following cervical stimulation. Lesions of the medial preoptic area trigger the release of nocturnal prolactin surges and induce repetitive, spontaneous pseudopregnancy (Clemens *et al.*, 1976; Wiegand *et al.*, 1980; Gunnet & Freeman, 1983, 1985). Conversely, electrochemical stimulation of the preoptic area reduces prolactin secretion perhaps by increasing the inhibitory action of this brain site (Clemens *et al.*, 1971). It has been proposed that the medial preoptic area may contain neurons which exert a chronic inhibitory effect on the prolactin surge (Gunnet & Freeman, 1983). Bilateral implantation of muscimol-mannitol mixture into the medial preoptic/anterior hypothalamic area increased blood prolactin level (Lamberts *et al.*, 1983; Bach *et al.*, 1992). SDN-POA neurons might inhibit the effect of other POA neurons and stimulate the release of prolactin through their GABAergic terminals. Indeed, the axons of the GABAergic neurons in the medial preoptic area often ramified into an apparent network of local collaterals, possibly forming local circuits in this region (Hoffman *et al.*, 1994).

Female-paced sexual behavior seems to maximize the induction of FOS-IR in the medial preoptic area including the SDN-POA. The induction of FOS-IR in the SDN-POA may be related to the termination of estrus, the release of prolactin for pregnancy and the

interruption of regular cycling of LH. The relation between A13 dopaminergic neurons and the medial preoptic area may serve as the link between sexual behavior and the neuroendocrine systems that mediate implantation and the onset of pregnancy.

CHAPTER 6. EFFECTS OF PCBS ON MALES AND THE RELATION OF MALE SEXUAL BEHAVIOR TO THE MAJOR PELVIC GANGLIA IN NEONATALLY A1221-TREATED RATS

Introduction

I have discussed the effects of PCBs on the development of female sexual behavior and brain dopaminergic systems in the previous chapters since my studies were primarily focused on females. However, I also examined sexual behavior of the male littermates. Since I had a skewed sex ratio in the animal colony, I did not have a complete data set for all the experiments. While I did not observe striking PCB effects, the analysis did reveal an intriguing relation between male sexual behavior and measures of the major pelvic ganglion. In this chapter, I summarize the male behavioral data from the perinatal and neonatal PCB treatment groups. Since PCBs did not have strong effects on male sexual behavior, I did not undertake the studies of the brain dopaminergic systems in these subjects. In addition, I only examined the pelvic ganglia of the neonatally A1221-treated rats since the technique was not developed and available to use with prior treatment groups.

The pelvic plexus comprises a major pelvic ganglion and some satellite ganglia lying on either side of the dorsolateral lobes of the prostate in male rats. In female rats, these bilateral ganglia (no satellite ganglia) were located at the uterine cervix-vagina junction and were called paracervical or pelvic ganglia. For the convenience of discussion, I refer to the male major pelvic ganglia and female pelvic ganglia as the MPG. The MPG is a mixed autonomic ganglion composed of both parasympathetic and sympathetic elements (Dail *et al.*, 1975; Dail and Evans, 1978), which innervate

androgen sensitive accessory organs of male rats as well as the pelvic viscera in both male and female rats (Langworthy, 1965; Purington *et al.*, 1973; Quinlan *et al.*, 1989). The MPG receives innervation from the spinal cord at L6 and S1 levels via the pelvic nerve and from the thoracolumbar outflow via the hypogastric nerve (Quinlan *et al.*, 1989).

Nissl-stained or tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the MPG display a strong sexual dimorphism. There are about 2.5 times more neurons in the male than in the female MPG (Greenwood *et al.*, 1985). Many TH neurons are found in the male MPG, but very few in the female MPG (Vera and Nadelhaft, 1992). The cavernous (penile) nerve from the MPG, which courses along the urethra and innervates the corpus cavernosum, is essential for penile erection in male rats (Quinlan *et al.*, 1989; Sachs and Liu, 1991). Pelvic and pudendal nerves from the MPG, on the other hand, are important sensory pathways for female sexual behaviors (Erskine, 1992). Here I report the behavioral data of the effect of neonatal A1221 treatment on sexual differentiation of the MPG in rats.

Materials and Methods

Animals

PCB treatments were described in Chapter 3 (perinatal) and Chapter 4 (neonatal). Female offspring from PCB-treated female rats were tested as described previously. Male offspring were tested intact at 90 days of age. They were placed with sexual experienced female rats over night at the testing arena two weeks before real tests (day 76). One more pretest was observed on day 83. Males were then tested for sexual behavior once per week for four weeks. Untreated rats of the same strain and age were purchased from the

breeder (Harlan Spraque Dawley, Inc., Indianapolis, IN) and tested with the same protocol.

Male Behavior Test

To test for male behavior, a sexually receptive female was placed with a male rat in a single-compartment testing chamber (56x44x49 cm). Male behaviors (mount, intromission, and ejaculation) were then recorded by a computer with a event recording program. Copulatory behavior of the male rats was characterized by a series of mounts, with or without vaginal insertion, then eventually accumulated in ejaculation. Intromission patterns (mount with vaginal insertion) could be distinguished behaviorally from mounts without penetration by the presence of a deep thrust and a springing dismount. An ejaculation occurred after several intromissions and followed by a refractory period.

Histochemistry for the Major Pelvic Ganglia

Rats were anesthetized with sodium pentobarbital (75 mg/Kg). The abdominal wall was opened and the MPG were removed and fixed with 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) over night at 4°C. The MPG was then dehydrated through an ethanol series (50%, 70%, 90%, 100%, 30 min, 2 times each) and finally transferred into ethanol/xylene (1:1, 30 min) then 100% xylene (30 min, 2 times). After incubating with xylene/paraffin (1:1) two times (30 min each) at 60°C, the MPG was infiltrated with pure paraffin at 60°C (1 hr, 2 times) and embedded into a paraffin cube. The MPG was sectioned at 30 μm with a microtome. Paraffin ribbons were mounted onto gelatin-coated slides, stained with thionine as described in Chapter 2, and covered with a

coverslip in DPX mounting media (Electron Microscopy Sciences). Neurons were quantified on every tenth section in one side of the MPG using camera lucida. Slides were number-coded and the person responsible for counting was not aware of the experimental design.

Statistics

The analyses of female behavioral data were described in previous chapters. To assess the temporal pattern of male sexual behavior, four latencies were measured: mount latency, intromission latency, ejaculation latency, and postejaculatory interval. Mount latency was the time interval measured from the introduction of the female to the first mount or intromission performed by the male. Similarly, intromission latency was the time interval measured from the introduction of the female to the first intromission performed by the male. Ejaculation latency, however, was the time interval from the first intromission to the following ejaculation performed by the male. Postejaculatory interval was the time interval measured from the first ejaculation to the following intromission. The mean of each behavioral parameter in each test was calculated by the computer program. The mean of four behavior tests were then used to calculate the mean for each litter and for further statistical analyses using StatView (Abacus Concepts, Inc., Berkeley, CA). Mount, intromission and ejaculation latencies and postejaculatory intervals were analyzed by one-way ANOVA. The frequencies of different copulatory events (mount frequency and intromission frequency) were also recorded and analyzed by the Kruskal-Wallis Test. The hit rate, defining as the intromission frequency divided by the total number of mounts and intromission, was also calculated and analyzed by the Kruskal-Wallis Test. Interintromission interval, the average interval between

intromission rate, the rate of intromissions per min, were analyzed by the Kruskal-Wallis Test as well. Since the sample size for perinatal A1221 treatment groups was not sufficient for statistical analyses, data were discarded.

In order to understand why male noncopulators had fewer cells in the MPG, I examined the relation between the number of cells in the MPG and male sexual behavior. Since there was no difference among treatment groups, I pooled the data of PCB-treated rats and did the correlation analyses using MPG cell number as the independent variable and the behavioral parameters as dependent variables.

The number of neurons in the MPG was analyzed by both one-way ANOVA and the Kruskal-Wallis Test. Fisher's LSD post hoc test was performed when there was a significant difference among treatments ($p<0.05$). Correlation analysis was performed between behavior parameters (dependent variables) and the number of neurons in the MPG (independent variable). Linear regression analysis was followed if significant correlations were found ($p<0.05$). F tests were performed between PCB-treated and purchased rats in both sexes.

Results

There was no difference in male sexual behavior or the cell number of the MPG among neonatal A1221 treatment groups. However, there was a sex difference in the number of neurons in the MPG with control males having more cells than control females. Within control males, the number of cells in the MPG was significantly different between copulators and noncopulators (Figure 23). The cell counts of non-copulators' MPG were significantly fewer than those of the control male copulators but significantly greater than those of control females.

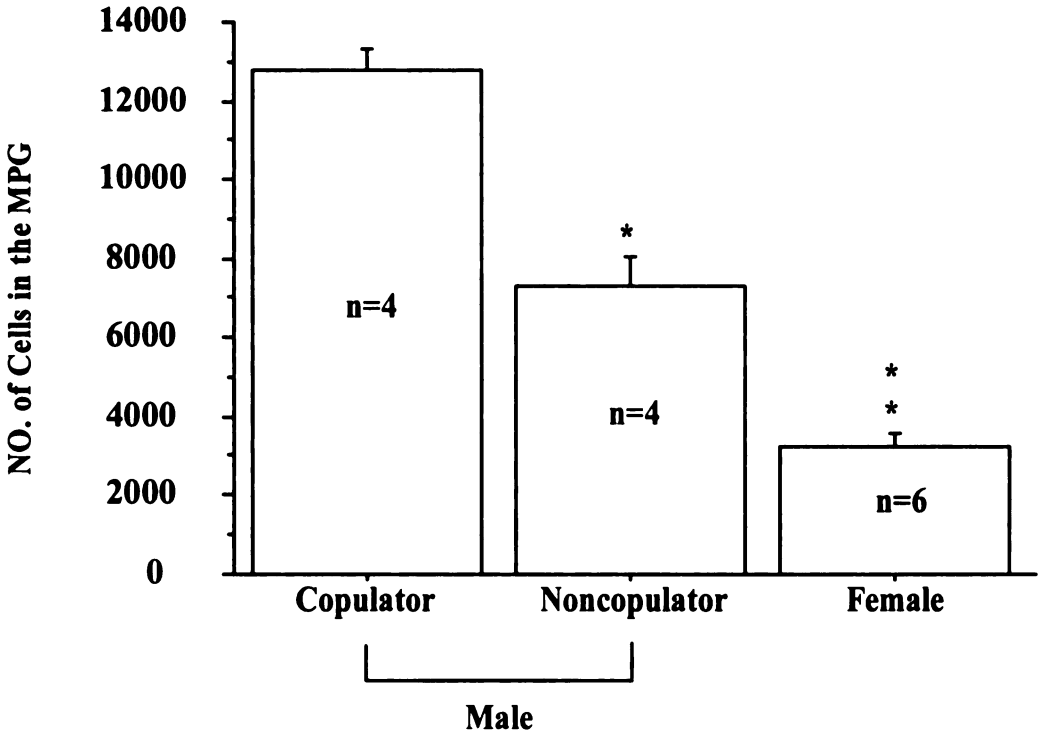

Figure 23. Sexual allomorphism in the number of cells in the major pelvic ganglia (MPG).

The mount frequencies of PCB-treated male rats were significantly correlated with the number of neurons in the MPG (correlation coefficient = 0.77, $p<0.01$). Linear regression analysis showed a negative linear relationship between male mount frequencies and the number of neurons in the MPG ($p<0.01$, Figure 24).

The ejaculation latencies of PCB-treated male rats were significantly correlated with the number of neurons in the MPG (correlation coefficient = 0.79, $p<0.01$). Linear regression analysis showed a negative linear relationship between male ejaculation latencies and the number of neurons in the MPG ($p<0.01$, Figure 25).

The interintromission intervals of PCB-treated male rats were significantly correlated with the number of neurons in the MPG (correlation coefficient = 0.82, $p<0.01$). Linear regression analysis showed a negative linear relationship between male interintromission intervals and the number of neurons in the MPG ($p<0.01$, Figure 26).

The intromission rates of PCB-treated males were significantly correlated with the number of neurons in the MPG (correlation coefficient = 0.73, $p<0.05$). Linear regression analysis showed a positive linear relationship between male intromission rates and the number of neurons in the MPG ($p<0.05$, Figure 27).

The hit rates of PCB-treated males were significantly correlated with the number of neurons in the MPG (correlation coefficient = 0.81, $p<0.01$). Linear regression analysis showed a positive linear relationship between male hit rate and the number of neurons in the MPG ($p<0.01$, Figure 28).

We also examined the MPG of newly purchased non-treated rats to confirm these correlations between male sexual behavior and the neuron number of the MPG. However, we did not find any significant correlations in the non-PCB treated rats. Further analyses revealed that the distribution of cell numbers in the PCB-treated group

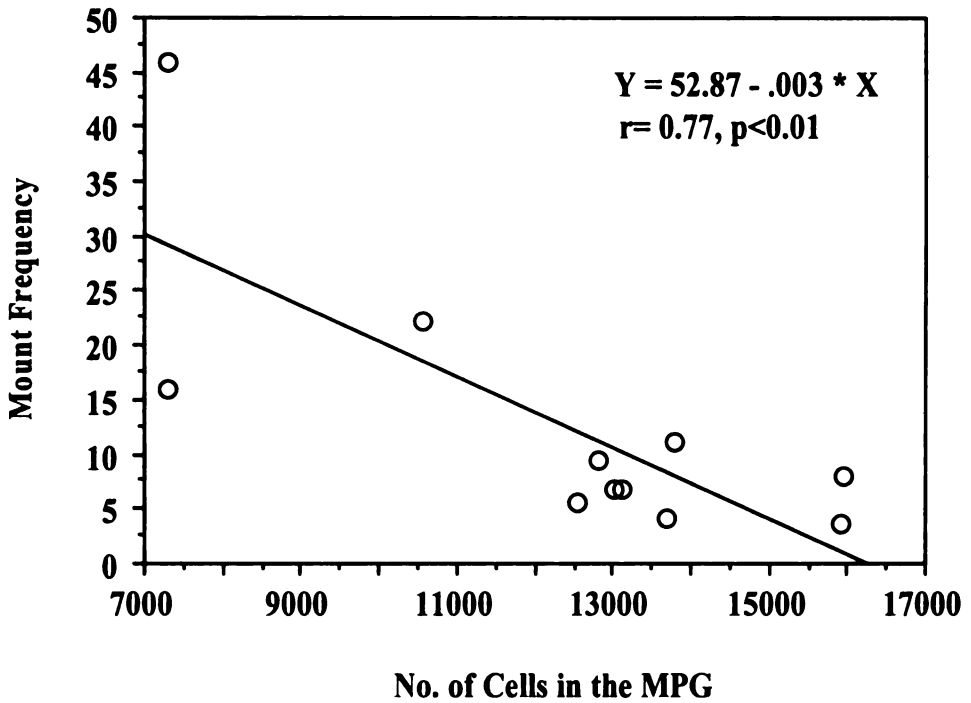

Figure 24. Linear relation between male mount frequency and the number of cells in the major pelvic ganglia (MPG). Correlation coefficient = 0.77, $p < 0.01$.

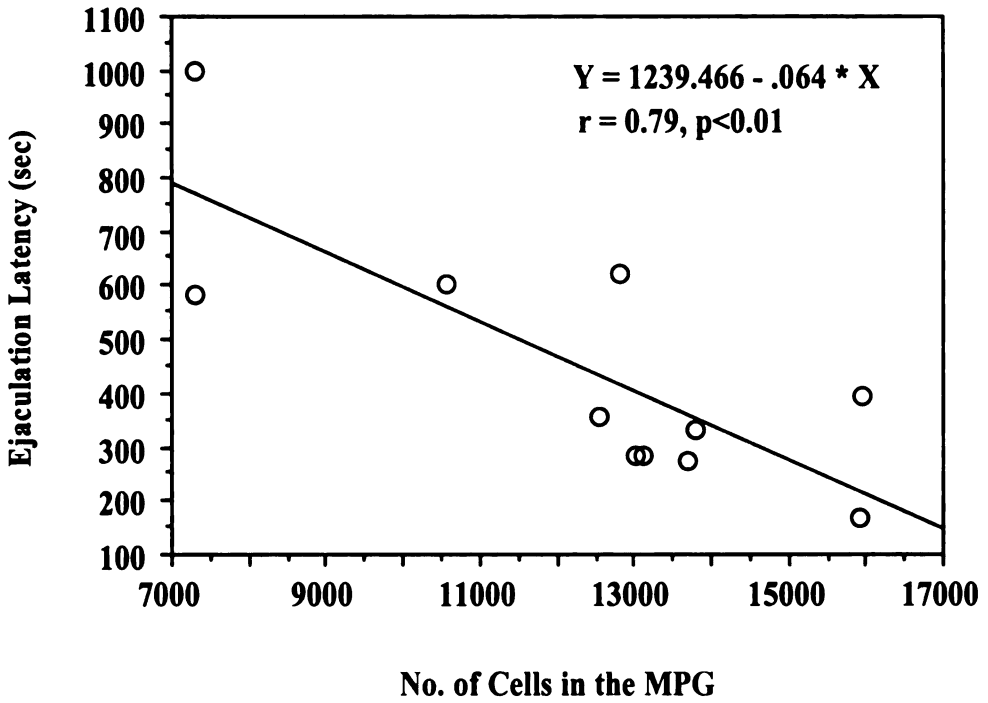

Figure 25. Linear relation between male ejaculation latency and the number of cells in the major pelvic ganglia (MPG). Correlation coefficient = 0.79, $p < 0.01$.

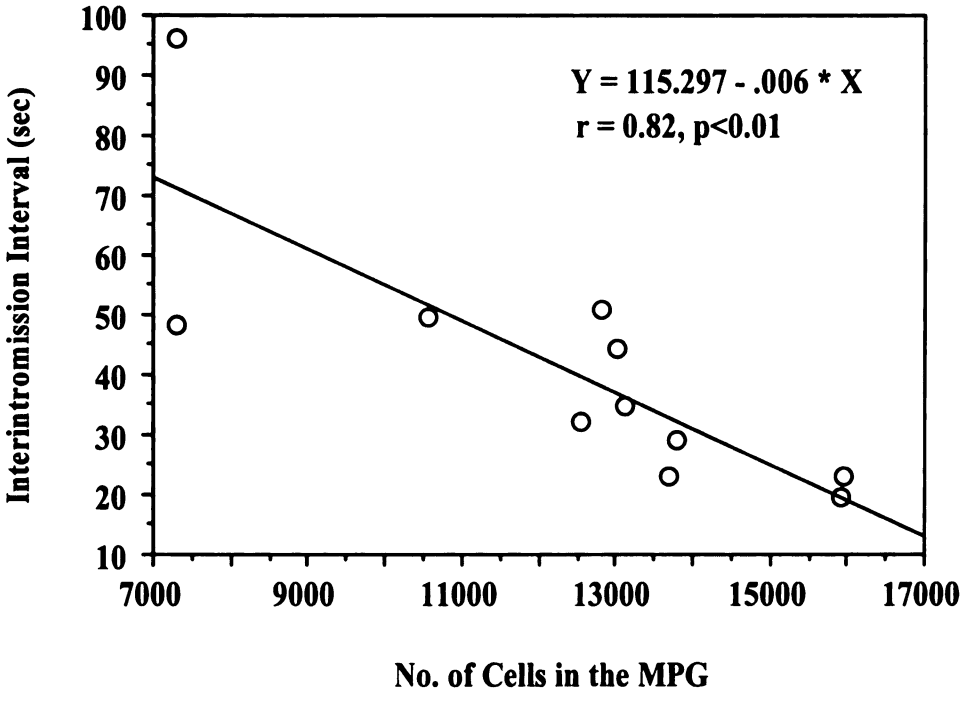

Figure 26. Linear relation between male interintromission interval and the number of cells in the major pelvic ganglia (MPG). Correlation coefficient = 0.82, $p < 0.01$.

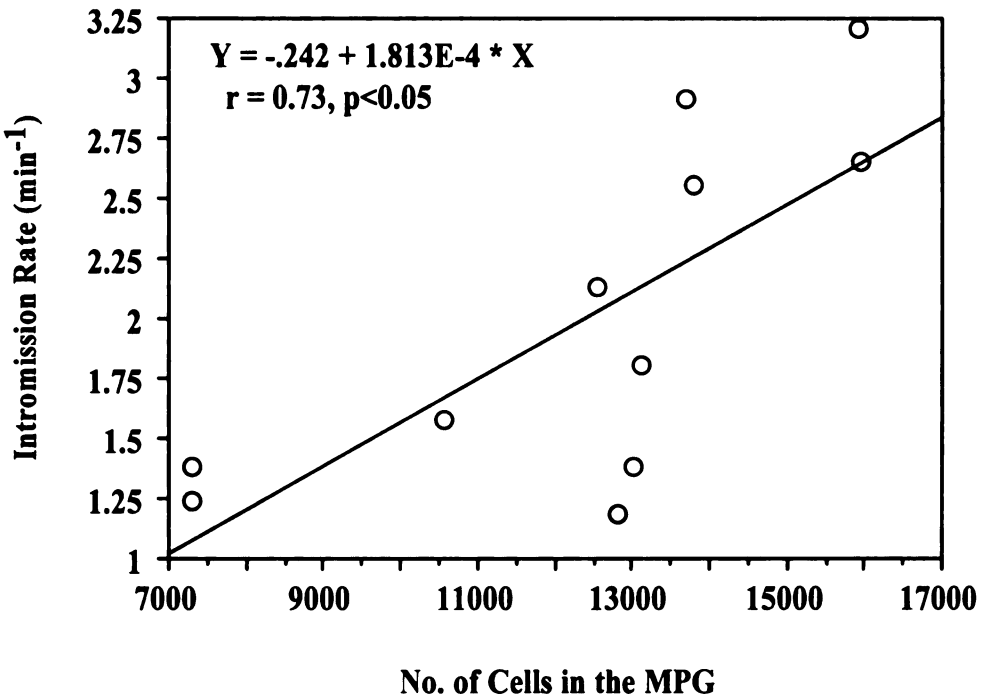

Figure 27. Linear relation between male intromission rate and the number of cells in the major pelvic ganglia (MPG). Correlation coefficient = 0.73, $p < 0.05$.

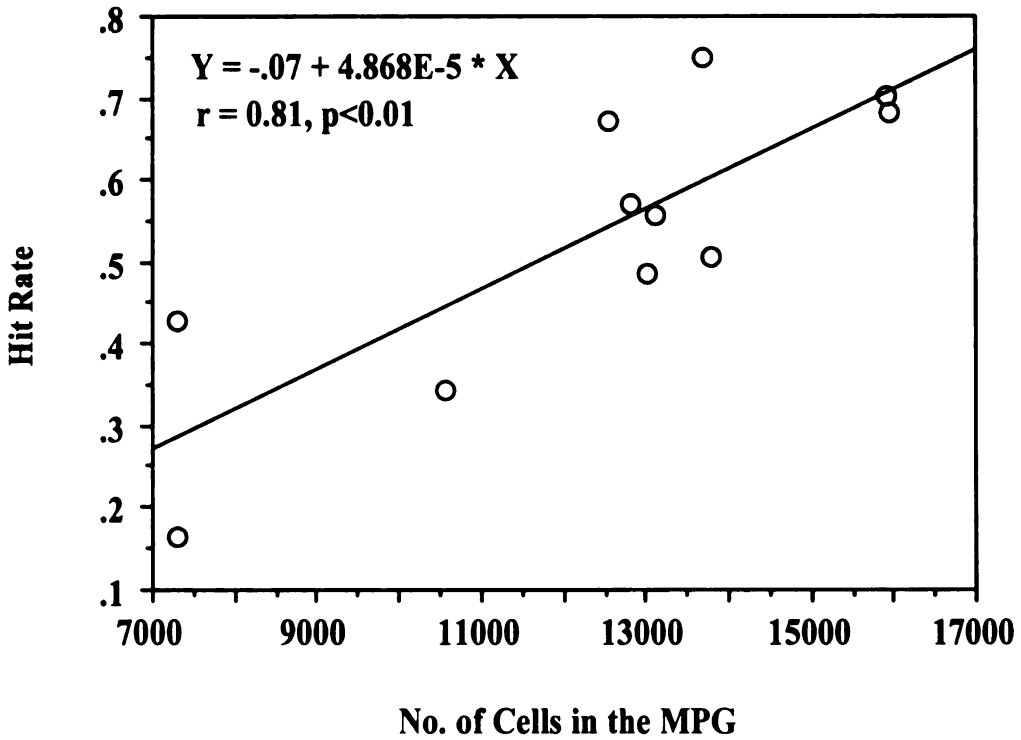

Figure 28. Linear relation between male hit rate and the number of cells in the major pelvic ganglia (MPG). Hit rate = intromission frequency (IF) divided by the sum of mount frequency (MF) and IF [IF/(MF+IF)]. Correlation coefficient = 0.81, $p < 0.01$.

and the untreated group was very different. The F test showed that these two groups in both sexes had significantly different variances ($p<0.0001$). The non-PCB treated rats were more homogeneous than the PCB-treated rats (Figure 29), although the mean number of neurons in the MPG did not differ for the two groups.

Discussion

I was able to reproduce the sex difference in the cell number of the MPG (Greenwood *et al.*, 1985) in the control rats. Moreover, I found that the number of neurons in the MPG was significantly different between copulators and non-copulators within control males. In addition, MPG cell number of PCB-treated rats had significantly greater variance than that of the untreated rats.

One possible explanation for the difference in the MPG was the various somatosensory inputs from their mothers during development. It is well known that lactating rats spend a great deal of time licking the genital area of their male pups which ensures the proper development of male sexual behavior (Moore & Morelli, 1979). Mother rats displayed significantly more anogenital licking towards male pups as opposed to female pups over the first 18 postpartum days (Moore & Morelli, 1979; Richmond & Sachs, 1984). Administering testosterone neonatally to female pups increased the amount of anogenital licking received from mothers (Moore, 1982). Additional experiments suggested that testosterone stimulates the production of a chemosensory cue by the preputial glands. This preputial pheromone is excreted in the urine, and is preferentially ingested by mothers (Moore, 1981; Moore & Samonte, 1986). Depriving rat pups of normal levels of maternal anogenital licking by making mothers anosmic (Moore, 1984) or by applying perfume to pups' anogenital region (Birke &

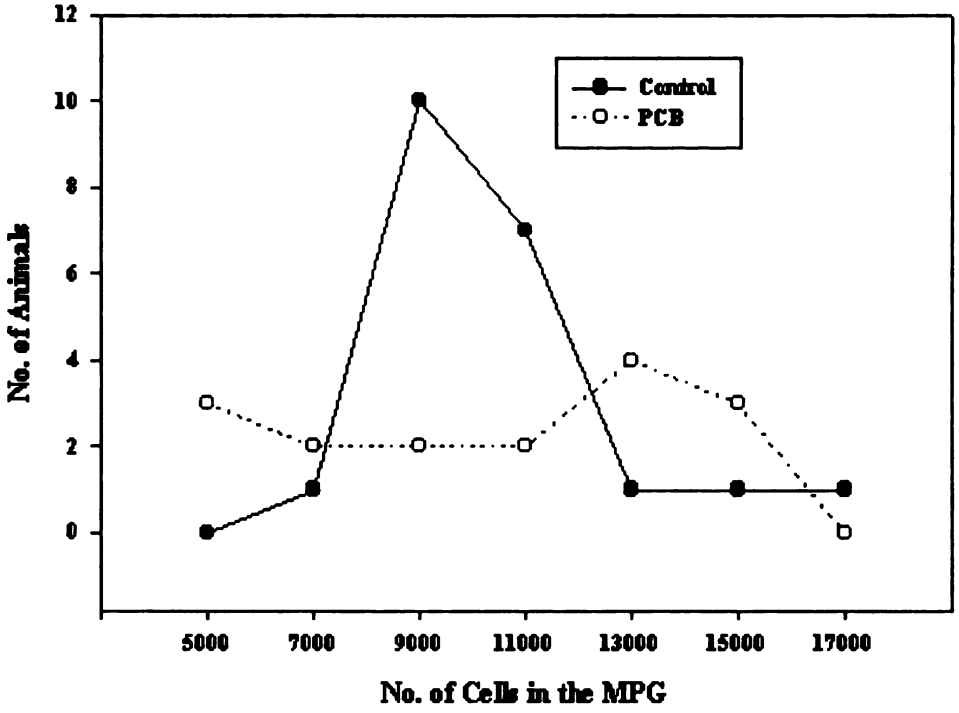

Figure 29. Distribution of the MPG cell number. MPG: major pelvic ganglia;PCB: animals from neonatal A1221 experiment.

Sadler, 1987) led to deficits in later masculine copulatory behavior of both male and female offspring. These behavioral deficits were correlated with a significant reduction in the number of motor neurons in the sexually dimorphic spinal nucleus of the bulbocavernosus muscle in male rats whose mothers were made anosmic over the first 14 postpartum days (Moore *et al.*, 1992). It is also possible that the deficits of male sexual behavior are also correlated with a significant reduction in the number of neurons in the MPG.

Males deprived of maternal anogenital licking later displayed significantly longer ejaculation latencies and longer interintromission intervals than controls (Moore, 1984). We found that longer ejaculation latency and longer interintromission intervals are correlated with fewer neurons in the MPG of PCB-treated animals. It is possible that maternal anogenital licking is correlated with the development of the MPG. Possibly noncopulators do not receive enough anogenital licking thus resulting in under-development of the MPG. Since reproduction is not essential to animal survival, it is likely that the neurons in the MPG of the noncopulators were largely those innervating the colon or bladder while those neurons innervating the penis for erectile mechanisms may well be reduced. More somatosensory inputs by neonatal maternal anogenital licking may induce more neuronal growth and/or prevent the neuronal death in the MPG during the development, hence the more neurons in the MPG and more neurons innervating the penis resulting in better performance in male sexual behavior. However, further investigation would be needed to draw such conclusions.

The possible explanation for the large variance in PCB-treated rats is that PCBs disrupt the maternal recognition of male rats by disrupting testosterone metabolism (Derr and Dekker, 1979) in the male pups or by masking the maternal recognition of their

urine. Alternatively, PCBs *per se* may disrupt the development of the MPG. It is known that tyrosine hydroxylase (TH), DOPA decarboxylase, and choline acetyltransferase (CAT) activities in the MPG are significantly reduced by postnatal castration on day 10-11. Testosterone and dihydrotestosterone replacement reversed all developmental enzyme- activity deficits (Melvin & Hamill, 1987 & 1989). In castrated animals, estrogen therapy reversed the deficits in CAT activity, but was ineffective in reversing the alterations in TH activity (Melvin & Hamill, 1989). Neonatal Aroclor 1221 treatment may disrupt the balance of gonadal hormones and cause the variation in the cell number of the MPG. Another alternative is that the PCB affected the mothers' behavior directly.

I found correlations among five measures of male sexual behavior and the neuron number in the MPG. The most important correlation is probably the relation between the hit rate and the MPG cell count. That result indicates that all the correlations we found may be due to the ability to achieve penile erection. Rats with fewer MPG neurons had more unsuccessful attempts (mounts), copulated slower and took a longer time to achieve ejaculation than rats with more MPG neurons. A brief review of the physiology of penile erection may provide insight into those correlations.

Three autonomic centers participate in the innervation of the penis (Figure 30). The sacral cord gives rise to preganglionic parasympathetic fibers that travel in the pelvic nerve to innervate ganglion cells in the pelvic plexus. This is the major pathway responsible for penile vasodilation. Although the mechanism of action is uncertain, the neurotransmitters of the postganglionic neurons are acetylcholine, vasoactive intestinal peptide (VIP) and nitric oxide (NO) (Burnett *et al.*, 1992; Dail, 1993). An alternate pathway for penile vasodilation is centered in the lumbar cord. This sympathetic pathway reaches penile neurons in the pelvic plexus via the hypogastric nerve. Evidence suggests

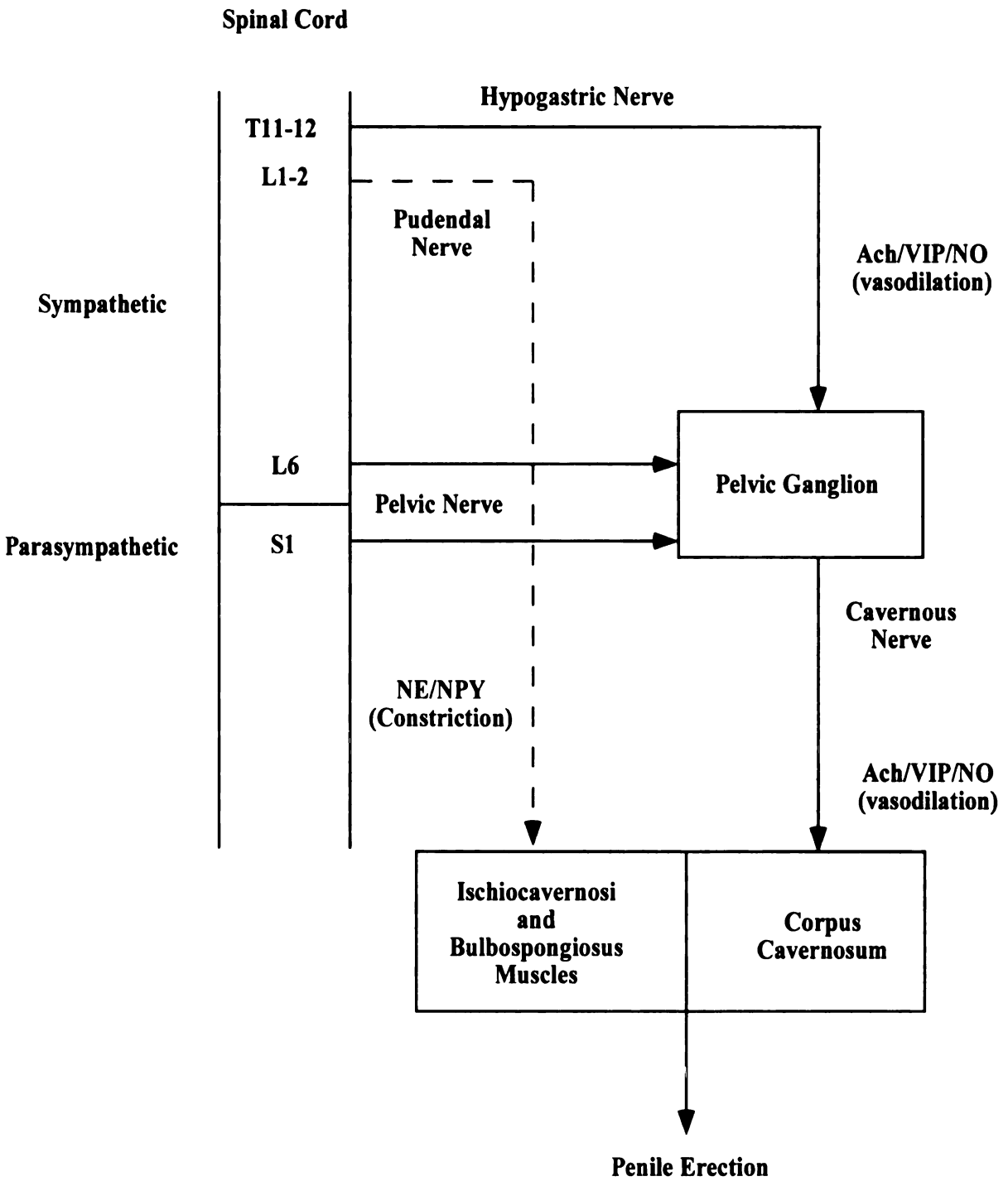

Figure 30. Neural control of penile erection. Ach: acetylcholine; L: lumbar; NE: noradrenaline; NPY: neuropeptide Y; NO: nitric oxide; S: sacral; T: thoracic; VIP: vasoactive intestinal polypeptide.

that this alternate pathway (the supra-sacral erector outflow) is also cholinergic-, VIP-ergic and/or possibly NO-ergic (Alm *et al.*., 1995; Domoto & Tsumori, 1994). Vasoconstriction in the penis is mediated by noradrenergic-neuropeptide Y fibers of sympathetic chain origin. Noradrenergic fibers probably reach the penis via the pudendal nerve. The pudendal nerve, a somatic nerve, innervates skeletal muscle at the base of the penis. Once autonomic mechanisms have increased blood flow to the penis, a further rise in pressure in erectile tissue may be obtained by contraction of the ischiocavernosi and bulbospongiosus muscles (Dail, 1993). Therefore, stimulation of the pudendal nerve or sympathetic trunk resulted in constriction and retraction of the penis of the cat and rabbit (Sjöstrand & Klinge, 1979; Langley & Anderson, 1985), whereas the erectile response could be produced by electrical stimulation of either the pelvic or cavernous nerve (Quinlan *et al.*, 1989).

One possible mechanism involved in the correlation between male sexual behavior and the MPG is that with more MPG neurons, the number of VIP/NO neurons in the MPG was increased resulting in a greater release of VIP/NO during copulation, hence faster and better penile erection. VIP and NO are potent vessel dilators that can facilitate penile erection via vascular mechanisms in the penis (Andersson & Wagner, 1995). In my most recent study, female rats treated with testosterone propriate neonatally had more neurons in the MPG, and the increase in the MPG neurons could be accounted for an increase in VIP neurons. It is possible that male rats with more MPG neurons may have more VIP/NO neurons as well, but further investigation is needed to confirm that. I would expect an even higher correlation between VIP/NO neuron number and male sexual behavior.

One important message from the correlations is that local circuits in the MPG may be sufficient to produce penile erection because the interintromission intervals, intromission rates and hit rates are highly correlated with the neuron number in the MPG. Indeed, the somatic component in the erectile response was not essential because there was no difference between curarized and control rats. Other evidence has shown that testosterone enhances the erectile response of cavernous nerve stimulation and acts peripherally to the spinal cord, but not in the central nervous system. These results all support that a local circuit peripheral to the spinal cord is sufficient and androgen also acts on the local circuit around the MPG.

The sites of testosterone action appear to be situated on neurons rather than on penile erectile tissue, and proerectile postganglionic parasympathetic neurons in the MPG seem to be the likely target sites for gonadal steroids (Giuliano *et al.*, 1993). However, the efferent control from the central nervous system also inhibits the efficiencies of penile erection because pelvic nerve transection increases intromission rate in male rats (Lodder & Zeilmaker, 1976a).

Electric stimulation of the sympathetic preganglionic or postganglionic fibers innervating the genital organs can produce ejaculation without erection (Langley & Anderson, 1985). Tracing studies in cats, rats, dogs, and monkeys have shown that the preganglionic neurons for the MPG are located in the intermediolateral nucleus of the spinal cord and send dendritic projections into areas of laminae V-VII and the dorsal commissure that receive afferent input from the penis. Thus the afferent and efferent components of the reflex pathway are in close proximity. In rats, penile afferents also project into the ventral horn and appear to make contacts with the soma and dendrites of motor neurons. These connections could be involved in the somatic reflex mechanisms

involved in copulation and in reflex responses such as the bulbocavernous reflex (Andersson & Wagner, 1995).

According to the results from PCB-treated male rats, as well as previous reports (Giuliano *et al.*, 1993; Andersson & Wagner, 1995), the MPG seemed to play an important role in penile erection in addition to other basic autonomic functions. Female rats were not expected to see similar results because they lacked the erectile tissues. Since the pelvic plexus innervates the female cervix, vagina and uterine horns, it is more important for the pelvic nerve to carry the afferents responsible for the induction of pseudopregnancy or for all phases of pregnancy, from activation of corpora luteal through and including normal parturition (Carlson & DeFeo, 1965). In this sense, we would expect the intromission return latency or the postejaculatory refractory period of female rats to be more likely related to the number of preganglionic neurons receiving the afferent information. An interesting finding (Berkley *et al.*, 1990) from pelvic nerve recording showed that the rat detected the vaginal stimulus at very low levels of distension by brief orienting movements toward the stimulus at its onset. They made escape responses only when distension levels were great enough to exceed vaginal compliance. This is very similar to the responses of female rats in the female-paced sexual behavior. Females escape from males after receiving vaginal cervical stimuli (intromissions or ejaculations), and the return latency is longer after greater stimulation (Erskine, 1992). In fact, our recent experiment, showed that the VIP neuron number, not the total cell number, in the MPG is highly correlated to female intromission return latency and postejaculatory refractory period (Fang *et al.*, 1998).

I found a significant difference in the variance analysis of the MPG neurons between PCB-treated animals and those not treated with PCBs. It would seem that during

normal development, sex hormones (estrogen or testosterone) act as the developmental restraint to create a homogeneous population with the MPG neuron number of most animals located within a normal range. PCBs, by disrupting sex hormone metabolism or maternal care during development resulted in a heterogeneous population. The increased variance may explain why I was able to find strong correlations between male sexual behavior and the cell counts of the MPG.

CHAPTER 7: GENERAL DISCUSSION

Permanent changes were seen in female sexual behavior after developmental treatment with the PCB mixtures, A1221 or A1254. Perinatal A1221 treatment lowered female receptivity and increased the latency for the female to approach the male during copulation and increased avoidance of the male by the female. Perinatal A1254 treatment, on the other hand, only increased the avoidance component of female-paced sexual behavior. Neonatal A1254 treatment, however, decreased female receptivity as well as the latency for the female to approach the male after receiving an intromission; neonatal A1221 treatment had no effect on these measures. Thus, different PCB mixtures appeared to act during different critical periods in development. The estrogenic A1221 seemed to act prenatally while the nonestrogenic A1254 was disruptive only neonatally.

Some of the changes in female sexual behavior were associated with the disruption of the developing A13 dopaminergic system. Examination of the incertohypothalamic dopaminergic systems revealed a functional brain asymmetry wherein the presence of some dopaminergic cells was only seen following copulatory stimulation. The brain appeared to restructure itself in relation to the environmental context. When the brain was stimulated during copulation, more incertohypothalamic dopaminergic neurons could be recruited to process information and to perform the task.

Further analysis in normal females showed the emergence of neuronal activity in the sexually dimorphic nucleus of the preoptic area (SDN-POA) in sexually active females. This activity coincided with the sensory and temporal factors associated with the release of prolactin and the induction of the progestational state of pregnancy. When

given the opportunity to pace her copulation, the female was able to maximize the effect of copulatory stimulation and thereby facilitate the onset of activity in the SDN-POA.

PCBs also disrupted the development of the major pelvic ganglion (MPG) in the male. Analysis of these changes revealed strong correlations between several measures of male sexual behavior and cell number in the MPG. In the normal male these relations appear more elusive due to the restricted variance in cell number.

Neural Control of Female-Paced Sexual Behavior

Based on the findings reported in the previous chapters, I have formulated a hypothesis concerning the events that bring about the female's timing of copulation (Figure 31). This hypothesis, while preliminary, is summarized below and is followed by a review of existing anatomical and functional studies that lend support to this concept.

Systems Involved in Female-Paced Sexual Behavior: A Working Hypothesis

The incertohypothalamic dopaminergic systems are seen as areas that integrate motor and sensory, as well as limbic information, and play important roles in the coordination of neuroendocrine processes with motor function in female-paced sexual behavior. Such a functional system might act in the following manner. When the female first encounters the male, visual, auditory, and olfactory information are relayed to the zona incerta and the medial preoptic area. The decision to approach or avoid may reflect processes in the medial preoptic area. The zona incerta, with its extensive connections to the brain areas involved in motor functions, enables the female to perform the behavior of approaching or avoiding the male. Sensory information from the first mount or

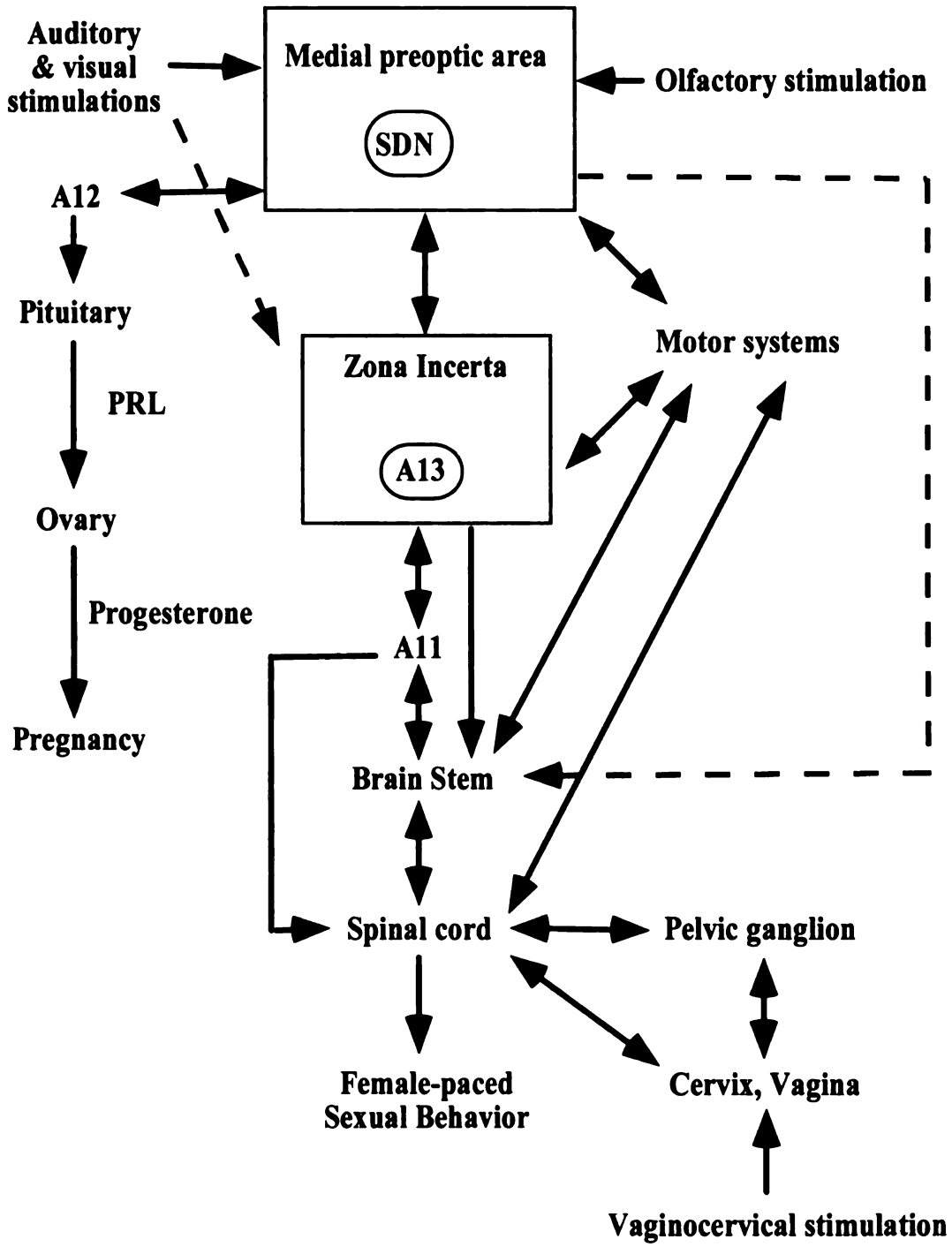

Figure 31. Hypothetical diagram for systems involved in female-paced sexual behavior. PRL: prolactin; SDN: sexually dimorphic nucleus of the preoptic area.

intromission the female receives from the male brings about increased activity in A11 dopaminergic neurons which are associated with modulation of potentially noxious somatic sensation associated with copulation, e.g. vaginal distention beyond compliance (Berkeley *et al.*, 1988). The notion that the MPOA is involved in the decision to approach the male is strengthened by the finding that MPOA has been implicated in the initiation of goal-oriented or motivated behaviors associated with reproduction (Swanson & Mogenson, 1981). Neurons in the MPOA may influence locomotor activity through a direct pathway via caudal regions of the zona incerta, and then continuing on to the pedunculopontine nucleus (a major component of the mesencephalic locomotor region) (Swanson *et al.*, 1987). Lesions of the MPOA have been shown to disrupt the temporal pattern of sexual behavior (Whitney, 1986; Yang & Clemens, 1996) in female rats. It seems clear that a number of goal-oriented behaviors, including female-paced sexual behavior, can be influenced by manipulation of the MPOA (Swanson *et al.*, 1987).

The number of intromissions and their timing can be directly related to activity in the SDN-POA. When ejaculation occurs, I suggest that the sexually dimorphic nucleus of the preoptic area initiates the processes that terminate the estrus, stop the cyclic release of gonadotropin and facilitate prolactin release. PCB treatment during development may disrupt the balance among these systems in a number of ways. While this hypothesis is probably far too simple, in the following section anatomical, physiological and behavioral data will be used to support the main elements of this concept and indicate areas of weakness.

The Anatomy and the Function of Incertohypothalamic Dopaminergic System in Female-paced Sexual Behavior

The incertohypothalamic system is the link between the spinal cord and the medial preoptic area in the hypothesis. It (Figure 32) was first described by Björklund *et al.* in 1975. On the basis of fiber distribution, a caudal and a rostral part can be discriminated: the caudal part extends from the area of the dopamine-containing cell bodies in the caudal thalamus, the posterior hypothalamic area and the medial zona incerta (the A11 and A13 cell groups) into the dorsal part of the dorsomedial nucleus and the dorsal and anterior hypothalamic areas; the rostral part extends from the area of the rostral periventricular dopaminergic cell system (the A14 cell group) into the medial preoptic area and the periventricular and suprachiasmatic preoptic nuclei. The system also extends into the most caudal portion of the lateral septal nucleus. The projection areas of these neurons signify an involvement in the control of pituitary hormone secretion (Björklund *et al.*, 1975). These anatomical data support but do not necessarily prove our hypothesis about the function in gonadotropin and prolactin release.

A11 Dopaminergic Neurons

The A11 dopaminergic neurons are the link between the spinal cord and the zona incerta, and they may modulate the analgesia that occurs during female-paced copulation in our hypothesis. This cell group is known to be the major source of fibers running in the periventricular catecholamine projection system, which innervates, e.g., medial and midline thalamus and several hypothalamic nuclei (Lindvall & Björklund, 1974). These cell bodies are located in the dorsal and posterior hypothalamus, zona incerta, and caudal

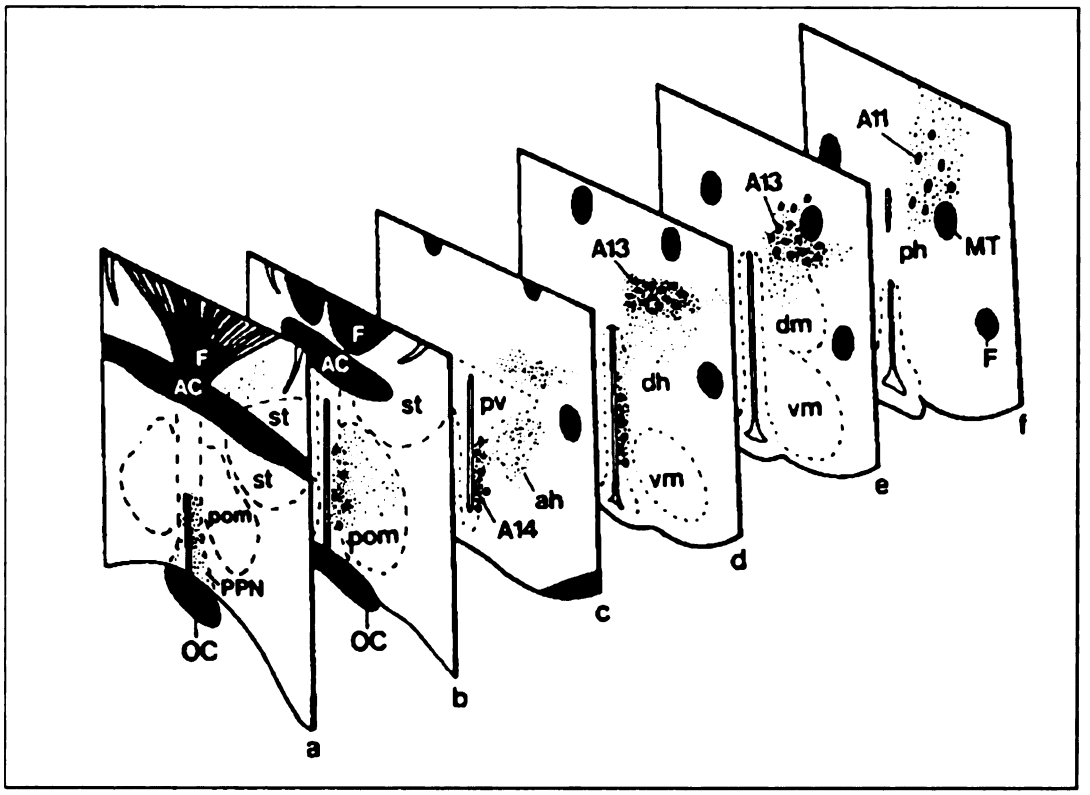

Figure 32. Locations of the cell bodies and terminals of the incertohypothalamic dopaminergic system are schematically depicted on coronal sections. AC: anterior commissure; ah: anterior hypothalamic area; A11, 13, 14: dopaminergic cell groups; dh: dorsal hypothalamic area; dm: dorsomedial nucleus; F: fornix; MT: mammilothalamic tract; OC: optic chiasm; ph: posterior hypothalamus; pom: medial preoptic area; PPN: preoptic periventricular nucleus; pv: paraventricular nucleus; st: bed nucleus of stria terminalis (from Björklund *et al.*, 1975)

thalamus (Figure 33). The A11 fiber system is a component of the dorsal longitudinal fasciculus of Schütz, which is a bidirectional tract interconnecting the lower brain stem and spinal cord with the periaqueductal gray and hypothalamic and thalamic areas.

The A11 dopaminergic projections in the spinal cord are located in the dorsal gray of the cervical, thoracic, lumbar, and sacral cord, and in the intermediate gray of the thoracic cord. In the dorsal horn, the dopaminergic fibers are most abundant in the lateral parts of the superficial layers and in the adjoining reticular nucleus. The highest density of the dopaminergic fibers is found in the intermediolateral cell column and in the area surrounding the central canal at thoracic and upper lumbar levels. This dopaminergic innervation is not uniform along the intermediolateral column but forms clusters or patches of varicose fibers corresponding to the clustering of preganglionic sympathetic neurons along the intermediolateral column (Skagerberg *et al.*, 1982; Lindvall & Björklund, 1983), which indicates important functions for this system involving sympathoexcitatory function (Bernthal & Koss, 1979; Gebber & Snyder, 1979; Simon & Schramm, 1983; Smith & Devito, 1984; Skagerberg & Lindvall, 1985) important for sexual behavior and sperm transport (Carlson & DeFeo, 1965; Erskine, 1992; Traurig & Papka, 1993).

The concentration of dopaminergic terminals in the dorsal horn suggests a modulatory role for the diencephalospinal A11 dopaminergic system in the processing of sensory information, such as pain (Lindvall & Björklund, 1983). Available evidence from studies with various nociceptive tests suggests that spinal dopaminergic mechanisms have an inhibitory effect on noxious input to the spinal cord (Jensen & Smith, 1982, 1983; Jensen & Yaksh, 1984). In fact, stimulation-produced analgesia has been elicited

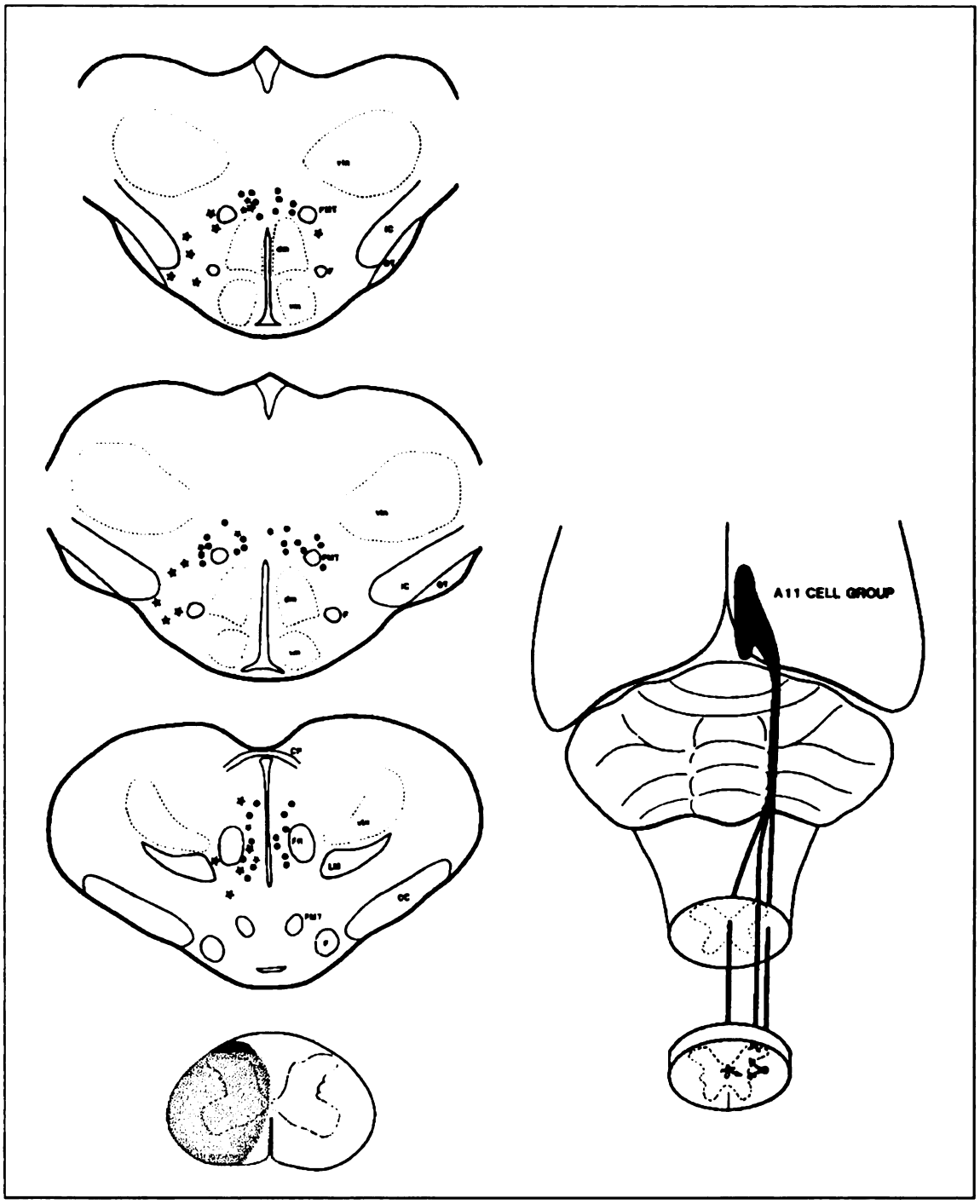

Figure 33. The location of A11 cell bodies and their spinal projections (from Skagerberg *et al.*, 1982).

from part of the area of the A11 cell group (; Rhodes & Liebeskind, 1978; Fleetwood-Walker *et al.*, 1988). Together with the pharmacological data, it has been suggested that the diencephalospinal A11 dopaminergic neurons might constitute an endogenous pain inhibitory system (Skagerberg & Lindvall, 1985), which may be important to sustain female receptivity during copulation.

Zona Incerta and A13 Dopaminergic Neurons

The A13 dopaminergic neurons within the zona incerta are the integration center of motor, sensory and limbic information in the hypothesis. The zona incerta can be subdivided into a ventral zone (ventral sector of pars caudalis and pars ventralis) and a dorsal zone (pars dorsalis). The somesthetic (somatosensory cortex, trigeminal complex, and dorsal column nuclei), collicular, and cerebellar projections terminate at the ventral zone. Limbic input (cingulate cortex), on the other hand, is directed into a dorsal zone. The contingent of somatosensory afferents is relatively large and there is a high degree of overlapping between the different somatosensory terminal fields within the ventral zona incerta. This suggests a participation of zona incerta in the treatment of somesthetic information and/or in the transmission of noxious stimuli (Roger & Cadusseau, 1985; Shammah-Lagnado *et al.*, 1985) during copulation.

The efferent connections of the zona incerta terminate mainly in an ipsilateral system of descending and ascending fibers distributed to reticular structures of the brain stem, precerebellar nuclei, the middle and deep layers of the superior colliculus, the pretectum, perioculomotor, the parvocellular portion of the red nucleus, the central gray substance, the nucleus tegmenti dorsalis lateralis, the ventral horn of the cervical spinal

cord, non-specific thalamic nuclei, basal ganglia, hypothalamic structures and a subpallidal district of the substantia innominata (Ricardo, 1981). Taken together, the zona incerta is the main tecto-subthalamic target regions and it also projects to the superior colliculus (Watanabe & Kawana, 1982; Araki *et al.*, 1984; Kim *et al.*, 1992; Kuenzle, 1996) and ipsilateral cortical fields, including sensory, limbic and association areas, with more incertal neurons projecting to the SI/MI cortices than to any other cortical area. The pathway orients toward the visual or frontal cortex and the projections possibly involved in limbic and circadian mechanisms, and may be important during copulation.

For example, based on the input-output organization of the zona incerta, this nucleus may act as an important integrative center linked to the reticular activating system in the midbrain (MacKenzie *et al.*, 1984), and involved in motor functions including drinking, visual discrimination, locomotion, muscular response to morphine and posture (Sinnamon, 1984; Mogenson & Wu, 1986; Nakanishi *et al.*, 1992). In addition, it is also important in the control of nociception (Wardas *et al.*, 1987). Therefore, the zona incerta may be involved in the motor component of female-paced sexual behavior. Electrical stimulation of the zona incerta leads to motor responses (Kaelber & Smith, 1979). Microelectrode recording in the monkey disclosed the existence of zona incertal units that respond to passive limb manipulations and to visual stimuli, are activated when the animal reaches for objects of interest and fire during the performance of a visuomotor tracking task (Crutcher *et al.*, 1980). Other evidence suggests that the zona incerta might be a link in pathways involved in the transfer of visual information to motor circuits (Legg, 1979). Considering the anatomical afferents

of zona incerta, the information about limb movement might reach the zona incerta via the projections from the dorsal column nuclei, whereas visual input could conceivably be conveyed to zona incerta by way of pathways originating from the ventral lateral geniculate nucleus, the visual areas of the cerebral cortex and the pretectum. In the context of motor integration, zona incerta receives substantial projections from the primary motor and retrosplenial cortices, cerebellum as well as apparently minor projections from other motor-related structures, such as the entopeduncular and red nuclei. Considering zona incertal efferents, they can be traced to several motor-related structures, such as precerebellar nuclei, the nucleus tegmenti pedunculopontinus pars compacta, the ventral horn of the spinal cord, many brain stem territories that send fibers to the spinal cord, both segments of the globus pallidus and the thalamic nuclei whose efferents reach the motor cortex; even a sparse direct projection from zona incerta to the primary motor cortex has been suggested (Jackson & Crossman, 1981; Ricardo, 1981; Roger & Cadusseau, 1985; Shammah-Lagnado *et al.*, 1985).

Zona incerta may be involved in the escape component of female-paced sexual behavior. Electrical stimulation of zona incerta in cats leads to typical escape responses (Kaelber & Smith, 1979); moreover, zona incertal units in the cat can be driven by noxious stimuli over wide body regions, whereas monkeys will work to terminate electrical stimulation of zona incerta (Berkley, 1980). These functional observations suggest the involvement of the zona incerta in nociceptive behavior which is, in part, important during copulation. Indeed, several structures which serve as sources of zona incertal afferents have been implicated in pain mechanisms. These structures include the periaqueductal gray substance, the trigeminal sensory complex, the nucleus reticularis

gigantocellularis, the nucleus raphe magnus and dorsalis, the pretectum, the superior colliculus and the reticular nucleus of the thalamus (Shammah-Lagnado *et al.*, 1985).

The zona incerta may also be involved in the motivational component of female-paced sexual behavior. Apparently, the diffusely organized projection from zona incerta to the cerebral cortex and spinal cord may play a role in the general arousal associated with a variety of motivated behaviors, such as self-stimulation and sexual behavior (Köhler *et al.*, 1984). Indeed, afferent projections to points of self-stimulation in the medial prefrontal cortex of the rat include the zona incerta (Vives *et al.*, 1983). Subthalamic lesions centered on the caudal zona incerta just dorsal to the subthalamic nucleus eliminated sexual behavior in males (Maillard & Edwards, 1988). Subthalamic lesions produced no obvious impairment in locomotion, posture, limb use, muscle tone or sensory motor orientation. Even so, the fact that electrical stimulation of the subthalamus elicits coordinated stepping suggests that the region is linked with systems directly concerned with movement and locomotion. These links could be particularly important in the process by which sexual motivation is translated into sexual behavior (Maillard & Edwards, 1988). In addition, the zona incerta may be involved in the initiation of female-paced sexual behavior since it is involved in the initiation of locomotor activity (Milner & Mogenson, 1988) and the generation of orienting movements (Kim *et al.*, 1992).

A13 Dopaminergic neurons with perikarya (Dahlström & Fuxe, 1964) located in the rostral portion of the medial zona incerta have been implicated in a variety of functions including the regulation of gonadotropin secretion (Wilkes *et al.*, 1979; MacKenzie *et al.*, 1984; James *et al.*, 1987; Sanghera *et al.*, 1991). Indeed, the extensive projections of A13 dopaminergic neurons were identified in hypothalamic areas

important for gonadotropin secretion (Wagner *et al.*, 1995).

The Medial Preoptic Nucleus and Female-paced Sexual Behavior

The medial preoptic nucleus acts as a coordinating unit for controlling sexual behavior and reproduction in the hypothesis. It plays an important neuroendocrine role since it has extensive reciprocal connections to almost all parts of the periventricular zone of the hypothalamus, including the anteroventral periventricular nucleus, anterior part of the periventricular, paraventricular and arcuate nuclei, and contains receptors for estrogen, progesterone and testosterone. Its involvement in the autonomic mechanisms is indicated by the connections to dorsal and lateral parvicellular parts of the paraventricular nucleus, the parabrachial nucleus, and the nucleus of the solitary tract. Other connections of the medial preoptic nucleus suggest participation in the initiation of specific motivated behaviors. For example, connections to the ventromedial and dorsomedial hypothalamic nuclei may be related to the control of reproductive and ingestive behaviors, respectively. The execution of these behaviors may involve activation of somatomotor regions via connections to the substantia innominata, zona incerta, ventral tegmental area, and pedunculopontine nucleus. Connections to other regions that project directly to the spinal cord, such as the periaqueductal gray, the laterodorsal tegmental nucleus, certain medullary raphe nuclei and the magnocellular reticular nucleus may also be involved in modulating somatic and/or automonic reflexes. Finally, the medial preoptic nucleus may influence a wide variety of physiological mechanisms and behaviors (including female sexual behavior) through its massive connections to the ventral part of the lateral septal nucleus, the bed nucleus of the stria

terminalis, the lateral hypothalamic area, the supramammillary nucleus, and the ventral tegmental area, all of which have extensive connections with regions along the medial forebrain bundle (Simerly & Swanson, 1988).

Future Research Directions

To better understand the mechanisms of PCB toxicity in the dopaminergic neurons during development, pure PCB congeners should be used. The comparison between pure coplanar and ortho-substituted PCBs is especially important since they may have diverse effects. The antagonistic or synergistic effects between coplanar and ortho-substituted PCBs are of equal significance.

To determine the critical period, prenatal, neonatal, adult as well as perinatal treatments are equally important since the same compound may have different effects in each developmental stage because of different sets of molecules and different cellular environment triggered in each developmental step may result in various outcomes.

The effect of PCBs on A14 dopaminergic neurons should be examined because of their vicinity to the MPOA and possible sexual dimorphism of these dopaminergic neurons. A12 dopaminergic neurons should be examined as well because of its involvement in the control of pituitary function. The effect of PCBs on A9 and A10 dopaminergic systems and their relation to sexual behavior are also worth tackling. The efferent areas of A9 and A10, the striatum and nucleus accumbens, are especially important since they are involved in female paced sexual behavior and the effect of PCBs on these sites may be related to the behavioral changes.

The ontogeny of the incertohypothalamic dopaminergic system is virtually unknown. Its involvement in the sexual differentiation and the control of sexual behavior

needs further investigation. The sex difference in the susceptibility of this system to PCBs is also interesting. The sex difference in the induction of TH-IR in the incertohypothalamic dopaminergic system by copulation is an exciting finding that calls for further investigation. Finally, the suggestion that the SDN is a dynamic, context-dependent nucleus needs further examination and testing.

APPENDIX

Table 1. The effect of perinatal Aroclor 1221 (A1221) treatment on female sexual behavior and A11 or A13 dopaminergic neurons.

	Control (sesame oil)	A1221 (5 mg)	A1221 (15 mg)
Approach Latency (sec)	7.594±0.983 (n=8)	22.719±7.149[b] (n=8)	3.792±0.568[c] (n=6)
Mount Return Latency (sec)	12.375±2.568 (n=8)	15.363±1.959 (n=8)	8.067±0.772 (n=6)
Intromission Return Latency (sec)	18.950±2.670 (n=8)	36.938±8.772[a] (n=8)	11.133±1.602[c] (n=6)
Postejaculatory Refractory Period (sec)	58.350±13.900 (n=8)	85.238±13.014 (n=8)	61.983±9.881 (n=6)
Percentage of Mount Leave	36.676±4.824 (n=8)	35.628±6.615 (n=8)	27.736±5.341 (n=6)
Percentage of Intromission Leave	55.040±7.129 (n=8)	73.104±2.754[a] (n=8)	49.466±4.554[c] (n=6)
Lordosis Quotient	83.125±5.083 (n=8)	48.125±8.014[b] (n=8)	44.167±15.352[b] (n=6)
A11 TH-IR Neurons (total)	1020.6±101.0 (n=5)	1274.0±91.1 (n=3)	876.0±105.0 (n=5)
A11 TH-IR Neurons (major)	545.4±52.2 (n=5)	686.0±16.5 (n=3)	464.4±58.0 (n=5)
A11 TH-IR Neurons (minor)	475.2±52.6 (n=5)	588.0±74.8 (n=3)	411.6±48.9 (n=5)
A13 TH-IR Neurons (total)	2898.000±419.006 (n=5)	2321.000±435.087 (n=3)	2409.600±199.082 (n=5)
A13 TH-IR Neurons (major)	1609.200±233.566 (n=5)	1302.000±211.544 (n=3)	1302.600±132.308 (n=5)
A13 TH-IR Neurons (minor)	1288.800±189.011 (n=5)	1019.000±224.007 (n=3)	1107.000±76.703 (n=5)

Data were represented as mean±S.E.
[a]: significantly different from control ($p<0.05$)
[b]: significantly different from control ($p<0.01$)
[c]: significantly different from 5 mg group ($p<0.01$)
TH-IR: tyrosine hydroxylase immunoreactive

Table 2. The effect of perinatal Aroclor 1254 (A1254) treatment on female sexual behavior and A11 or A13 dopaminergic neurons.

	Control (sesame oil)	A1254 (5 mg)	A1254 (15 mg)
Approach Latency (sec)	7.594±0.983 (n=8)	6.583±0.823 (n=6)	7.333±1.794 (n=5)
Mount Return Latency (sec)	12.375±2.568 (n=8)	11.350±2.010 (n=6)	9.880±2.805 (n=5)
Intromission Return Latency (sec)	18.950±2.670 (n=8)	27.433±4.167 (n=6)	17.000±3.044 (n=5)
Postejaculatory Refractory Period (sec)	58.350±13.900 (n=8)	74.883±11.858 (n=6)	80.000±11.312 (n=5)
Percentage of Mount Leave	36.676±4.824 (n=8)	57.250±3.880[a] (n=6)	55.763±8.437[a] (n=5)
Percentage of Intromission Leave	55.040±7.129 (n=8)	77.873±4.771[b] (n=6)	77.701±4.990[b] (n=5)
Lordosis Quotient	83.125±5.083 (n=8)	78.333±6.412 (n=6)	92.000±2.550 (n=5)
A11 TH-IR Neurons (total)	1020.600±101.049 (n=5)	N.D.	835.500±252.813 (n=4)
A11 TH-IR Neurons (major)	545.400±52.207 (n=5)	N.D.	451.500±121.814 (n=4)
A11 TH-IR Neurons (minor)	475.200±52.604 (n=5)	N.D.	411.600±48.886 (n=4)
A13 TH-IR Neurons (total)	2898.000±419.006 (n=5)	N.D.	2488.500±560.727 (n=4)
A13 TH-IR Neurons (major)	1609.200±233.566 (n=5)	N.D.	1353.750±330.262 (n=4)
A13 TH-IR Neurons (minor)	1288.800±189.011 (n=5)	N.D.	1134.750±241.344 (n=4)

Data were represented as mean±S.E.
N.D.: not determined
[a]: significantly different from control ($p<0.05$)
[b]: significantly different from control ($p<0.01$)
TH-IR: tyrosine hydroxylase immunoreactive

Table 3. The effect of neonatal Aroclor 1254 (A1254) treatment on female sexual behavior.

	Control (sesame oil)	A1254 (2.5 mg)	A1254 (5 mg)
Approach Latency (sec)	7.754±0.933 (n=15)	9.600±2.968 (n=15)	5.445±0.885 (n=15)
Mount Return Latency (sec)	9.882±0.960 (n=15)	11.591±1.441 (n=15)	12.273±3.359 (n=15)
Intromission Return Latency (sec)	24.729±5.557 (n=15)	14.469±1.655* (n=15)	12.863±1.014* (n=15)
Postejaculatory Refractory Period (sec)	52.643±6.822 (n=15)	43.896±4.676 (n=15)	41.635±4.447 (n=15)
Percentage of Mount Leave	39.353±4.157 (n=15)	40.643±4.584 (n=15)	32.480±3.920 (n=15)
Percentage of Mount Stay	60.647±4.157 (n=15)	59.357±4.584 (n=15)	67.520±3.920 (n=15)
Percentage of Interintromission Leave	61.178±4.669 (n=15)	51.895±4.031 (n=15)	64.127±4.422 (n=15)
Percentage of Interintromission Stay	38.822±4.669 (n=15)	48.105±4.031 (n=15)	35.873±4.422 (n=15)
Lordosis Quotient (Nonpacing)	85.779±2.444 (n=15)	40.223±4.730* (n=15)	56.444±8.122* (n=15)
Lordosis Quotient (Pacing)	84.757±3.652 (n=15)	52.684±5.657** (n=15)	60.383±5.630** (n=15)

Data were represented as mean±S.E.
* significantly different from control ($p<0.05$)
** significantly different from control ($p<0.001$)

Table 4. The effect of neonatal Aroclor 1221 (A1221) treatment on female sexual behavior.

	Control (sesame oil)	A1221 (2.5 mg)	A1221 (5 mg)
Approach Latency (sec)	8.690±1.373 (n=14)	7.474±0.857 (n=13)	7.679±0.776 (n=14)
Mount Return Latency (sec)	11.697±1.788 (n=14)	21.288±9.641 (n=13)	7.606±1.070 (n=14)
Intromission Return Latency (sec)	18.912±2.503 (n=14)	16.802±1.581 (n=13)	13.470±2.191 (n=14)
Postejaculatory Refractory Period (sec)	43.143±7.416 (n=14)	62.077±6.855 (n=13)	45.631±4.792 (n=14)
Percentage of Mount Leave	30.947±7.583 (n=14)	50.031±6.858 (n=13)	29.181±5.577 (n=14)
Percentage of Mount Stay	69.053±7.583 (n=14)	49.969±6.858 (n=13)	70.819±5.577 (n=14)
Percentage of Interintromission Leave	52.712±6.091 (n=14)	67.882±6.757 (n=13)	62.976±5.207 (n=14)
Percentage of Interintromission Stay	47.288±6.091 (n=14)	32.118±6.757 (n=13)	37.024±5.207 (n=14)
Lordosis Quotient (Nonpacing)	81.429±5.108 (n=14)	91.795±2.155 (n=13)	84.286±3.346 (n=14)
Lordosis Quotient (Pacing)	74.048±3.697 (n=14)	80.976±2.588 (n=13)	76.976±3.439 (n=14)

Data were represented as mean±S.E.

Table 5-1. The effect of adult polychlorinated biphenyl treatment on female sexual behavior.

	Test	Control (sesame oil)	Aroclor 1254 (2.5 mg)	Aroclor 1254 (5 mg)
Approach Latency (sec)	Pre	3.833±0.548 (n=12)	5.583±1.819 (n=12)	4.417±0.543 (n=12)
	Post	4.750±0.986 (n=12)	3.556±0.818 (n=9)	5.083±1.300 (n=12)
Mount Return Latency (sec)	Pre	6.291±1.401 (n=11)	10.500±1.734 (n=10)	4.550±0.673 (n=10)
	Post	6.955±1.013 (n=11)	9.814±3.394 (n=7)	9.064±1.453 (n=11)
Intromission Return Latency (sec)	Pre	7.208±0.768 (n=12)	12.717±2.449 (n=12)	10.317±1.727 (n=12)
	Post	13.917±4.709 (n=12)	23.700±4.951 (n=9)	16.433±3.097 (n=12)
Postejaculatory Refractory Period (sec)	Pre	24.917±6.543 (n=12)	34.083±8.670 (n=12)	34.833±6.292 (n=12)
	Post	77.917±22.586 (n=12)	54.889±14.690 (n=9)	71.000±10.392 (n=12)
Percentage of Mount Leave	Pre	31.284±4.781 (n=12)	46.875±8.777 (n=12)	44.701±9.401 (n=12)
	Post	48.592±8.828 (n=12)	42.200±15.049 (n=7)	50.190±7.940 (n=12)
Percentage of Interintromission Leave	Pre	62.615±8.385 (n=12)	63.469±5.493 (n=12)	51.899±6.598 (n=12)
	Post	71.267±7.246 (n=12)	72.610±6.140 (n=9)	82.124±6.103 (n=12)
Lordosis Quotient (Nonpacing)	Pre	70.833±7.732 (n=12)	80.000±7.177 (n=12)	74.167±7.120 (n=12)
	Post	87.500±4.286 (n=12)	79.167±4.994 (n=12)	80.833±6.088 (n=12)
Lordosis Quotient (Pacing)	Pre	82.083±5.641 (n=12)	84.364±2.909 (n=11)	79.273±3.628 (n=11)
	Post	83.250±4.085 (n=12)	87.091±6.231 (n=11)	82.182±3.858 (n=11)

Data were represented as mean±S.E.

Table 5-2. The effect of adult polychlorinated biphenyl treatment on female sexual behavior (continued).

		Control (sesame oil)	Aroclor 1221 (2.5 mg)	Aroclor 1221 (5 mg)
Approach Latency (sec)	Pre	3.833±0.548 (n=12)	6.167±1.392 (n=12)	5.833±1.272 (n=12)
	Post	4.750±0.986 (n=12)	4.583±1.033 (n=12)	4.083±0.633 (n=12)
Mount Return Latency (sec)	Pre	6.291±1.401 (n=11)	15.700±4.992 (n=10)	6.909±1.112 (n=11)
	Post	6.955±1.013 (n=11)	9.627±1.636 (n=11)	7.709±1.258 (n=11)
Intromission Return Latency (sec)	Pre	7.208±0.768 (n=12)	21.008±6.764 (n=12)	10.291±2.339 (n=11)
	Post	13.917±4.709 (n=12)	16.083±4.914 (n=12)	13.583±2.990 (n=12)
Postejaculatory Refractory Period (sec)	Pre	24.917±6.543 (n=12)	30.000±9.823 (n=12)	32.417±5.562 (n=12)
	Post	77.917±22.586 (n=12)	52.273±12.857 (n=11)	47.250±11.058 (n=12)
Percentage of Mount Leave	Pre	31.284±4.781 (n=12)	44.789±10.365 (n=11)	39.417±7.309 (n=12)
	Post	48.592±8.828 (n=12)	56.717±10.023 (n=12)	49.478±5.120 (n=11)
Percentage of Interintromission Leave	Pre	62.615±8.385 (n=12)	55.492±7.680 (n=12)	54.425±9.300 (n=12)
	Post	71.267±7.246 (n=12)	78.620±5.555 (n=12)	70.502±6.954 (n=12)
Lordosis Quotient (Nonpacing)	Pre	70.833±7.732 (n=12)	76.667±5.551 (n=12)	79.167±6.450 (n=12)
	Post	87.500±4.286 (n=12)	75.000±6.455 (n=12)	80.000±6.629 (n=12)
Lordosis Quotient (Pacing)	Pre	82.083±5.641 (n=12)	82.000±3.238 (n=12)	71.583±4.458 (n=12)
	Post	83.250±4.085 (n=12)	83.583±5.610 (n=12)	78.500±5.479 (n=12)

Data were represented as mean±S.E.

Table 6. The effect of neonatal Aroclor 1221 (A1221) treatment on A11 and A13 dopaminergic neurons.

	Control (sesame oil)	A1221 (2.5 mg)	A1221 (5 mg)
A11 TH-IR Neurons (total)	903±138.818 (n=4)	1086.4±59.351 (n=5)	861.333±256.794 (n=3)
A11 TH-IR Neurons (major)	483.000±69.902 (n=4)	596.800±47.521 (n=5)	461.333±138.974 (n=3)
A11 TH-IR Neurons (minor)	435.000±57.093 (n=4)	489.600±17.463 (n=5)	400.000±118.006 (n=3)
A11 Asymmetric Index1	1.110±0.044 (n=4)	1.219±0.081 (n=5)	1.143±0.041 (n=3)
A11 FOS/TH-IR Neurons (major)	97.000±28.862 (n=4)	82.400±16.570 (n=5)	178.667±52.679 (n=3)
A11 FOS/TH-IR Neurons (minor)	81.000±15.610 (n=4)	105.600±26.126 (n=5)	162.667±68.521 (n=3)
A11 Asymmetric Index 2	1.165±0.170 (n=4)	0.998±0.264 (n=5)	1.403±0.489 (n=3)
Ratio of A11 FOS/TH-IR (major)	19.182±3.283 (n=4)	14.754±3.910 (n=5)	45.327±14.044 (n=3)
Ratio of A11 FOS/TH-IR (minor)	18.265±1.653 (n=4)	21.280±4.958 (n=5)	42.353±18.383 (n=3)
A13 TH-IR Neurons (total)	1855.000±324.433 (n=4)	1790.400±168.236 (n=5)	1474.667±485.443 (n=3)
A13 TH-IR Neurons (major)	1075.000±180.742 (n=4)	1214.400±163.387 (n=5)	886.667±266.827 (n=3)
A13 TH-IR Neurons (minor)	780.000±158.114 (n=4)	576.000±72.078 (n=5)	588.000±223.762 (n=3)
A13 Asymmetric Index1	1.415±0.142 (n=4)	2.218±0.339 (n=5)	1.691±0.357 (n=3)
A13 FOS/TH-IR Neurons (major)	275.000±48.232 (n=4)	230.400±21.858 (n=5)	188.000±70.086 (n=3)
A13 FOS/TH-IR Neurons (minor)	188.000±51.562 (n=4)	123.200±17.591 (n=5)	182.667±49.817 (n=3)
A13 Asymmetric Index 2	1.638±0.241 (n=4)	1.940±0.158 (n=5)	1.077±0.258 (n=3)
Ratio of A13 FOS/TH-IR (major)	25.693±2.052 (n=4)	19.910±2.505 (n=5)	20.380±1.388 (n=3)
Ratio of A13 FOS/TH-IR (minor)	23.035±2.465 (n=4)	21.328±1.576 (n=5)	33.813±5.747 (n=3)

Data were represented as mean±S.E.
Asymmetric Index 1 = TH-IR Neurons (Major) / TH-IR Neurons (Minor)
Asymmetric Index 2 = [FOS/TH-IR Neurons (Major)] / [FOS/TH-IR Neurons (Minor)]
TH-IR: tyrosine hydroxylase immunoreactive

Table 7. The effect of neonatal Aroclor 1254 (A1254) treatment on A11 and A13 dopaminergic neurons.

	Control (sesame oil)	A1254 (2.5 mg)	A1254 (5 mg)
A11 TH-IR Neurons (Total)	609.000±129.207 (n=4)	688.000±115.703 (n=5)	643.200±153.745 (n=5)
A11 TH-IR Neurons (Major)	389.000±61.911* (n=4)	389.600±58.468 (n=5)	358.400±79.162 (n=5)
A11 TH-IR Neurons (Minor)	220.000±67.469 (n=4)	298.400±61.013 (n=5)	284.800±75.274 (n=5)
A11 Asymmetric Index 1	2.111±0.443 (n=4)	1.432±0.217 (n=5)	1.673±0.459 (n=5)
A11 FOS/TH-IR Neurons (Major)	58.000±13.216* (n=4)	60.000±23.152 (n=5)	61.600±12.998 (n=5)
A11FOS/TH-IR Neurons (Minor)	26.000±11.372 (n=4)	46.400±13.775 (n=5)	41.600±10.852 (n=5)
A11 Asymmetric Index 2	2.081±0.504 (n=4)	2.094±1.000 (n=5)	1.652±0.208 (n=5)
Ratio of A11 FOS/TH-IR (Major)	14.600±1.257 (n=4)	16.732±7.856 (n=5)	17.246±1.635 (n=5)
Ratio of A11 FOS/TH-IR (Minor)	14.465±8.881 (n=4)	14.091±3.693 (n=5)	16.744±2.092 (n=5)
A13 TH-IR Neurons (Total)	1214.000±200.749 (n=4)	1096.000±212.119 (n=4)	1258.000±362.018 (n=4)
A13 TH-IR Neurons (Major)	975.000±227.569* (n=4)	641.000±82.662 (n=4)	721.000±203.098 (n=4)
A13 TH-IR Neurons (Minor)	239.000±32.099 (n=4)	455.000±130.705 (n=4)	543.000±158.420 (n=4)
A13 Asymmetric Index 1	4.687±1.741 (n=4)	2.004±0.724 (n=4)	1.363±0.113 (n=4)
A13 FOS/TH-IR Neurons (Major)	256.000±28.752* (n=4)	81.000±20.290 (n=4)	251.000±67.278 (n=4)
A13 FOS/TH-IR Neurons (Minor)	81.000±6.191 (n=4)	74.000±42.411 (n=4)	138.000±36.679 (n=4)
A13 Asymmetric Index 2	3.251±0.508 (n=4)	1.807±0.510 (n=4)	1.845±0.135 (n=4)
Ratio of A13 FOS/TH-IR (Major)	28.602±4.197 (n=4)	14.182±5.056 (n=4)	35.138±2.872 (n=4)
Ratio of A13 FOS/TH-IR (Minor)	34.850±2.783 (n=4)	23.823±12.409 (n=4)	25.933±2.206 (n=4)

Data were represented as mean±S.E.
Asymmetric Index 1 = TH-IR Neurons (Major) / TH-IR Neurons (Minor)
Asymmetric Index 2 = [FOS/TH-IR Neurons (Major)] / [FOS/TH-IR Neurons (Minor)]
The major side is significantly different from the minor side ($p<0.05$)
TH-IR: tyrosine hydroxylase immunoreactive

Table 8. Time course of A13 FOS and tyrosine hydroxylase immunoreactivities.

Treatment	No. of TH-IR cells (major)	No. of TH-IR cells (minor)	No. of FOS/TH-IR cells (major)	No. of FOS/TH-IR cells (minor)
Control (n=4)	453 ± 42	408 ± 47	52 ± 9	59 ± 14
FP-10 min (n=3)	977 ± 17[ac]	828± 40[c]	155 ± 13[a]	149 ± 42[a]
FP-30 min (n=6)	739 ± 101[bc]	641 ± 108[bc]	109 ± 11[ab]	95 ± 16[bc]
FP-1 h (n=5)	1167 ± 133[a]	1050 ± 134[a]	173 ± 30[a]	171 ± 25[a]
MP-10 min (n=3)	832 ± 42[c]	696 ± 37[c]	85 ± 35[bcd]	65 ± 9[bcd]
MP-30 min (n=6)	899 ± 120[ac]	744 ± 148[c]	103 ± 8[bc]	105 ± 9[bc]
MP-1 h (n=4)	1421 ± 247[a]	1287 ± 216[a]	158 ± 17[a]	175 ± 22[a]

Data were represented as mean±S.E.
[a]: significantly different from the control ($p<0.05$)
[b]: significantly different from FP-1 h ($p<0.05$)
[c]: significantly different from MP-1 h ($p<0.05$)
[d]: significantly different from FP-10 min ($p<0.05$)
FP: Female-paced
MP: Male-paced
TH-IR: tyrosine hydroxylase immunoreactive

Table 9. Time course of A11 FOS and tyrosine hydroxylase immunoreactivities.

Treatment	No. of TH-IR cells (major)	No. of TH-IR cells (minor)	No. of FOS/TH-IR cells (major)	No. of FOS/TH-IR cells (minor)
Control (n=4)	575 ± 45	441 ± 50	118 ± 20	98 ± 28
FP-10 min (n=3)	689 ± 19	645 ± 20	172 ± 10[c]	149 ± 24
FP-30 min (n=6)	527 ± 30	469 ± 15	73 ± 15[bc]	63 ± 20[bc]
FP-1 h (n=5)	716 ± 49	616 ± 94	238 ± 39[a]	206 ± 53
MP-10 min (n=3)	684 ± 45	637 ± 29	101 ± 17[bc]	84 ± 17[c]
MP-30 min (n=6)	563 ± 50	456 ± 73	69 ± 16[bc]	43 ± 13[bc]
MP-1 h (n=4)	677 ± 106	567 ± 77	334 ± 96[a]	284 ± 87[a]

Data were represented as mean±S.E.
[a]: significantly different from the control ($p<0.05$)
[b]: significantly different from FP-1h ($p<0.05$)
[c]: significantly different from MP-1h ($p<0.05$)
[d]: significantly different from FP-10 min ($p<0.05$)
FP: Female-paced
MP: Male-paced
TH-IR: tyrosine hydroxylase immunoreactive

Table 10. Time course of FOS immonoreactive cells in the medial preoptic nucleus.

Treatment	Plate 20	Plate 21	Sum of Plate 18 to Plate 22
Control (n=3)	74 ± 2	311 ± 25	758 ± 85
FP-10 min (n=5)	204 ± 65[c]	421 ± 97[c]	1144 ± 242[c]
FP-30 min (n=3)	254 ± 68[ad]	681 ± 133[ad]	1717 ± 301[ad]
FP-1 h (n=8)	267 ± 37[a]	471 ± 52	1369 ± 108
MP-10 min (n=6)	116 ± 12[bc]	353 ± 57[c]	928 ± 128[bc]
MP-30 min (n=3)	196 ± 52[c]	468 ± 54	1410 ± 307
MP-1 h (n=8)	323 ± 13[a]	686 ± 83[ab]	1815 ± 133[ab]

Data were represented as mean±S.E.
[a]: significantly different from the control ($p<0.05$)
[b]: significantly different from FP-1 h ($p<0.05$)
[c]: significantly different from MP-1 h ($p<0.05$)
[d]: significantly different from MP-10 min ($p<0.05$)
FP: Female-paced
MP: Male-paced

Table 11. A11 FOS and tyrosine hydroxylase immunoreactivities under various sexual stimulation.

Treatment	No. of TH-IR cells (major)	No. of TH-IR cells (minor)	No. of FOS/TH-IR cells (major)	No. of FOS/TH-IR cells (minor)
Control (n=4)	575± 45	441 ± 50	118 ± 20	98 ± 28
FP-1E (n=3)	689 ± 19[a]	645 ± 20	172 ± 10	149 ± 24
FP-2E (n=3)	621± 91[b]	539 ± 100	164 ± 101	153 ± 118
FP-3E (n=3)	539 ± 46[b]	516 ± 55	136 ± 78	111 ± 63
FP-4E (n=3)	595 ± 61[b]	557 ± 52	128 ± 37	129 ± 39
MP-1E (n=3)	684 ± 45[b]	637 ± 29	101 ± 17	84 ± 17
MP-2E (n=3)	629± 38[a]	564 ± 23	52 ± 13	41 ± 16
MP-3E (n=3)	535 ± 51[b]	419 ± 110	123 ± 10	79 ± 22
MP-4E (n=4)	615 ± 123[a]	413 ± 113	224 ± 107	162 ± 93

Data were represented as mean±S.E.
[a]: significantly different from the control ($p<0.05$)
[b]: significantly different from MP-4E ($p<0.05$)
E: ejaculatory series
FP: Female-paced
MP: Male-paced
TH-IR: tyrosine hydroxylase immunoreactive

Table 12. A13 FOS and tyrosine hydroxylase immunoreactivities under various sexual stimulation.

Treatment	No. of TH-IR cells (major)	No. of TH-IR cells (minor)	No. of FOS/TH-IR cells (major)	No. of FOS/TH-IR cells (minor)
Control (n=4)	453 ± 42	408 ± 47	52 ± 9	59 ± 14
FP-1E (n=3)	977 ± 17	828 ± 40	155 ± 13	149 ± 42
FP-2E (n=3)	847 ± 195	752 ± 221	119 ± 7	112 ± 22
FP-3E (n=3)	877 ± 217	769 ± 214	127 ± 31	91 ± 24
FP-4E (n=3)	892 ± 255	804 ± 223	159 ± 59	167 ± 55
MP-1E (n=3)	832 ± 42b	696 ± 37	85 ± 35	65 ± 9
MP-2E (n=3)	1063 ± 176[a]	967 ± 217[a]	117 ± 6	121 ± 5
MP-3E (n=3)	640 ± 22[b]	504 ± 98[b]	120 ± 37	133 ± 45
MP-4E (n=4)	1353 ± 203[a]	1113 ± 221[a]	141 ± 22	139 ± 23

Data were represented as mean±S.E.
[a]: significantly different from the control ($p<0.05$)
[b]: significantly different from MP-4E ($p<0.05$)
E: ejaculatory series
FP: Female-paced
MP: Male-paced
TH-IR: tyrosine hydroxylase immunoreactive

Table 13. FOS-immunoreactive cells in the medial preoptic nucleus under various sexual stimulation.

Treatment	Plate 20	Plate 21	Plate 22	Sum of Plate 18 to Plate 22
Control (n=3)	74 ± 2	311 ± 25	282 ± 42	758 ± 85
FP-1E (n=5)	204 ± 65	421 ± 97[bc]	309 ± 64[bc]	1144 ± 242[bc]
FP-2E (n=4)	228 ± 47	402 ± 78[bc]	350 ± 135[bc]	1237 ± 152[bc]
FP-3E (n=3)	287 ± 101[ad]	544 ± 61	258 ± 25[bc]	1456 ± 204
FP-4E (n=3)	320 ± 4[ad]	714 ± 120[a]	618 ± 86[a]	1948 ± 204[a]
MP-1E (n=6)	116 ± 12[bc]	353 ± 57[bc]	240 ± 51[bc]	928 ± 128[bc]
MP-2E (n=3)	176 ± 33	468 ± 54	297 ± 63[bc]	1185 ± 90[bc]
MP-3E (n=3)	255 ± 16[ad]	559 ± 58	352 ± 70[bc]	1608 ± 288[ad]
MP-4E (n=4)	311 ± 21[a]	727 ± 151[a]	674 ± 92[a]	1968 ± 206[a]

Data were represented as mean±S.E.
[a]: significantly different from the control ($p<0.05$)
[b]: significantly different from FP-4E ($p<0.05$)
[c]: significantly different from MP-4E ($p<0.05$)
[d]: significantly different from MP-1E ($p<0.05$)
E: ejaculatory series
FP: Female-paced
MP: Male-paced

Table 14. The effect of perinatal Aroclor 1254 (A1254) treatment on male sexual behavior.

	Control (sesame oil)	A1254 (5 mg)	A1254 (15 mg)
Mount Latency (sec)	11.836±3.675 (n=14)	10.880±2.868 (n=5)	15.838±5.492 (n=8)
Intromission Latency (sec)	22.664±5.572 (n=14)	19.780±3.428 (n=5)	53.963±20.445 (n=8)
Ejaculation Latency (sec)	435.421±48.348 (n=14)	560.360±79.972 (n=5)	512.925±109.679 (n=8)
Postejaculatory Interval (sec)	322.200±15.059 (n=14)	338.720±18.857 (n=5)	359.850±21.460 (n=8)
Mount Frequency	13.957±1.538 (n=14)	12.360±1.836 (n=5)	14.688±3.605 (n=8)
Intromission Frequency	13.971±1.121 (n=14)	12.160±0.851 (n=5)	13.575±1.073 (n=8)
Inter-intromission Interval (sec)	31.771±2.496 (n=14)	49.600±5.022* (n=5)	39.075±6.717 (n=8)
Intromission Rate (min^{-1})	2.073±0.144 (n=14)	1.347±0.081* (n=5)	2.026±0.365 (n=8)

Data were represented as mean±S.E.
significantly different from control ($p<0.05$)

Table 15. The effect of neonatal Aroclor 1254 (A1254) treatment on male sexual behavior.

	Control (sesame oil)	A1254 (2.5 mg)	A1254 (5 mg)
Mount Latency (sec)	9.760±1.806* (n=32)	9.500±1.867* (n=32)	17.438±3.838 (n=32)
Intromission Latency (sec)	45.333±17.440 (n=32)	28.031±11.530 (n=32)	32.750±9.389 (n=32)
Ejaculation Latency (sec)	493.021±38.194 (n=32)	489.771±51.251 (n=32)	401.750±30.274 (n=32)
Postejaculatory Interval (sec)	388.250±22.003 (n=12)	365.667±20.683 (n=12)	302.450±9.116 (n=20)
Mount Frequency	11.875±1.113 (n=32)	15.594±2.590 (n=32)	8.625±0.962 (n=32)
Intromission Frequency	9.760±1.806 (n=32)	9.500±1.867 (n=32)	17.438±3.838 (n=32)
Inter-intromission Interval (sec)	12.250±0.687 (n=28)	12.357±0.891 (n=28)	10.917±0.802 (n=12)
Intromission Rate (min^{-1})	1.986±0.164 (n=24)	2.254±0.301 (n=20)	1.698±0.147 (n=12)

Data were represented as mean±S.E.
* significantly different from A1254 5 mg group ($p<0.05$)

Table 16. There is no effect of neonatal Aroclor 1221 (A1221) treatment on male sexual behavior.

	Control (sesame oil)	A1221 (2.5 mg)	A1221 (5 mg)	Untreated
Mount Latency (sec)	17.0±6.3 (n=12)	10.4±1.6 (n=12)	9.9±1.3 (n=12)	3.8±0.1 (n=21)
Intromission Latency (sec)	55.1±22.0 (n=12)	19.7±3.9 (n=12)	46.8±21.5 (n=12)	15.1±4.6 (n=21)
Ejaculation Latency (sec)	542.7± 109.4 (n=12)	413.5±66.6 (n=12)	578.3±136.0 (n=11)	480.4±66.7 (n=21)
Postejaculatory Interval (sec)	378.3±25.2 (n=12)	328.4±11.9 (n=12)	374.0 ±31.2 (n=11)	355.2±11.4 (n=21)
Mount Frequency	15.9±3.0 (n=12)	12.8±3.5 (n=12)	16.1±4.4 (n=11)	11.4±2.0 (n=21)
Intromission Frequency	11.1±1.1 (n=12)	11.2±0.9 (n=12)	12.4±0.8 (n=11)	10.7±0.6 (n=21)
Inter-intromission Interval (sec)	48.7±8.2 (n=12)	38.5±6.7 (n=12)	48.1±13.4 (n=11)	43.8±4.5 (n=21)
Intromission Rate (min^{-1})	1.6±0.2 (n=12)	2.2±0.3 (n=12)	1.9±0.2 (n=11)	1.7±0.1 (n=21)
Hit Rate	0.4±0.0 (n=12)	0.5±0.1 (n=12)	0.5±0.0 (n=11)	0.6±0.0 (n=21)

Data were represented as mean±S.E.
Note: The data of untreated rats are included but cannot be used to compare with A1221-treated rats because of different breeding condition and homogeneity (F test $p<0.0001$).

Table 17. There is no effect of neonatal Aroclor 1221 (A1221) treatment on the cell number of the major pelvic ganglia.

	Control (sesame oil)	A1221 (2.5 mg)	A1221 (5 mg)	Untreated
Male	10783.3±94.0 (n=6)	13038.6±1102.4 (n=7)	12084.0±1546.6 (n=5)	11430.5± 616.9 (n=21)
Female	3251.7±304.5 (n=6)	6244.0±1791.1 (n=5)	3708.0± 442.3 (n=5)	2938.3±186.2 (n=8)

Data were represented as mean±S.E.
Note: The data of untreated rats are included but cannot be used to compare with A1221-treated rats because of different homogeneity (F test $p<0.0001$).

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