NEUROENDOCRINE BASIS OF THE SEX DIFFERENCES IN THE SOCIAL PLAY OF JUVENILE NORWAY RATS

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A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfillment of the Requirements

for the degree of Doctor of Philosophy at

Concordia University

Montreal, Quebec, Canada

August 1981

Abstract

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Among juvenile Norway rats, as in many species of primates, there are sex differences in social play behaviour. Juvenile males engage in more play-fighting (or rough-and-tumble play) than do their female peers. In this thesis are presented the results of six experiments designed to investigate the neuroendocrine basis of this sex difference in the social play of Norway rat pups. From the time of weaning animals were housed in mixed-sex, peer-groups of six to eight , composed of some treated and some untreated animals. Observations were made of these animals in these groups each day between Days 26 and 40 of life in Experiments 1,3,4,5, and 6, and between Days 31 and 40 in Experiment 2. In Experiment 1 it was found that males castrated on Day 1 of life engaged in less play-fighting than did intact males, and did not differ from normal females. In Experiment 2

castration carried out at 23 days of age had no effects on the frequency with which males engaged in play-fighting. In Experiment 3 it was found that neonatal ovariectomy had no effect on the frequency with which female pups engaged in play-fighting. In Experiment 4, females treated on Days 1 and 2 of life with either 250 ug of testosterone propionate or 250 ug of dihydrotestosterone engaged in play-fighting at rates that were comparable to those of normal males, whereas treatment with 5 ug of estradiol benzoate had no such effect. In Experiment 5 it was found that neither the reduction of testosterone-derived estradiol (by implants of the aromatization blocker, androst-1,4,6,-triene-3,17-dione) nor that of testosterone-derived dihydrotestosterone (by implants of the 5 α-reductase blocker, testosterone 17β-carboxylic acid) during the early neonatal period (Days 1 to 10) changed the frequency of play-fighting in intact males. The results of these experiments indicate that the sex difference in the social play of prepubertal Norway rats is dependent upon the neonatal exposure to testosterone or its 5 α-reduced metabolite, dihydrotestosterone. The reduction of testosterone to dihydrotestosterone, however, would not appear to be a necessary step. The final experiment was designed to examine the effects of lesions of the amygdala, a prominent neuroendocrine control area, on the play-fighting of prepubertal males and females. Amygdaloid lesions, made on Days 21 or 22, suppressed play-fighting in males rats to

levels that were indistinguishable from those of females. In contrast to males, amygdaloid lesions had no effect on the play-fighting of females. Androgen-dependent sex differences in the anatomy and physiology of the amygdala are discussed as a possible explanation of its apparent differential influence on the social play of male and female prepubertal Norway rats.

ACKNOWLEDGEMENTS

Experiments 1 through 5 were supported by a grant from the Natural Sciences and Engineering Research Council of Canada to Dr. Jane Stewart. Experiment 6 was supported by grant HD 12620-01 from NICHHD to Dr. William W. Beatty. The author was supported by a predoctoral fellowship from the Ministere de l'Education, Province de Quebec.

It occurs to me that the problems associated with writing an acknowledgements section are not to be underestimated. First, you must identify what has actually contributed to your ability to complete a doctoral dissertation and, second, you must somehow assess the magnitude of that contribution. These problems notwithstanding, this section offers a rare opportunity for self-indulgence and I plan to make the most of it (as you can probably already gather).

Given that this paper is concerned with behavioural development, it seems appropriate to begin with some reference to my parents, Lawrence and Dorothy Meaney. This is certainly a reasonable choice, since if anyone can justifiably claim to have kindled my interest in education it is my parents. However long and silly my arguments may have been they listened (no mean feat, I assure you), and forever encouraged me to think for myself. With love and appreciation

I acknowledge their efforts.

Authors (I mean real authors) have spent the better portions of their lives in an attempt to describe the feelings I hold for the following two people: Gloria Waters (friend and lover) and Jane Stewart (friend and teacher). It is somehow the talent of such people to make even the most common moment seem memorable, the most tedious task, tolerable, if not enjoyable, and to awaken an appreciation of quality, my own talents (and faults) included. My skills are simply not adequate to describe at length their contribution without succumbing to phrases that are embarassingly cliched. These are people to whom careers, and not merely theses, are dedicated.

In addition, it has been my good fortune to work in a lab with fellow graduate students Rudy Eikelboom and David Sandberg. People who were equally capable of constructive criticism and friendly support, and sensitive enough to know when one and not the other was needed. This is a rare talent.

Thanks also to Bill Beatty for a rather remarkable summer of collaboration at North Dakota State University. No finer a compliment can be paid to a host than to say that they made spending 4 months in Fargo, North Dakota an extremely pleasant experience. In this regard thanks also to the folks at NDSU (Pat Beatty, Jerry Holzer, Ron Roller, Tony and Laura Dodge, Kathy Traylor, Russ Glasgow, Akela, Otter, Natasha, Lighia, and Cindor). Special thanks to Jerry and Ron

for introducing me to American beer(?), frisbee golf, the badlands, and behavioural ecology.

For the past 6 years I have worked on the 10th and 11th floor in the Psychology Department. This area is occupied predominantly by students in physiological psychology; people whose dedication to good research is matched only by their complete lack of respect for sane and "proper" social behaviour; who could ask for more. To David Sinyor, Lorne Switzman, George Foriezos, Ron Skelton, Cathy Bielajew, Susan Schenk, Rodney John, Ivan Kiss, Dale Corbett, Lawrence Sklar, Rick Blair, Franc Rogan, Steve Vallentyne, Carol Washer, Nicole Milhomme, Gabi Galler, Paule Poulin, and to 5th floor refugees Patrica Baker and Steve Stober, it's been fun. (Thanks to David Sinyor for his help in preparing this thesis.)

Thanks also to Kali Sakell, administrative secretary (a.k.a. the real boss), for her help in rescuing me from the jaws of the bureaucracy on more than one occasion.

I would also like to acknowledge the very instructive comments and encouragement of Drs. Barbara Woodside and Lisa Serbin in the preparation of this thesis; and to Dr. Robert Goy, my external examiner, for provocative comments and encouragement.

These are times when university education is viewed with considerable cynicism, and not without reason. The people mentioned above, however, each in their way, have made my

studies the type of experience that only the most idealistic of educators have envisioned.

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NEUROENDOCRINE BASIS OF THE SEX DIFFERENCES IN THE SOCIAL PLAY OF JUVENILE NORWAY RATS

Sex differences have been described in the social behaviour of several social-living mammalian species. These sex differences appear to be related to a sex-dependent division of labour in reproductive behaviour and territorial defense, as well as to differences in the formation of dominance hierarchies. In several primate species, particularly the well-studied terrestrial monkeys (e.g. the rhesus, Japanese, and vervet monkeys, and several species of baboons), the care of infants is performed primarily by the adult females, while territorial defense appears to be the domain of the adult males (e.g. Eaton, 1976; Gartlan, 1968; Kummer, 1968). In primate troops females assume a rank that is usually dependent on the rank of the mother (i.e. a dependent rank), whereas males establish their rank independent of the status of their mother (i.e. basic rank; see Kawai, 1958; Sade, 1967).

Interestingly, in these and related species there are also sex differences in the social behaviour of the juveniles. Juvenile males engage in more play-fighting than do females (see Table 1), while juvenile females engage in more social grooming (e.g. Owens, 1975), and in some species,

Table 1

A Summary of Studies of Sex Differences in Play-fighting (♂ > ♀ = studies in which males were observed to engage in play-fighting more frequently than were females; ♂ ≈ ♀ = studies in which males and females were observed to engage in play-fighting with similar frequencies. Play-fighting has never been observed to occur more often in females than in males).

Species Studied	Finding ♂ > ♀	♂ ≈ ♀
Apes		
Humans (*Homo sapiens*)		Blurton-Jones (1972); Braggio, Nadler, Lance & Miseyko (1978)
Chimpanzees (*Pan troglodytes*)		van Lawick-Goodall (1968)
Gorilla (*Gorilla gorilla*)		Freeman & Alcock (1973)
Orangutan (*Pongo pymaeus*)		Nadler & Braggio (1974)
Old World Monkeys		
Crab-Eating monkey (*Macaca cynomologous*)		Seay, Schlottman & Gandolfo (1972)

Table 1 (Cont'd)

Species Studied	Finding ♂ > ♀	♂ = ♀
Old World Monkeys		
Bonnet monkey (*M. radiata*)	Simonds (1974)	
Barbary ape (*M. sylvanus*)	Burton (1972); MacRoberts (1970)	
Pigtail monkey (*M. nemestrina*)	Bernstein (1967)	
Rhesus monkey (*M. mulatta*)	Harlow (1969); Harlow & Lauersdorf (1974); Hinde & Spencer-Booth (1967);	
Stumptail monkey (*M. arctoides*)	Bertrand (1976)	
Olive baboon (*Papio anubis*)	Chalmers (1980); Owens (1975a)	
Hamadryas baboon (*P. hamadryas*)	Kummer (1968)	

Table 1 (Cont'd)

Species Studied	Finding ♂ > ♀	Finding ♂ ≈ ♀
Old World Monkeys		
Vervet monkey (*Cercopithecus aethiops*)	Bramlett (1978); Raleigh, Flannery & Ervin (1979)	
Sykes monkey (*C. albogularis*)	Dolan (cited in Mitchell 1979)	
Talapoin monkey (*C. talapoin*)	Wolfheim (1977)	
Patas monkey (*Erythrocebus patas*)	Bramlett (1973); Seay et al. (1972)	
Hanuman langur (*Presbytis entellus*)	Hrdy (1977); Jay (1963)	
Lutong langur (*P. cristatus*)	Bernstein (1968)	
Nilgiri langur (*P. johnii*)	Poirer (1972)	

Table 1 (Cont'd)

Species Studied	Finding ♂ > ♀	Finding ♂ = ♀
New World Monkeys		
Squirrel monkey (*Saimiri sciureus*)	Baldwin & Baldwin (1974)	
Common marmoset (*Callithrix jacchus*)	Abbott (1978)	
Canids		
Beagle (*Canis familiaris*)		Bekoff (1974)
Coyote (*C. latrans*)		Bekoff (1974)
Timber wolf (*C. lupis*)		Bekoff (1974)
Felidae		
Domestic cat (*Felis catus*)		Barrett & Bateson (1978)

Table 1 (Cont'd)

Species Studied	Finding ♂ > ♀	Finding ♂ ≈ ♀
Rodentia		
Norway rat (*Rattus norvegicus*)	Meaney & Stewart (1981a; in press); Olioff & Stewart (1978); Poole & Fish (1976)	
Golden hamster (*Mesocricetus auratus*)	Goldman & Swanson (1975)	
Ungulate		
Domestic sheep (*Ovis aries*)	Sachs & Harris (1978)	
Pinnipod		
Stellar sea lion (*Eumetopias stelleri*)	Gentry (1974)	

Table 2

A Summary of Studies of Sex Differences in Play-Mothering (♀ > ♂ = studies in which females were observed to engage in play-fighting more frequently than were males; ♀ ≈ ♂ = studies in which males and females were observed to engage in play-mothering with similar frequencies).

	Finding	
Species Studied	♀ > ♂	♀ ≈ ♂
Apes		
Humans	Berman (1980, for a review)	
Old World Monkeys		
Rhesus monkeys	Chamove, Harlow & Mitchell (1967)	
Olive baboons	Owens (1975a)	
Vervet monkeys	Gartlen (1968); Lancaster (1971)	
Barbary ape		Burton (1972)
New World Monkeys		
Squirrel monkeys	Baldwin (1969)	

females engage in more play-mothering (see Lancaster, 1971). These sex differences in the social behaviour of the juveniles seem to preview those observed among adults.

Although they are less-well studied in other species, these patterns of sex differences in social behaviour are not unique to primates. In rodents, for instance, in both the Norway rat (Barnett, 1963; Brown, 1980; Calhoun, 1962) and the roof rat (Ewer, 1971) the parental care of infants is performed almost exclusively by the adult females, while the maintenance of territorial integrity (Calhoun, 1962; Ewer, 1971) is primarily a male activity. Among Norway rats, females engage in more social grooming than do males (Meaney & Stewart, 1979).

Among juvenile Norway rats, as well as adults, there are sex differences in social behaviour. Juvenile males engage in more play-fighting than do their female peers (Meaney & Stewart, 1981; Poole & Fish, 1976), while juvenile females engage in more social grooming than do males (Meaney & Stewart, 1981). Thus, the pattern of sex differences observed in both juvenile and adult Norway rats is similar to that observed in several species of old world monkeys. Of particular interest here is the similarity in the pattern of sex differences in social play.

The range of species in which sex differences in socal play have been observed (literally from pinnipods to primates; see Tables 1 and 2) is impressive. These sex

differences in social play suggest that the opportunities for early social learning in these species depend upon the gender of the young and may be related to the sex roles of the adults. In play-mothering, for example, where early interactions with infants appear to serve a motor-learning function (see Lancaster, 1971), sex differences in social play might contribute to sex differences in the infant-care skills of the adults. Sex differences in social play also seem to determine, in part, with whom young animals interact. In old world monkeys juvenile males form play groups where, in the course of their play-fighting, they interact with peers and adults of the same sex. In contrast juvenile females avoid the play groups of the males. Females, possibly as a function of their attraction towards infants, tend to remain in close contact with their mother and with other adult females and their infants (see Cheney, 1978; Seyfarth, Cheney, & Hinde, 1978; Simonds, 1974;1977). These early social interactions with animals of the same sex appear to facilitate the integration of the juveniles into the gender-specific, dominance hierarchies within the group (Cheney, 1978; Harcourt & Stewart, 1981; Simonds, 1977). Thus, sex differences in social play may allow the animals to form relationships with animals of the same sex that in turn allow for the formation of stable male and female hierarchies.

One question that emerges from these considerations

concerns the determinants of sex differences in social play. In this thesis I describe a series of experiments that examine the effects of gonadal hormones on the sex differences in the social play of juvenile Norway rats. The results of these experiments suggest that perinatal hormones, apart from their influence on adult social behaviour, serve to predispose the social play behaviour of juvenile males and females.

Play-fighting

The focus of this thesis is on one particular form of social play - that of play-fighting. Play-fighting among juvenile rats involves a fixed sequence of behaviours. This sequence is depicted in Figure 1. The behaviour referred to as POUNCING can be considered as a play-initiation act since it precedes any other behaviour in the sequence (Meaney & Stewart, 1981; Poole & Fish, 1976). If the animal that is pounced on is responsive, then a WRESTLING bout ensues that is often accompanied by BOXING. The endpoint of the play-bout is the DOMINANCE/SUBMISSION relation between the two animals. Even after "submitting", however, the animal on the bottom often continues to kick at the other animal. This is especially true of younger animals (i.e. animals less than 35 days of age, Meaney & Stewart, 1981).

The actual definition of play-fighting as a behavioural category is complicated by two problems. First, the

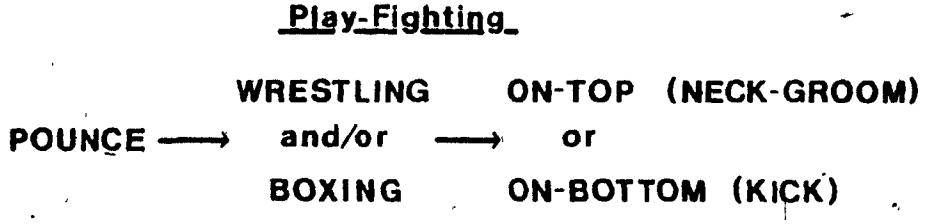

Figure 1. Depicted here are the behavioural components of a play-fight in Norway rat pups, and the sequence in which they occur.

behavioural components of play-fighting are, topographically, the same as many of those involved in the fighting of adults. Thus a strict behavioural definition of play-fighting is not possible. Second, since one is dealing with maturing animals, a definition of play-fighting for animals at one age may differ from that for older animals. Nevertheless, the real concern here is to distinguish between the play-fighting of juveniles and the agonistic encounters of adults.

Traditionally, play-fighting has been distinguished from the fighting of adult animals by its apparent lack of seriousness. While this is a nebulous and unsatisfying description it is not hard to find evidence for the difference between play-fighting and intraspecies aggression. In the play-fights of Norway rat pups, the distress vocalisations that are common in the agonistic encounters of adults are rarely recorded (Calhoun, 1962; Meaney & Stewart, 1981). This is not surprising since biting occurs only in an inhibited form that does not result in the wounding of an opponent (Poole & Fish, 1976). Another distinguishing feature of play-fighting in rats is that, unlike adult agonistic encounters, roles (i.e., attacker/target) are frequently reversed; an animal that is dominated during a play-fight will often immediately pounce on the other animal and then dominate it (Poole & Fish, 1976).

Additional examples of the distinction between play-fighting and adult-like aggression come from the work of

Poole (1966) with polecats (Mustella putorius). Poole reports that 5 out of 7 of the behaviours that appear in the attack component of the agonistic encounters of adults also appear in the play-fighting of juveniles, as do 3 out of 4 of the behaviours that comprise the defensive components. The two attack components that were absent in play-fighting (sustained neck-biting and sideways attack) are the behaviours that serve the function of inflicting injury on an opponent. The defensive component not seen in play-fighting was that of defensive threat. The "screaming" vocalisations recorded from attacked adults were also not heard during play-fighting. Thus, in the play-fighting of polecats the more extreme forms of both the attack and the defensive components of adult aggression are not observed.

In rhesus monkeys Symons (1974) has reported that the facial expressions that characterize the combatants in an aggressive encounter are not seen in the play-fighting of juveniles. Particularly noteworthy is that in the play-fighting of rhesus monkeys there are no gestures of threat or submission.

Thus, play-fighting contains only the milder forms of attack and defense that are seen in the fighting of adult animals. Most notably absent in play-fighting are the behavioural components that lead to the infliction of injury in an opponent and, consequently the components associated with "defeat." It is probably the case in the play-fighting

of all species that although one animal may gain the upper hand in the course of a wrestling bout, that there is no defeat or complete submission. Thus, the immediate function for the participants of a play-fight is apparently different from that for animals involved in an adult-like, agonistic encounter.

Sex Differences in the Play-Fighting of Juvenile Norway Rats

Presented in this section is a summary of the work describing the sex differences in the play-fighting of Norway rat pups. With the exception of one study (i.e., Poole & Fish, 1976) these studies have been conducted by the author in collaboration with Jane Stewart. Each of these studies varies in its methodology and setting, and it should be noted that the basic finding of a sex difference in play-fighting emerges in each study despite these differences.

In a first study we sought to examine the ontogeny of play-fighting and to describe the frequency of play-fighting in juvenile rats between the ages of 6 and 60 days of life. In this study a time-sampling technique was used to score the behaviour of pups that were observed in intact litters with the mother present. The results (see Figure 2) showed that play-fighting emerges by about 17 days of life and begins to decline at about the time of puberty. The sex difference in play-fighting is not clear until about Day 25, and is present thereafter. It should be noted that this sex difference was

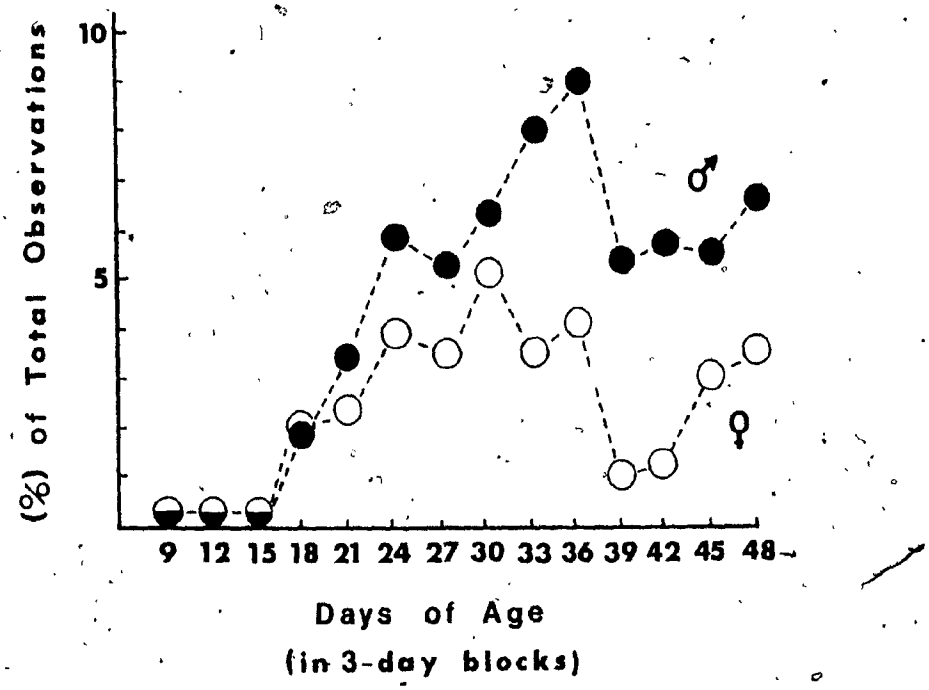

Figure 2. The frequency of play-fighting for male and female pups over days of age. Scores are expressed as a percentage of the total number of observations.

not due to any greater amount of general activity in male pups. In the same study we found that there was no sex difference in general locomotor behaviour.

In a second study (Meaney & Stewart, 1981) we examined in more detail the play-fighting of male and female Norway rat pups. In this study observations were made of pups while in intact peer-groups. The social behaviour of each pup was scored for thirty 20-second periods a day. The results of this study confirmed the existence of the sex difference that was observed in the first study (see Figure 3). In addition we found that males initiated more play-fights than did females, and that between 21 and 35 Days of age males initiated more play-fights with males than with females. Between 36 and 40 Days of age, however, this play-partner preference was reversed; males initiated and became involved in more play-fights with females than with males. This period between 36 and 40 Days of age marks the onset of the sexual attraction of the male towards females (Meaney & Stewart, 1980), but is a period in which very little overt sexual behaviour is observed. The onset of this sexual attraction in males, then, appears to result in a play-partner preference for females, and about 5 to 7 days later it is followed by the onset of sexual behaviour. Once this transition has occurred the pubertal males (between 50 and 55 Days of age) once again direct more play-fighting towards males than females.

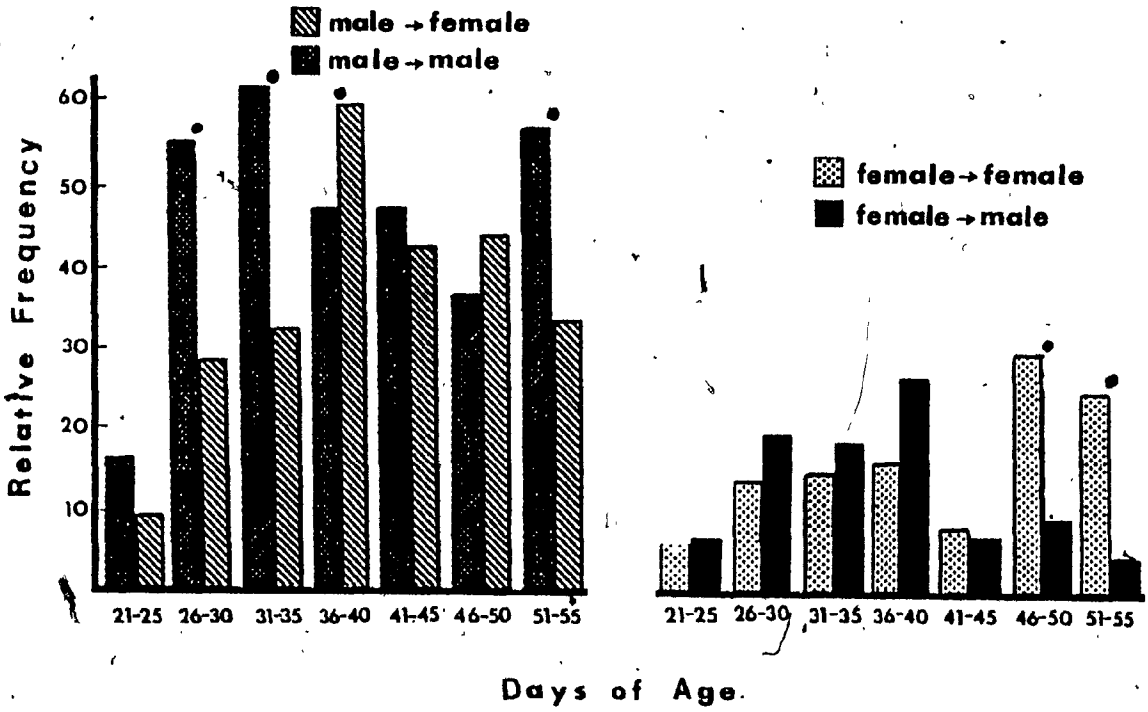

Figure 3. The relative frequency of pouncing (play initiation) in each age period for each of the four possible male-female dyads. The left half of the figure refers to play bouts that were initiated by males (towards either males or females), while the right half refers to play bouts that were initiated by females. Significant sex-of-play-partner preferences are indicated by the closed circles.

The behavioural components of play-fighting are not sex dependent. In the Norway rat, as in the rhesus monkey (Goy, 1974), when females do engage in a play-fight, they do so in a manner that is no different from that of their male peers. The behavioural components of play-fighting and the temporal order in which they occur are presented in Figure 1. This pattern is the same for both male and female pups.

While the results of this second study provide detailed information about the behavioural components of play-fighting, the "resolution" afforded the observer is limited in that the social play of rats is extremely fast-paced, and the data must be recorded by hand. This is particularly true of the movements made by female pups. In order to obtain more detailed information a third study was conducted in which the behaviour of pups between 25 and 50 days of age was scored using a frame by frame analysis of video-tape recordings. The speed of the tape was set at about 65 frames per second. This allowed the observer to make detailed records of the play-fights as well as to record data concerning the duration of the individual components of play-fighting.

An analysis of these tapes revealed a second form of play-fighting that we have termed "hit-and-run" play. This form of play is very similar to the "approach/avoidance" play that Harlow (1969) has observed in rhesus monkeys. In hit-and-run play one animal pounces on another and then

quickly (i.e., in less than 5 seconds) retreats. The retreat, or run component seems to involve two leaps away from the animal that has been pounced on. This stereotypic movement is reminiscent of the solicitation pattern of the adult female rat (see McClintock & Adler, 1978). This form of social play is also sex dependent. Hit-and-run play was observed more often in females than in males. Harlow (1969), interestingly, has found that female rhesus monkeys engage in more approach/avoidance play than did males.

Female pups were not only more likely to withdraw following a pounce (the hit-and-run pattern), but also withdrew more often than did males prior to the formation of a dominance relation. This finding is consistent with that of the previous study (see Figure 4) showing that play-fights involving females and males were less likely to progress to a dominance relation than were fights involving males only.

In summary, then, the play-fighting of Norway rat pups has been found to be sex dependent. This finding has emerged from a number of studies using various observational techniques and settings. The sex difference in the play-fighting of juvenile Norway rats is a difference of degree and not one of kind: Females do not differ from males in their potential to play-fight. The sex difference in play-fighting is due to (a) the fact that males initiate and become involved in more play-fights than do females, and (b) to the fact that females withdraw from play-fights more than do males (see Figure 5).

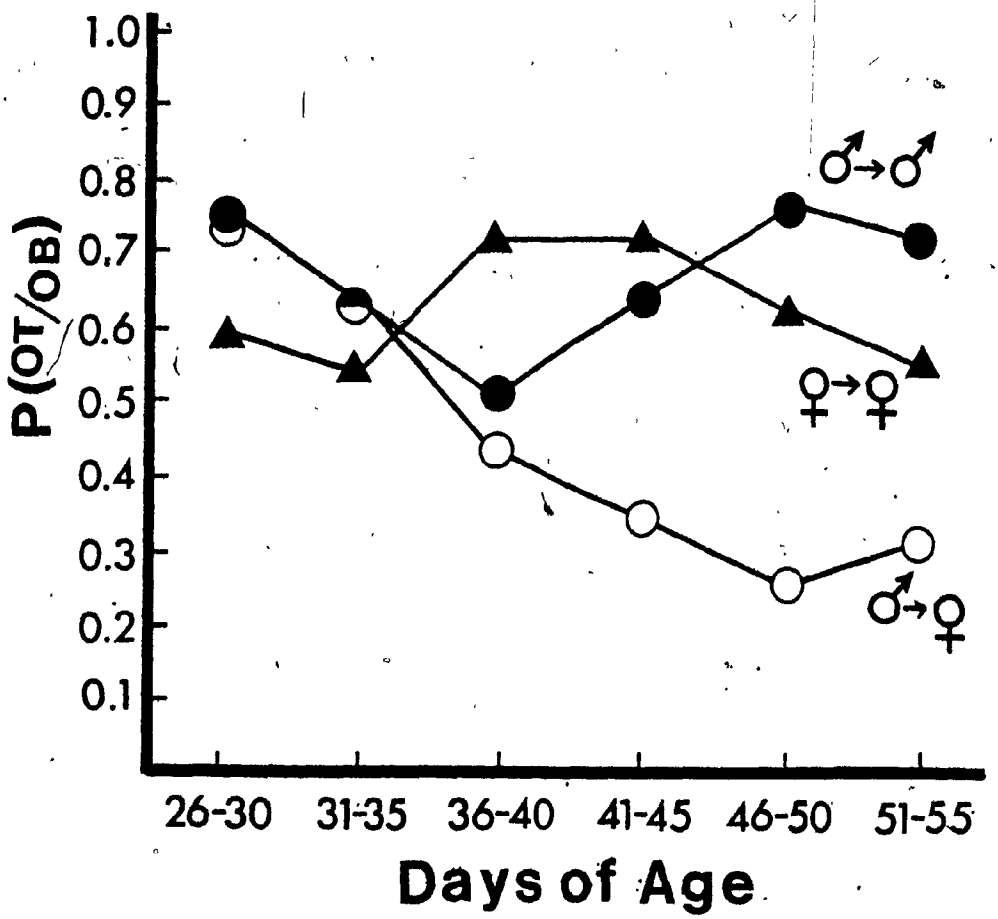

Figure 4. The probability of a play-fight resulting in an On-Top/On-the-Back relation (P(OT/OB)) for ♂ - ♀, ♂ - ♂, and ♀ - ♀ dyads over age. Female - male play-fights did not occur frequently enough to permit analysis.

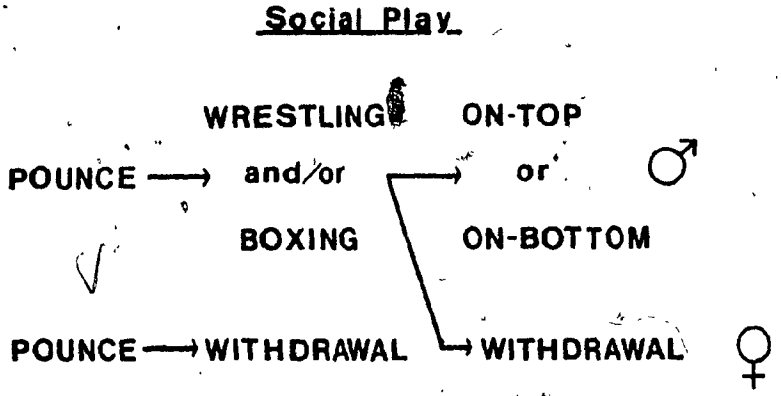

Figure 5. Presented in this figure is a summary of the sex differences in the social play of Norway rat pups. Depicted on the upper portion is a play-fight sequence. While males are more likely than females to initiate a play-fight, females (as seen in the lower portion of the figure) are more likely to withdraw either before a dominance relation is formed or immediately after having pounced on another animal (i.e., hit-and-run play).

Hormonal Determinants of Sex Differences in Play-Fighting

Working with rhesus monkeys Goy (1970) found that females exposed to exogenous testosterone propionate (testosterone is the principal secretory product of the testes) during gestation engaged in play-fighting at rates that were comparable to those of their male peers. In the rat, neonatal (i.e., postnatal Days 1 and 2) treatment with testosterone also eliminates the sex difference in play-fighting (Olioff & Stewart, 1978). (With respect to these findings it is important to note that the prenatal period in the monkey and the neonatal period in the rat are critical periods for neurogenesis and for the hormone-dependent, sexual differentiation of mating behaviours). In both of these species, then, perinatally androgenized females did not differ from males in the frequency with which they engaged in play-fighting. Thus, sex differences in social play appear to be related to the early actions of androgens in both the rat and the rhesus monkey. These results also suggest that the sex difference in play-fighting is not directly dependent on the presence or absence of the Y chromosome. In both of the experiments described above genetic females treated with testosterone at the appropriate period did not differ from genetic males in the frequency with which they engaged in play-fighting. Rather, male-typical levels of play-fighting appear to be

related to the androgenic secretions of the Y chromosome-dependent gonad (i.e., the testes).

The experiments that follow have been designed to examine further the relationship between testosterone and play-fighting. These experiments deal with two basic questions concerning this relationship. The first concerns the temporal characteristics of the androgenic influence on play-fighting. This question is seminal to an understanding of the nature of the androgenic action. The literature on hormone-behaviour interaction (see Beatty, 1979; Goy & McEwen, 1978 for reviews) demonstrates that there are, generally speaking, two ways in which hormones influence behaviour. The first concerns the activational effects of hormones on behaviour. Instances in which the presence or absence of a particular hormone at or near the time of testing can be related to the probability with which a behaviour will be observed represent an activational effect. That is the hormone is considered to "activate," or in some cases to "deactivate" a particular behaviour. The effects of adult castration on male or female sexual behaviour in the rat (e.g., Beach, 1956) are classical examples of activational effects. The second way in which hormones are said to influence behaviour is by "organizing" the CNS during some early developmental period in such a way that an individual is predisposed to respond to a particular stimulus in a certain way (Phoenix, Goy, Gerall, Young, 1959).

Instances in which the probabilty of the occurrence of a particular behaviour can be related to the presence or absence of a hormone during some previous period of development (i.e., an identifiable critical period) represent an organizational effect. The sterility of adult female rats treated neonatally with testosterone (e.g., Gorski, 1973) is an example of an organizational effect. It should be noted that both organizational and activational effects are not defined by the processes by which they influence behaviour, since in all cases hormonal effects are mediated by other events (both intracellular and extracellular). Rather, they are defined by the temporal characteristics of the hormone effects. It is for this reason that a description of the temporal characteristics of a hormone effect represents a first step towards an understanding of the nature of that effect.

The second question concerns the metabolic pathways involved in the androgen influence on play-fighting. The issues involved in this question are described in detail prior to Experiment 4, so at this point I will only briefly outline the problem.

Testosterone is actually a prohormone. That is, once inside a cell, testosterone can (a) be aromatized into an estrogen, (b) be reduced to a 5α- or 3α-androgen (such as dihydrotestosterone), or (c) remain in its original form. The brains of animals castrated and adrenalectomized and then

treated with hydrogen-tritiated testosterone have been found to contain various amounts of labelled estrogen, reduced androgens, and testosterone (Lieberburg & McEwen, 1977). Therefore any testosterone effect, be it activational or organizational, can conceivably be due to testosterone-derived estrogen, testosterone-derived, reduced androgens, or to testosterone itself. Experiments 4 and 5 of this thesis were designed to identify the form in which testosterone exerts its influence on play-fighting.

In each of the experiments described the animals were studied during the prepubertal period from 26 to 40 Days of age, except in Experiment 2 in which the period studied was from days 31 to 40. A prepubertal period was chosen so as to avoid the potentially confounding endocrine changes that occur at the time of sexual maturation. Following weaning the animals used in any particular experiment were placed into mixed-sex groups of between six and eight animals. The animals remained in these groups throughout the experimental period. This allowed the experimenter to make detailed observations of the pups throughout the prepubertal period. Each group within each experiment was composed of a similar number of treated and untreated animals. This provided each animal with rearing companions of both sexes including normal, untreated animals.

Experiment 1

In this first experiment the frequency of social play of intact male and female pups was compared to that of male pups that had been castrated within 24 hours after birth. For while it had been found in an earlier experiment (Olioff & Stewart, 1978) that intact females exposed to testosterone injections on Days 1 and 2 of life engaged in more social play than did oil-treated females, the effects of removing the primary source of testosterone in males during the critical period for the organizational effects of gonadal hormones had not yet been studied.

Method

Subjects

The animals observed in this experiment were 16 male and 8 female Long-Evans hooded rats. These animals were selected from amongst the offspring of 8 dams that were obtained while pregnant from the Canadian Breeding Farms and Laboratories, St. Constant, Quebec. Within 24 hours following birth the animals were removed from their mother, sexed, and randomly assigned to treatment groups. Twelve males were castrated and 12 were sham operated. Surgery was performed under hypothermia anesthesia. The animals were then assigned to six

mothers. Each mother received 10 pups (the average litter size for this species) four of which were similarly-treated males and six of which were same-aged, untreated females. The animals were then left undisturbed until weaning. A 12L/12D light schedule was maintained in the animal colony (lights off at 09:00 h).

On Day 23 the animals were separated from their mothers and housed in four groups of six animals each. By this time five castrated males and one sham-operated, intact male had died. Therefore, three of the four groups were composed of two castrated males, two intact males, and two same-aged females, while the fourth was composed of three intact males, one castrated male, and two females making for a total of nine intact males, seven castrated males, and eight females. The eight females were chosen from those that had been added to the six litters. The animals were maintained in these groups throughout the experiment. The animals were marked for identification (coloured, felt-tipped pens) every 5 days and were not otherwise handled. Although they were housed in a different room the animals were maintained on the same feeding conditions and light schedule as in the animal colony.

Apparatus and Procedure

Each postweaning group was housed in a cage 51 x 33 x 26 cm, one wall of which was made of 1.25 cm plywood and had mounted on it a wire-mesh feeder and two waterbottles. The

remaining sides were made of 0.6 cm wire-mesh.

The animals were observed daily during the prepubertal period between Days 26 and 40. Each group of six animals was observed for 70 observation periods per day. Each lasted for 20 s, and during that period the behaviour of all six animals was scored. If, during the period an animal engaged in a play-bout it was given a score of "1." Animals that did not engage in a play-bout were given a score of "0." Thus, for any animal the possible range of scores for each day of observations was from 0 to 70. An animal that engaged in any of the behavioural components of a play-fight sequence (see below) was considered to have engaged in a play-bout. All observations were conducted between 12:00 and 16:00 hours.

Behavioural components of play-fighting. The following is a description of the behavioural components of the play-fight sequence (see Figure 1 and p.3). Pouncing. One pup lunges at another with its forepaws extended outward. It is the forepaws that first make contact with the other animal. Wrestling. Two animals roll and tumble with one another. Boxing. Two animals standing upright, facing one another, and making pawing movements towards one another. On-the-Back Posture. One animal lies on its back, fully exposing its ventral surface to another animal. On-Top. One animal positioned over another animal with its forepaws placed on the other animal. In the experiments described in this thesis play-fighting and social play were considered as being

synonomous. The hit and run form of social play, a sub-category of play-fighting, was scored as being an instance of play-fighting simply because it is extremely difficult to detect without the aid of video analysis. This does not present a serious confound since only about 3 to 5 % of all play-bouts involve the hit and run sequence (Meaney & Stewart, unpublished observation).

Data analysis. Total play scores were calculated for individual animals across all 15 observation days. The effects of treatment conditions were examined by comparing the scores of animals in each of the three conditions regardless of observation group using a Kruskal-Wallis H-test. Post-hoc, paired comparisons were made using Mann-Whitney U-tests. Non-parametric statistics were used because of the ease with which they are computed and because they do not appear to differ in power from parametric statistics (see Bonneau, 1976).

Note that the combining of scores across observation groups results in the addition of between group "variance" to the design. This confusion of between and within group "variance", however, adds to the conservativeness of the statistical analysis since it serves as a bias against finding between group treatment effects.

Results and Discussion

The results of Experiment 1 are summarized in Figure 6. Statistical analysis revealed a significant treatment effect ($H = 9.67$, $p < .01$). Post-hoc comparisons showed that intact males engaged in significantly more play-fighting than did castrated males ($U = 7$, $p < .005$) or intact females ($U = 8.5$, $p < .005$). The difference between the castrated males and the intact females was not significant. These results indicate that genetic males deprived of testicular hormones from birth show greatly reduced levels of play-fighting in the prepubertal period.

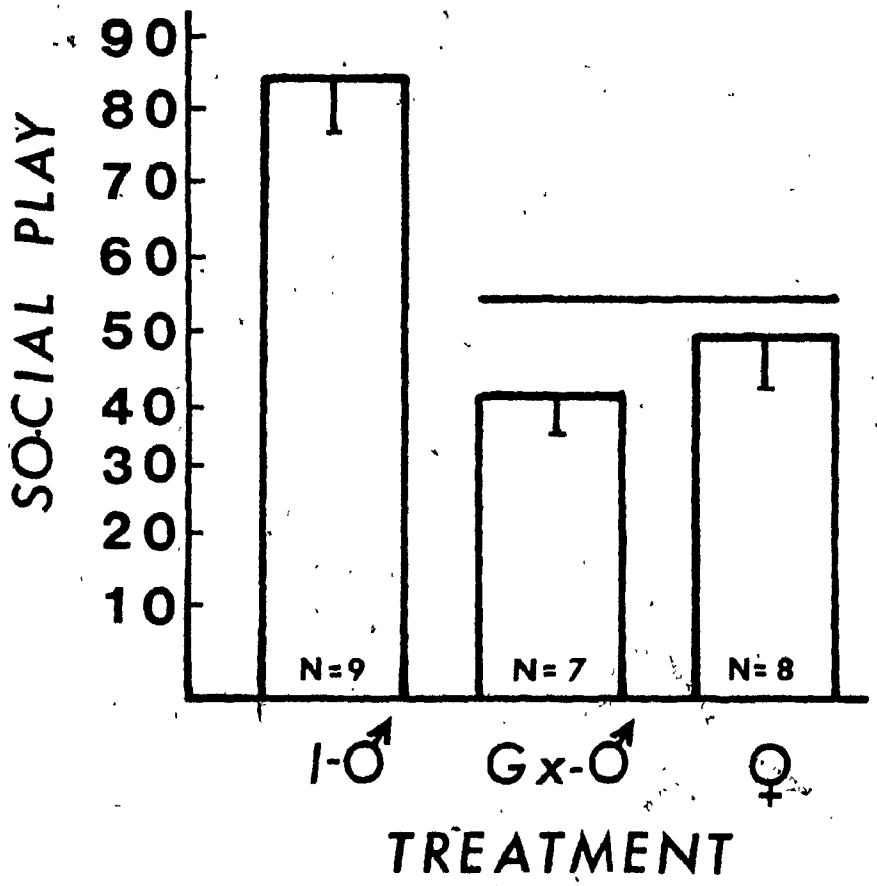

Figure 6. Mean (-SE) number of 20-sec sessions in which intact males (I-♂), Day 1-castrated males (Gx-♂), and intact females (♀) were observed to be engaged in

Experiment 2

Since the animals in the first experiment were deprived of testosterone from birth onward, it is not possible to specify the timing of the androgen effect. The Day 1-castrated males were deprived of testosterone not only during the neonatal period, but also during the time of testing. The results of Olioff and Stewart (1978), however, support the view that the androgen influence is limited to the neonatal period since females treated with testosterone, but which lacked, at least, the testicular source of androgens during the period of observation, did not differ from intact males in their frequency of play-fighting. This suggests that there is no activational influence of circulating androgens on play-fighting, and that the effects of testicular hormones is specific to the neonatal period.

Experiment 2 was designed to examine the possible effects of circulating androgens on the play-fighting of male pups. In this experiment the frequency of play-fighting in intact male and female pups was compared to that of males that were castrated on Day 23. Since Day-23 castrates were without testes throughout the period of observation (Days 31 to 40), but not during the early neonatal period, it was possible to determine whether the presence of testicular hormones at the time of testing contributes to the expression

of male-typical levels of play-fighting.

Method

Subjects

The animals observed in this experiment were 16 male and 8 female Long-Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. On Day 23 eight males were castrated and eight were sham operated. Surgery was performed under Nembutal (1cc/KG) anesthesia. Following surgery the animals were placed into groups of six same-aged animals. There were four such groups, each composed of two castrated males, two sham-operated, intact males, and two same-aged, untreated females.

Apparatus and Procedure

The apparatus and procedure were the same as in Experiment 1, with the exception that the period of observation was shortened to 10 days (Days 31 to 40). This was done to allow for recovery from surgery.

Data analysis. The data from this experiment were treated similarily to those from Experiment 1. Note, however, that there were 10 and not 15 days of observation in this experiment.

Results and Discussion

The results of Experiment 2 are summarized in Figure 7. The statistical analysis across all three groups revealed a moderate treatment effect ($H = 4.59$, $.10 > p > .05$). The post-hoc analysis showed that both intact males ($U = 13.5$, $p < .05$) and castrated males ($U = 17$, $p < .06$) engaged in more play-fighting than did intact females. There was no significant difference between the two male groups.

The fact that castration at 23 days of age did not affect play fighting in prepubertal males suggests that there is no activational influence of circulating androgens on social play. Together with the findings of Experiment 1 and those of Olioff and Stewart (1978) these results indicate that while the exposure to testicular hormones in the early neonatal period is crucial for the development of the sex difference in social play, the presence of testicular hormones at the time of testing does not contribute to the expression of the sex difference. This conclusion has received further support from the results of subsequent work. In one study (Beatty, Dodge, Traylor, & Meaney, 1981) it was found that castration on neither Day 10 nor Day 20 affected the frequency of play-fighting in male pups. In a second study Meaney and Stewart (Note 1) found that daily injections of 200 ug of testosterone propionate to male pups between 26 and 40 days of age had no affect on the frequency with which

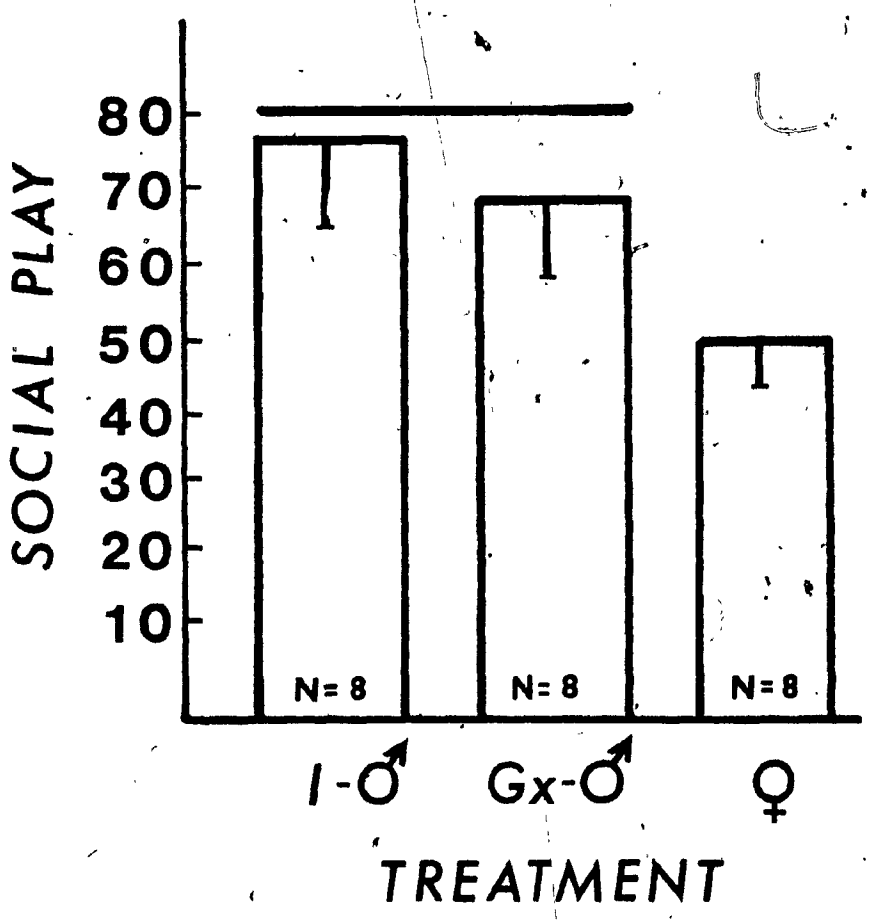

Figure 7. Mean (-SE) number of 20-sec sessions in which intact males (I-♂), Day 23-castrated males (Gx-♂), and intact females (♀) were observed to be engaged in play-fighting.

they engaged in play-fighting.

This pattern of androgenic influence on the play-fighting of the rat parallels that found in the rhesus monkey. As mentioned earlier, testosterone treatment during an apparent critical period for brain development masculinizes the play-fighting of genetic rhesus monkeys (Goy, 1970; Goy & Goldfoot, 1974) and Norway rats (Olioff & Stewart, 1978). Once beyond this period, however, testosterone treatment does not seem to influence play-fighting in either species. In the rat this conclusion is supported by the evidence presented above. In the rhesus monkey neither the postnatal castration of males (Goy, 1978) nor the postnatal testosterone treatment of females (Joslyn, 1973) was found to influence the frequency with which animals engaged in play-fighting. Thus, in neither species does testosterone serve to activate play-fighting.

Experiment 3

The results of Experiments 1 and 2 suggest that the sex difference in the social play of prepubertal rats is due, at least in part, to the influence of neonatal testicular androgens. It is possible, however, that this sex difference may also be influenced by a suppressive effect of ovarian hormones either at the time of testing or earlier. In Experiment 3 we examined the influence of ovarian secretions on the social play of prepubertal female rats. In this experiment the frequency of play fighting of intact male and female pups was compared to that of female pups that were castrated on Day 1 of life.

Method

Subjects

In this experiment the observations were performed on 16 female and 8 male Long-Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. Within 24 hours following birth the animals were removed from their mother, sexed, and randomly assigned to treatment groups. Ten females were ovariectomized and 10 were sham operated. Surgery was performed under hypothermia anesthesia. The animals were then reassigned to mothers as in Experiment

1.

On Day 23 the animals were separated into four groups of six animals. By this time two ovariectomized animals had died. Each group was comprised of two ovariectomized females, two sham-operated, intact females, and two untreated, same-aged males.

Apparatus and Procedure

The apparatus, procedure, and analysis of the data were the same as in Experiment 1.

Results and Discussion

The results of Experiment 3 are summarized in Figure 8. The statistical analysis across all three groups revealed a significant treatment effect ($H = 6.76$, $p < .05$). The post-hoc analysis showed that the intact males engaged in significantly more play-fighting than did either the intact females ($U = 10$, $p < .01$) or the ovariectomized females ($U = 16$, $p < .05$). The difference between the two female groups was not significant.

These results indicate that there is no detectable influence of ovarian hormones on the social play of prepubertal rats. Thus, the sex difference in the play-fighting of Norway rat pups does not appear to be due to any suppressive effects of ovarian hormones on the social play of females.

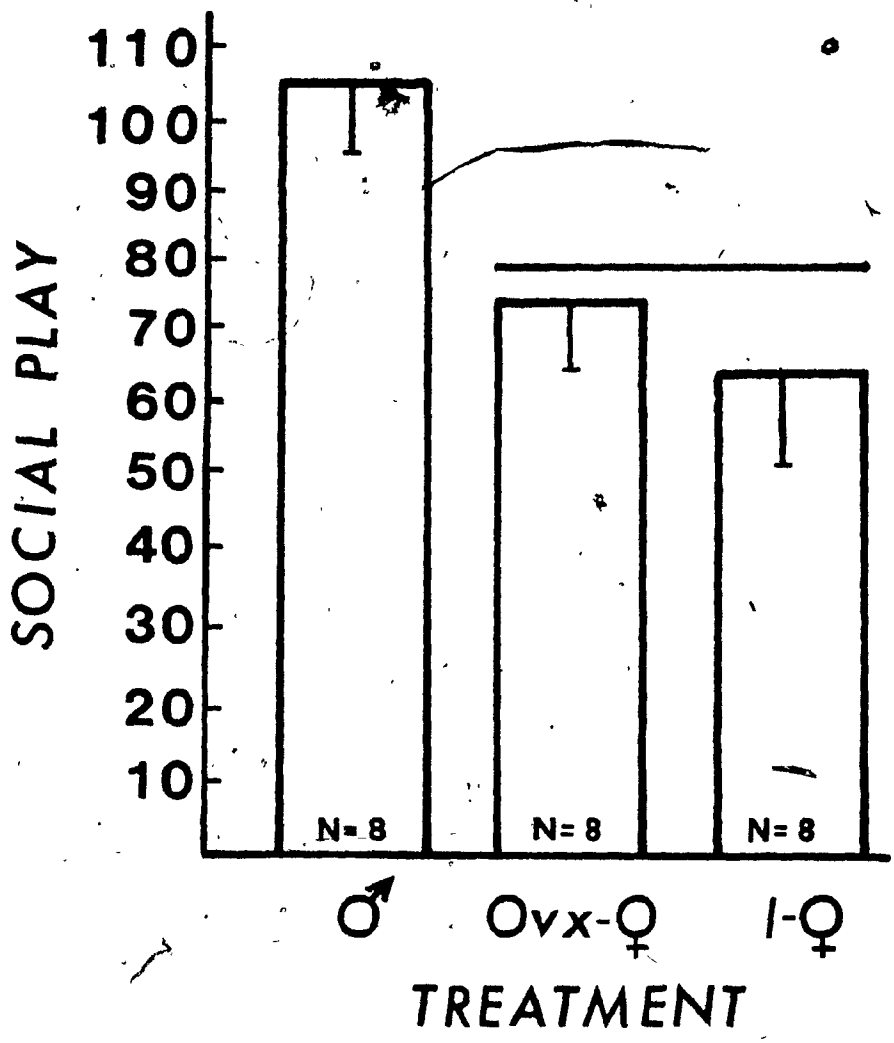

Figure 8. Mean (±SE) number of 20-sec sessions in which intact males (I-♂), Day 1-ovariectomized females (Ovx-♀), and intact females (♀) were observed to be engaged in play-fighting.

Experiment 4

The results of the first two experiments indicate that the sex difference in the social play of rat pups is dependent upon the presence of testicular hormones during the early neonatal period. In this experiment I sought to examine whether testosterone itself or one of its metabolites exerts this influence. It is known that within certain cells in the brain of the rat and other species, testosterone can be converted into either estradiol through the aromatization pathway (Naftolin, Ryan, & Petro, 1972) or into dihydrotestosterone through the 5α-reductase pathway (Denef, Magnus, & McEwen, 1974; Martini, 1978). Moreover, there are receptor sites, especially within the limbic system, for estradiol (Stumpf, Sar, & Keefer, 1974), for dihydrotestosterone and for testosterone (Sar & Stumpf, 1974; Sheridan, 1979). It is conceivable, then, that the androgen effect described in Experiments 1 and 2 may be due to the actions of testosterone itself, to the action of testosterone-derived estradiol, or to the action of testosterone-derived dihydrotestosterone.

The issue of which metabolite is primarily involved is of particular interest in view of the major role attributed to the estradiol metabolite (17β-estradiol) in the differentiation of the neural circuitry involved in

gonadotrophin release and in the defeminization and masculinization of sexual behaviour patterns in many rodents (McEwen, Lieberburg, Chaptal, & Krey, 1977). Estradiol is known to be converted from testosterone in many regions of the brain during the period of sexual differentiation and to bind with high affinity to estrogen receptors within specific brain cells (Lieberburg, MacClusky, Roy, & McEwen, 1978). It is this feature that has made it possible to begin to relate hormone action to specific changes in brain structure during this period. No such direct CNS action for testosterone itself or for its 5 α-reduced and non-aromatizable metabolite, dihydrotestosterone, has yet been determined, though it has been suspected. Thus, while it is well recognized that dihydrotestosterone plays the major role in promoting the growth of the male genitalia and body type, the evidence that exists for a direct androgenic action in the CNS during differentiation is considered equivocal (for a review see Goy & McEwen, 1978). One reason for this is that until recently receptors for testosterone or dihydrotestosterone were not known to be present in the brain of developing animals during critical periods for differentiation. The finding of androgen receptors in the limbic brain of one-day old rats, receptors that exhibit nuclear binding properties typical of androgen receptors in adult rats, is therefore of great interest (Fox, Vito, & Wieland, 1978; Lieberburg et al., 1978, Vito, Wieland, & Fox,

1979). Furthermore, the report of 5 -reductase activity in the brain of neonatal rats adds to the possibility that androgen metabolites of testosterone may play a role in the differentiation of neural tissue (Martini, 1978).

In Experiment 4 the frequency of play-fighting in genetic females treated neonatally (Days 1 and 2) with either testosterone propionate, estradiol benzoate, or dihydrotestosterone was compared to that of normal male and female pups.

Method

Subjects

The animals observed in this experiment were 24 female and 12 male Long-Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. On both Days 1 and 2 of life six females were injected subcutaneously with 250 ug of testosterone propionate, six with 250 ug of dihydrotestosterone, six with 5 ug of estradiol benzoate, and six with the oil vehicle alone. All steroids were dissolved in peanut oil and were delivered in .05 ml amounts. Collodion (Fisher Scientific Ltd.) was applied at the point of injection to prevent leakage. On Day 23 the animals were rehoused into six groups of six animals, each group containing one estradiol benzoate-treated female, one dihydrotestosterone-treated female, one testosterone

propionate-treated female, one oil-treated female, and two untreated, same-aged males.

Apparatus and Procedure

The apparatus, procedure, and analysis of the data were the same as in Experiment 1.

Results and Discussion

The results of Experiment 4 are summarized in Figure 9. As can be seen, testosterone propionate-treated females, dihydrotestosterone-treated females, and normal males did not differ in the frequency with which they engaged in play-fighting. All three groups were observed to engage in more play-fighting than did both the oil-treated females and the estradiol benzoate-treated females. The statistical analysis revealed that there was an overall treatment effect ($H = 21.24$, $p < .005$). Post-hoc analysis showed that there was no significant difference between the males and either the testosterone propionate-treated females or the dihydrotestosterone-treated females. Both testosterone propionate-treated females and dihydrotestosterone-treated females differed significantly from the estradiol benzoate-treated females ($U = 0$, $p < .001$; and $U = 5.5$, $p < .03$, respectively) and the oil-treated females ($U = 0$, $p < .001$ and $U = 1.5$, $p < .01$, respectively). There was no significant difference between the estradiol benzoate-treated females and

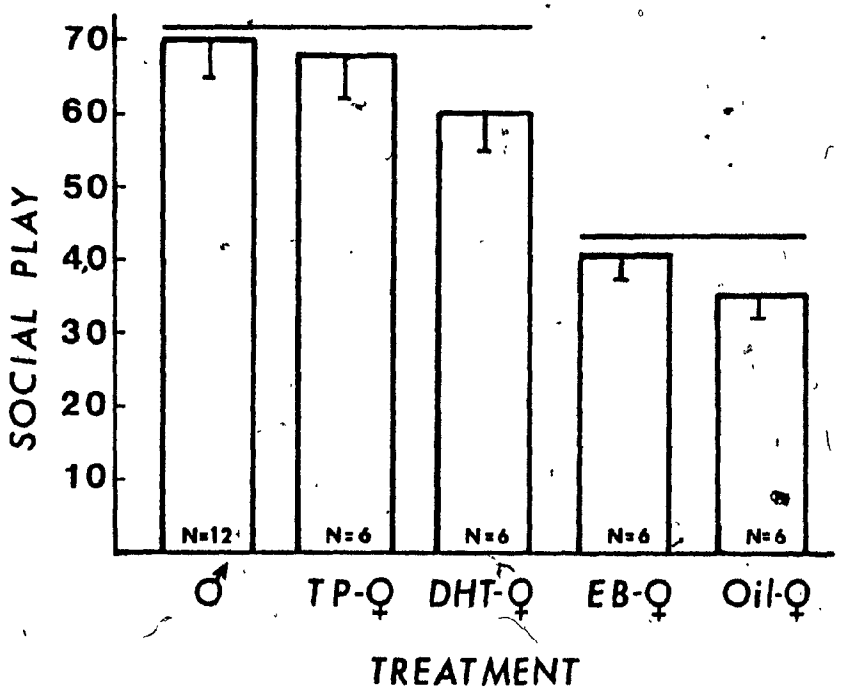

Figure 9. Mean (-SE) number of 20-sec sessions in which intact males (I-♂), testosterone propionate-treated females (TP-♀), dihydrotestosterone-treated females (DHT-♀), esradiol benzoate-treated females (EB-♀), and oil-treated females (oil-♀) were observed to be engaged in play-fighting.

the oil-treated females.

These results indicate that either testosterone or its 5α-reduced metabolite, dihydrotestosterone, administered early during the neonatal period is able to increase the frequency of play-fighting in prepubertal female rats and, in the doses used, to eliminate the sex difference in social play. In contrast, estradiol benzoate, even in doses sufficiently large to defeminize both open-field and lordotic behaviour (see Stewart, Vallentyne, & Meaney, 1979), has no such effect. This suggests that the influence of testosterone on the social play of rats is a true androgen effect.

Experiment 5

The results of Experiment 4 suggest that the high levels of play-fighting observed in male pups are due to the action of testosterone or its dihydrotestosterone metabolite in the neonatal period, and that there is no effect of testosterone-derived estrogen. In the present study we examined this hypothesis using intact males. While studies that simulate the process of masculinization in genetic females (e.g., Experiment 4) are highly informative, it was considered important to confirm these findings in genetic males whenever possible. The question here is not only whether androgens <u>can</u> masculinize social play, but also whether, under normal conditions (i.e., genetic males), this is the process by which male-typical social play develops.

In this study, then, I further examined the metabolic pathway involved in the androgenic influence on social play. This was done by implanting newborn male pups with silastic capsules containing either androst-1,4-6,-triene-3,17-dione (ATD) or 4-androsten-3-one 17β-carboxylic acid (testosterone 17 β -carboxylic acid). ATD is an aromatase inhibitor and is presumed to inhibit the conversion of testosterone to estradiol (Lieberburg, Wallach, & McEwen, 1977). ATD has been shown to attenuate the estradiol-mediated defeminizing effect of testosterone on female sexual behaviour (Booth, 1977;

Clemens & Gladue, 1978; McEwen et al., 1977; Vreeburg, van der Vaart, & van der Schoot, 1977). In the present study the ATD capsules were left in the animals from Day 1 to 10. Thus, the endogenous levels of testosterone-derived estradiol should have been substantially reduced during the period when estradiol normally acts to defeminize rat behaviour. If testosterone-derived estradiol were involved in the development of male-typical levels of play-fighting in the male rat, then ATD would be expected to reduce the observed levels of play-fighting. In the first part of this experiment (5a) the frequency of play-fighting in normal male and female pups was compared to that of males that were implanted with ATD during the early neonatal period.

Similarly, testosterone 17 β-carboxylic acid is a compound that inhibits the reduction of testosterone into dihydrotestosterone (Kao & Weisz, 1979; Luttge, Jasper, Sheets, & Gray, 1978). In the second part of this experiment (5b) male pups were implanted with silastic capsules containing testosterone 17 β-carboxylic acid from Day 1 to 10, thus reducing the endogenous levels of testosterone-derived dihydrotestosterone. If the metabolism of testosterone into dihydrotestosterone is necessary for the development of male-typical levels of play-fighting, then animals implanted with testosterone 17 β-carboxylic acid during the neonatal period should differ from normal males in the frequency with which they engage in play-fighting. In

experiment 5b the frequency of play-fighting in normal male and female pups was compared to that of dihydrotestosterone-treated males.

Method

Subjects

The animals observed in this experiment were 36 male and 18 female Long-Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. Within 24 hours of birth three groups of males were given silastic implants containing either ATD (n=10), cholesterol (n=18), or testosterone 17 β-carboxylic acid (n=8). The implants were inserted under the skin using hypothermia as anesthesia and were removed on Day 10 using ether anesthesia. The implants were made from silastic tubing .058 mm in inner diameter and .077 mm in outer diameter. Each implant contained 7 mm of steroid. The ends were sealed with 3 mm of silastic adhesive. The implants were then soaked in absolute alcohol for 1 hour to clean them and to check for leakage. They were then kept in 1% serum bovine albumin for at least 24 hours before use (see McEwen et al., 1977). In our laboratory we have used this procedure with ATD to prevent the defeminization of lordotic behaviour in male rats (see Stewart et al., 1979).

At 23 days of age the animals in Experiment 5a were placed into five groups of six animals, each group composed

of two ATD-treated males, two cholesterol-treated males, and two untreated, same-aged females. At the same age the animals in Experiment 5b were placed into four groups of six, each group composed of two testosterone 17 β-carboxylic acid-treated males, two cholesterol-treated males, and two untreated, same-aged females.

Apparatus and Procedure

The apparatus, procedure, and analysis of the data were the same as in Experiment 1.

Results and Discussion

The results of Experiment 5a are summarized in Figure 10. The statistical analysis of the data revealed a significant treatment effect ($H = 12.77$, $p < .005$). Post hoc analysis showed that both the ATD-treated males ($U = 20$, $p < .01$) and the cholesterol-treated males ($U = 19$, $p < .01$) engaged in significantly more play-fighting than did the females. There was no significant difference between the two groups of males. There was, then, no effect of ATD treatment between Days 1 and 10 on the frequency with which the male pups engaged in play-fighting. It might be argued that after the removal of the ATD implants on Day 10, the resulting normal levels of testosterone-derived estradiol might be capable of masculinizing social play after Day 10. Recently,

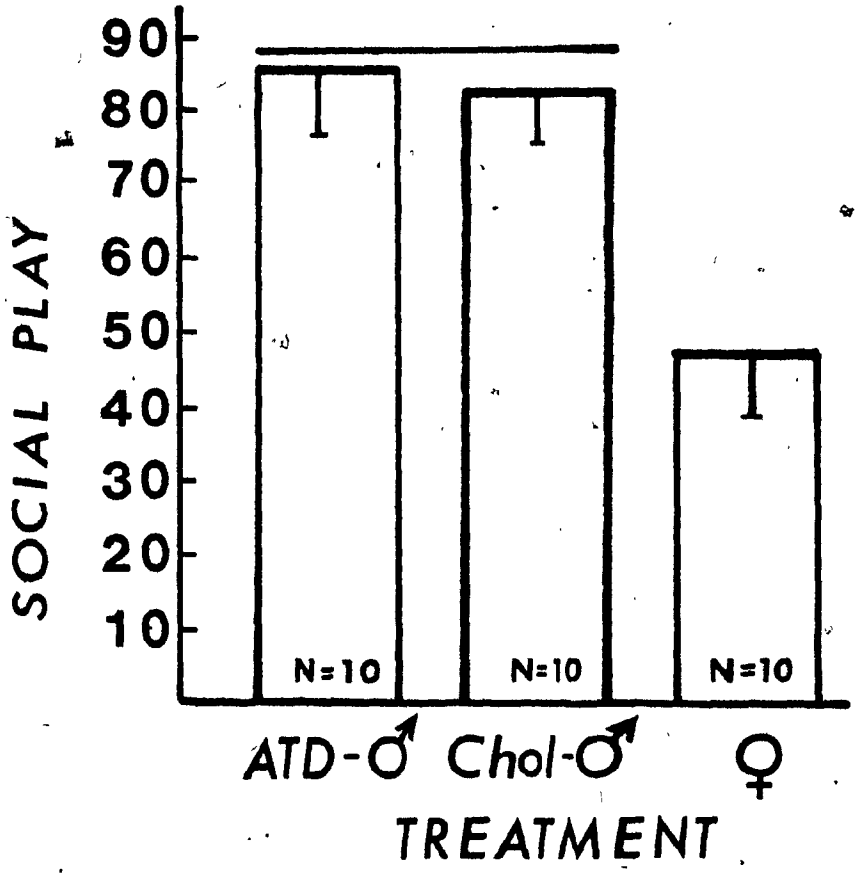

Figure 10. Mean (−SE) number of 20-sec sessions in which cholesterol-treated males (Chol-♂), ATD-treated males (ATD-♂), and intact females (I-♀) were observed to be engaged in play-fighting.

however, it has been found that castration of male pups as early as Day 10 does not influence the frequency with which male pups engage in play-fighting (Beatty et al., 1981). Thus, in male rats the masculinization of social play occurs before Day 10 and, with respect to the present experiment, before that time when the implants were removed. The results of this experiment, then, confirm and extend the findings of Experiment 4 that in the rat estradiol in the immediate postnatal period neither promotes nor is necessary for the development of male-typical levels of play-fighting.

The results of Experiment 5b are summarized in Figure 11. The statistical analysis of the data revealed a significant treatment effect ($H = 9.02$, $p < .02$). Post hoc analysis showed that both testosterone 17 β-carboxylic acid-treated males ($U = 2$, $p < .001$) and cholesterol-treated males ($U = 14.5$, $p < .04$) had higher play scores than did the females. There was no significant difference between the two groups of males.

The results of Experiment 5b would seem to indicate that the metabolism of testosterone into dihydrotestosterone is not a necessary step for the androgenic effect on social play. This conclusion must be somewhat tentative, however, in that we have no direct measure of the degree of inhibition produced by testosterone 17 β-carboxylic acid. Moreover, recent work in the laboratory of Michael Baum at the Massachusetts Institute of Technology (Baum and Bradshaw,

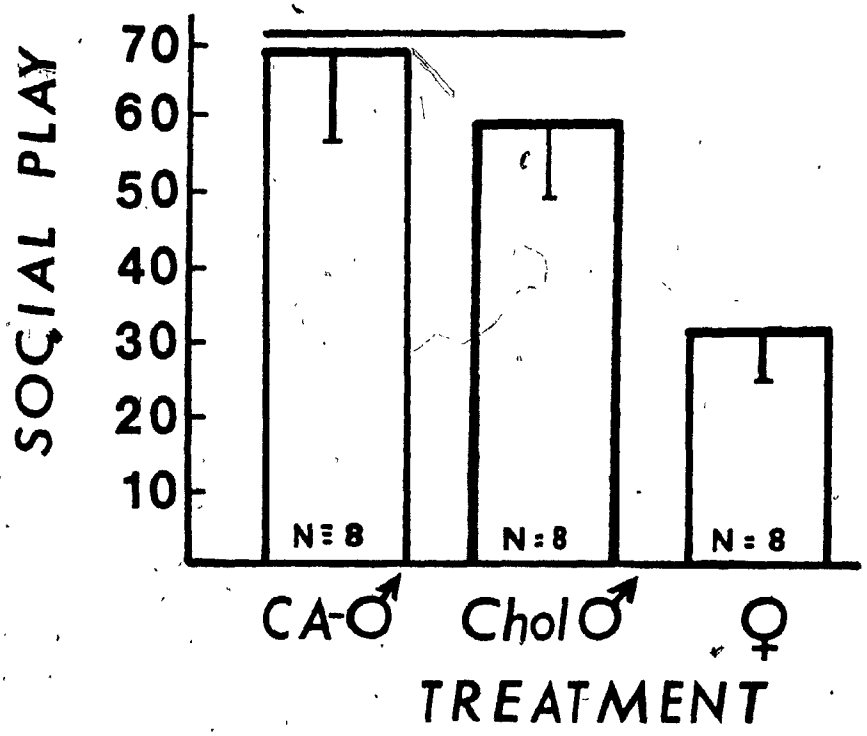

Figure 11. Mean (±SE) number of 20-sec sessions in which cholesterol-treated males (Chol-♂), testosterone 17β-carboxylic acid-treated males (CA-♂), and intact females (♀) were observed to be engaged in play-fighting.

personal communication) has suggested that testosterone 17β -carboxylic acid may be less effective an inhibitor of dihydrotestosterone than was originally assumed.

Experiment 6

The results thus far support the idea that the sex difference in the social play of prepubertal rats is an androgen-mediated effect, dependent on the neonatal exposure to either testosterone or dihydrotestosterone. The estradiol metabolite of testosterone does not appear to be directly involved in the development of male-typical social play.

The present experiments do not provide information about exactly how long into the neonatal period testosterone or dihydrotestosterone may be effective in influencing social play, but the results of Experiment 4 do indicate that exposure to either testosterone or dihydrotestosterone on Days 1 and 2 of life is sufficient to masculinize the social play of female pups. In addition the results of Experiments 2 and 4 demonstrate that circulating testicular androgens are not necessary for the expression of male-typical levels of play-fighting. Neither males castrated at 23 days of age nor females given testosterone neonatally, both of which lacked testicular androgens at the time of testing, differed from intact males in the frequency with which they engaged in play-fighting. Taken together these findings indicate that, while testicular androgens are not directly responsible for the expression of male-typical levels of play-fighting, their activity during the neonatal period contributes to the sexual

differentiation of some CNS function that in turn mediates this behavioural sex difference (i.e., an organizational effect).

It is possible that this androgen-dependent sexual differentiation is mediated by androgen receptor proteins in the CNS, most of which bind with both testosterone and dihydrotestosterone (Sar & Stumpf, 1974). Androgen receptors have been detected in the limbic brain of the rat as early as postnatal Day 1 (Fox et al., 1978; Lieberburg et al., 1978). Moreover, these neonatal androgen receptors exhibit properties typical of androgen receptors in adult rats; most notably they bind to DNA cellulose with the same affinity (Fox et al., 1978; Lieberburg et al., 1978). This characteristic is important because the intracellular effects and in particular the growth-related effects of steroid hormones are mediated by DNA (e.g., Salaman & Birkell, 1977). Thus, one possible explanation for the masculinizing influence of neonatal androgens on the social play of rat pups is that this early androgenic exposure promotes the formation of a sex-specific neural circuitry and that this circuitry serves to mediate the sex difference in social play that is seen in prepubertal rats. The question, of course, concerns the identity of this sex-specific circuitry.

The present study represents an initial attempt to identify the neural mechanisms involved in the social play of rat pups. Given the apparent endocrine involvement in social

play, the choice was made to examine the role of a prominent neural target area for steroid hormones, the amygdala, in the play-fighting of prepubertal rats. Moreover, since the behavioural components of play-fighting in the rat are similar to those of adult-like aggression, the well-documented effects of amygdalectomy on aggression (e.g., Clemente & Chase, 1973; Galef, 1970) were also suggestive.

In this study male and female pups were either amygdalectomized or sham operated. Surgery was performed on Days 21 and 22. On Day 25 the animals were placed into peer groups as previously described. The frequency of play-fighting observed in amygdalectomized male and female pups was compared to that of control males and females.

Method

Subjects

The animals observed in this experiment were 38 male and 24 female Holtzman albino rats born in the animal colony at North Dakota State University. (The change of strain in this experiment was due to the fact that Holtzman, the only supplier within a reasonable distance of North Dakota State University, did not breed hooded rats. Note, however, that the Olioff & Stewart study involved albino rats.) The animals were the offspring of 14 dams obtained while pregnant from the Holtzman Co., Madison, Wisconsin. The animals were housed

in the same way as in Experiment 1.

Surgery

Twenty-six males and 12 females were given bilateral electrolytic lesions under Chloropent anesthesia (1cc/KG) by passing a 1.5 mA current for 15 seconds between a rectal cathode and a No. 1 stainless steel insect pin that was insulated to the tip with Epoxylite. With the rat's head flat between bregma and lamda the coordinates were 2.0 mm posterior to bregma, 3.9 mm lateral to the midline, and 7.3 mm in depth from the surface of the brain. Six males and 6 females received sham lesions in which the electrode tip was lowered 6.0 mm from the surface of the brain. Six males and 6 females received the Chloropent anesthesia only. All animals were between 21 and 22 days of age at the time of surgery. Recovery seemed to be complete by about 2 to 3 days after surgery. By this time the animals were playing vigorously, and, through casual observation, seemed to be normally active. Note that the body weight data presented in Table 3 shows that there was no noticeable effect of the lesion on the growth of the animals.

Apparatus and Procedure

Following surgery the animals were placed into groups and housed in adjoining 41 x 61 x 38 cm cages made from wire mesh with a wooden frame for support. The groups were separated from each other by sheet metal dividers. The front of the cages was made from plexiglass. Six of the groups were

Table 3

Mean Body Weights on Day 25 (BW1) and Day 41 (BW2) for Lesioned and Control Animals (a)

Group	BW1	BW2
Amygdalectomized Males	66.6	174.1 (b)
Amygdalectomized Females	68.4	170.3
Control Males	68.0	153.0 (b)
Control Females	68.3	146.5

(a) All weights are expressed as grams

(b) Amygdalectomized Males Control Males Amygdalectomized Females Females Control Females ($p < .0001$)

comprised of eight animals and two were comprised of seven animals each. Each group contained some treated and some untreated animals and was balanced for sex. The animals were weighed on Day 25 (BW1) and Day 41 (BW2). The remainder of the procedure was the same as in Experiment 1, with the exception that the number of observations was increased from 70 to 105 per day.

Data analysis. The data were prepared for statistical analysis in the same way as in Experiment 1. This experiment yielded a two-way (sex x lesion) factorial design. The statistical analysis, then, was changed to a parametric analysis of variance to handle the factorial design. Post-hoc comparisons were made using t-tests.

Histology. Following the completion of the behavioural observations all rats with lesions and a representative sample of sham-operated animals were sacrificed under deep Chloropent anesthesia. After perfusion with physiological saline and 10% formalin the brains were sectioned on a cryostat at 40 u. Every fifth section through the lesion was saved and stained with formal thionin (Donovick, 1974). Each lesion was reconstructed with the aid of a microprojector and categorized with respect to the anterior-posterior plane of maximum extent, presence of optic tract damage (absent, unilateral, or bilateral), and size (3 = largest, 1 = smallest) by an observer who was unaware of the rat's performance or sex. Nearly all of the lesions were extensive

and bilaterally symmetrical. In three animals (two males and one female) the lesions were asymmetrical and posterior to the amygdala (two cases) or could not be detected (one case). Behavioural data from these animals were discarded.

Results and Discussion

Behavioural Data

Since there were no differences between the sham-operated males and the anesthetic-only males in the frequency of play-fighting, the data from these animals were pooled to form the control male group. Likewise the data from the sham-operated and the anesthetic-only females were pooled to form the control female group.

The data from Experiment 6 are summarized in Figure 12 where it can be seen that the control males engaged in more play-fighting than did either the amygdalectomized males, the amygdalectomized females, or the control females. Statistical analysis of the data revealed a significant effect of sex ($F = 8.96$; d.f. = 1, 55; $p < .005$) and a significant sex x lesion effect ($F = 5.75$; d.f. = 1, 55; $p < .02$). Post hoc analysis showed that the control males engaged in significantly more play-fighting than did the amygdalectomized-males ($t = 3.13$, $p < .01$), the amygdalectomized-females ($t = 3.04$, $p < .01$), and the control females ($t = 3.57$, $p < .01$). No other comparisons approached significance.

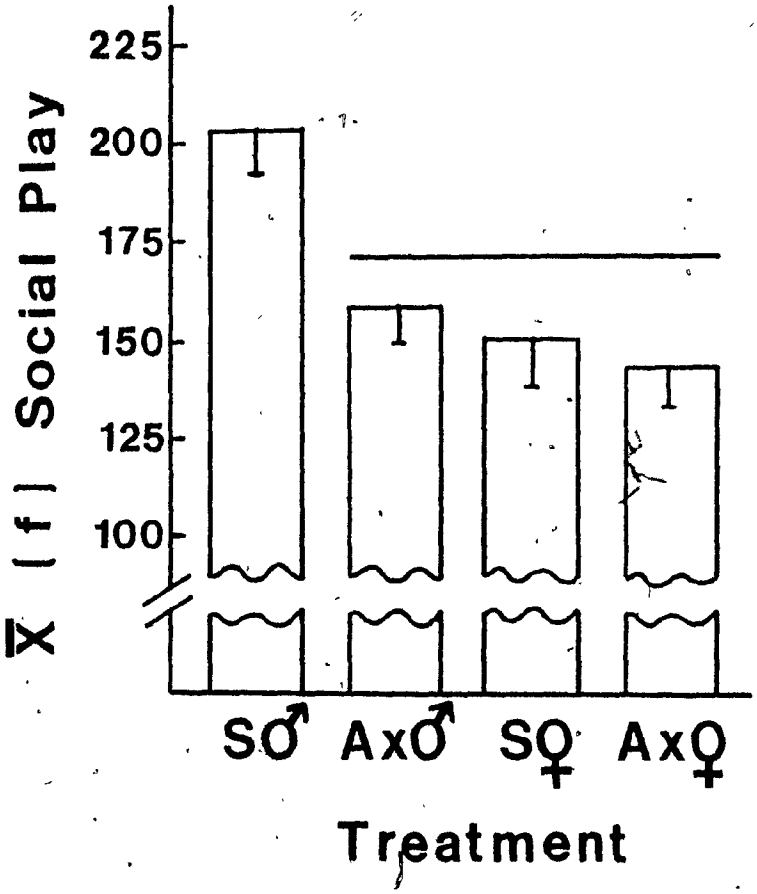

Figure 12. Mean (-SE) number of 20-sec sessions in which sham-treated males (SO♂), amygdalectomized males (AO♂), sham-treated females (SO♀), and amygdalectomized females (AO♀) were observed to engaged in play-fighting.

Presented in Table 3 are the body weight data from the weighings on Day 25 and Day 41. Statistical analysis of these data showed that there were no group differences for BW1, while there was, as to be expected, a significant sex difference ($F = 30.72$; d.f. = 1, 55; $p < .0001$) for BW2. There was no lesion effect on eiter BW1 or BW2.

Histological Findings

The lesions were large, bilaterally symmetrical, and centered in the medial portion of the basolateral nuclei. Damage was greatest in the posterior part of the amygdala. Destruction was most severe in the basolateral nuclei which were bilaterally involved in most cases. In the majority of cases there was bilateral damage to the cortical nucleus as well. The medial and posterior nuclei were damaged in some cases; often the invasion of these areas was unilateral. More posteriorly placed lesions invariably damaged the ventral hippocampus and the entorhinal cortex. The posterior portion of the caudate-putamen was damaged in some of the more dorsally placed lesions. Nine of the animals sustained unilateral damage to the optic tract and in three there was bilateral damage to the optic tract. There was no relationship between the extent of damage to the optic tract and the frequency of play-figting. Likewise neither lesion size nor location was correlated with the frequency of play-fighting. Overall the lesions in males and females were quite similar in anterior-posterior location, size, and

degree of optic tract damage.

In Figure 13 reconstructions of four lesions at the locus of the greatest extent are presented. Rat 2, a male, is typical of the more posteriorly placed lesions. Note the invasion of the ventral hippocampus. Rat 25, also a male, illustrates the appearance of the more anteriorly placed lesions. Rat 27, another male, and 47, a female, illustrate the variation in the size of the lesions at the anterior-posterior location where the majority of the lesions were largest. Rat 47 sustained the most severe damage to the optic tracts of any animal in the study. Despite the presumed visual impairment, its play frequency (194) was well above the mean for amygdalectomized-females (see Figure 12). By contrast, the male (rat 27) sustained more extensive damage to the amygdala without involvement of the optic tracts and played comparatively little (play frequency = 108).

The results of this study demonstrate a clear, sex-dependent effect of amygdaloid lesions on the play-fighting of prepubertal rats. Amygdalectomized males engaged in less play-fighting than did control males, whereas there was no such difference between the amygdalectomized and control females. The effect of amygdaloid lesions, then, was to eliminate the sex difference in social play.

This finding suggests that sex differences in the neural circuitry of some portion of the amygdala may underlie the

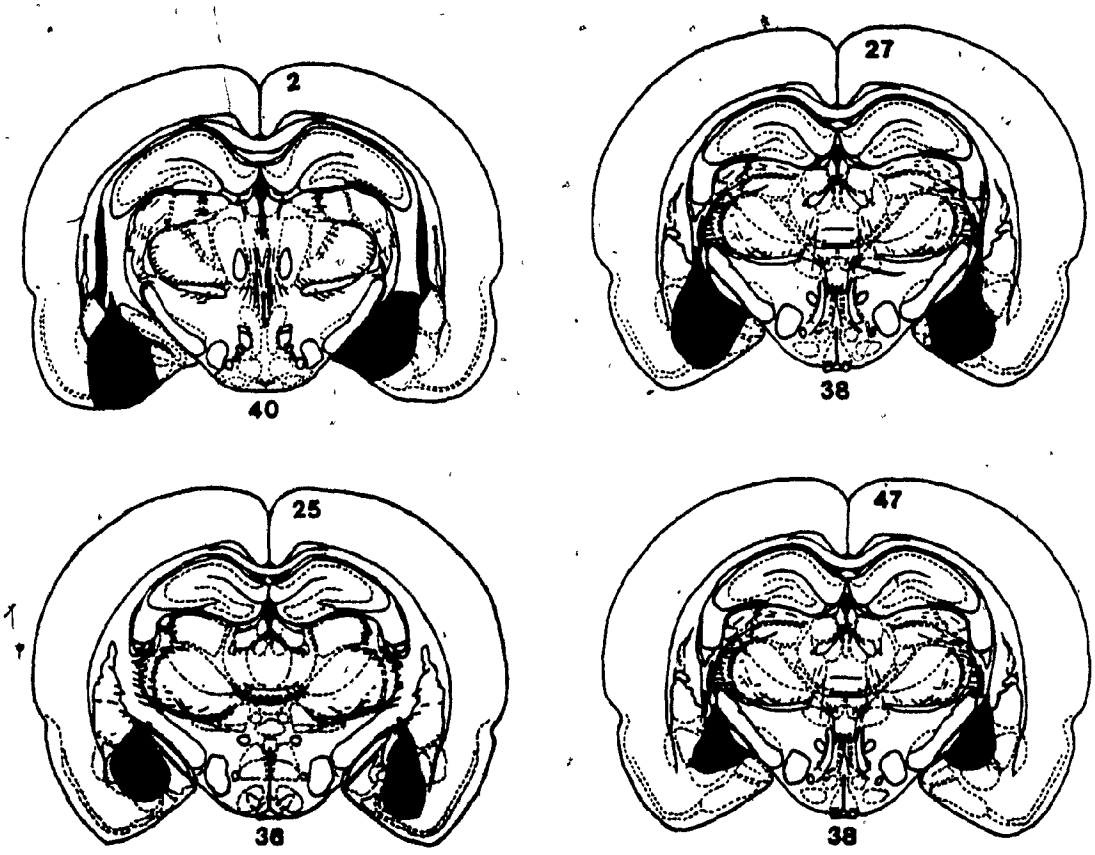

Figure 13. Reconstructions of four representative lesions at the anterior-posterior plane of maximum extent. Animal numbers are on the upper right side of each section. Numbers below the section are plate numbers from the Konig and Klippel (1963).

sex difference that is observed in the social play of prepubertal Norway rats. With respect to this suggestion it is interesting to note that there actually is evidence for androgen-induced sex differences in the amygdala. Staudt and Dorner (1976) have found a sex difference in the nuclear cell size in the central and medial nuclei of the amygdala that is dependent on the presence of androgens during the early neonatal period. Dyer, MacLeod, & Ellendorf (1976) found that when they stimulated the cortico-medial area of the amygdala and recorded from the medial preoptic and anterior regions of the hypothalamus (regions that receive projections from the cortico-medial portion of the amygdala), many more of these cells were driven by amygdaloid stimulation in males and in neonatally androgenized females than in either normal females or Day 1-castrated males. Nishizuka and Arai (1981) found a greater number of shaft synapses (i.e., synapses on the shafts of dendrites) in cells of the medial nucleus of male rats. Once again, the sex difference was dependent upon the presence of androgens during the early neonatal period. These findings suggest that there are sex differences in the neural circuitry of the amygdala that, like that in social play, are dependent upon the presence of neonatal androgens. It is also interesting to note that the amygdala of the neonatal rat contains androgen receptors (Sheridan, 1981). Thus, the mechanism for an androgen organizational effect exists in the amygdala of the neonatal rat.

Unfortunately, it is not clear whether changes in the anatomy of the developing amygdala are produced by a direct action of androgen (presumably involving the androgen receptor) or depend upon the aromatization of androgen to estradiol. Estradiol is known to stimulate the growth of neural processes (neurites) in cultures of hypothalamic tissue from the brains of neonatal mice (Toran-Allerand, 1978) and may be responsible for the differentiation of the sexually dimorphic nucleus in the preoptic area of the rat brain recently described by Gorski, Gordon, Shyrne, & Southam (1978). With this caveat in mind it is nevertheless tempting to consider the possibility that androgen-dependent changes in the development of the amygdala may be responsible, in part, for the sex differences in play-fighting (whether or not it is the sexually dimorphic "amusement center" awaits further work).

One further question that follows from this work concerns the role of the amygdala in social play and <u>why</u> its influence may be sex dependent. A possible explanation lies in the function of the amygdala as a "relay" for olfactory information to other limbic structures, particularly thalamic and hypothalamic structures (see Hamilton, 1976). Meaney and Stewart (1981) have found that prepubertal male rats show sex-dependent, play-partner preferences. Between Days 21 and 35 male pups directed more play-fighting towards male than towards female littermates. Between Days 36 and 40, however,

this preference is completely reversed; male pups during this period direct more play-fighting towards female than towards male littermates. Interestingly, this period (Days 36 - 40) is also the age at which males begin to show a preference for the olfactory cues of same-aged females. Meaney & Stewart (Note 2) have found that male pups between 35 and 55 days of age show a preference for the urine (an odour cue) taken from same-aged females compared to urine taken from same-aged males or to a neutral substrate. Male pups tested prior to 35 days of age showed no such preference. In contrast, prepubertal females showed no sex-dependent, play-partner preference (Meaney & Stewart, 1981), nor did they show any urine preference in the same test described above (Meaney & Stewart. Note 3). Thus, it may be that the use of olfactory cues in social play is unique to males, and that the disruption of this information by amygdaloid lesions, then, affects the play of males and not that of females.

These considerations imply that the influence of neonatal androgens on play-fighting may be related to this amygdaloid processing of olfactory cues. Neonatal androgens might act to induce a sex difference in the anatomy of cells within the amygdala that receive projections from olfactory areas. The function of these amygdaloid cells might be to determine the "salience" of certain olfactory cues. Thus, it might be that the males, being more responsive (attracted?) to the olfactory cues of conspecifics, are more likely to

initiate a social interaction with peers, and therefore are more likely to become involved in a play-fight. While this hypothesis is highly speculative, it might explain how the actions of androgens during the neonatal period influence behaviour at a later age.

General Discussion

A major point that can be made concerning the findings presented in this thesis is that the neuroendocrine basis of sex differences in social play in the Norway rat appears to parallel that of sex differences in the social play in the rhesus monkey. In both species male-typical levels of play-fighting result from exposure to androgens during an early period of neurogenesis (i.e. an organizational effect). Furthermore, in neither the Norway rat nor the rhesus monkey is the expression of male-typical levels of play-fighting dependent on the presence of androgens during the period when the sex differences in social play are observed (i.e., no activational effect). In addition, in both species the androgenic effect appears to be mediated by testosterone itself or or its 5α-reduced metabolite, dihydrotestosterone. In this sense it can be said to be a true androgen effect.

In humans data relevant to the present question have been obtained from children with an adrenal abnormality that results in a high exposure to adrenal androgens. This

condition has been referred to as congenital adrenal hyperplasia (CAH). It is a genetic abnormality in which the adrenal fails to produce cortisone. Instead there is an excessive secretion of adrenal androgens from the prenatal period until the disorder is detected. CAH females are born with masculinized genitalia and unless the condition is detected early they are often reared as males. The population of interest here, however, is that of CAH females in which the detection has occurred early. There are two advantages to studying these children. First, when the detection occurs early in postnatal life, the endocrine condition can be rectified, thus limiting the period of excessive androgen exposure primarily to the prenatal period. Second, these females are reared as females. Such girls have been found to engage in male-typical forms of play and to be identified as "tomboys" more often than control subjects (usually unaffected, same-sex siblings) (Ehrhardt, Epstein, & Money, 1968; Ehrhardt & Baker, 1974). These results are, of course, similar to those of Goy (1970; 1978) and to those described in the thesis.

One further similarity between the results of the present work with Norway rats and work with primates, concerns the sex-dependent effects of amygdaloid lesions. In several species of old world monkeys (Macaca mulatta, M. speciosa, Cercopithecus aethiops) amygdaloid lesions decrease the frequency of aggressive behaviour in both immature and

adult males. In contrast, amygdaloid lesions in females often increase aggressive behaviour (see Kling, 1974 for a review). Thus, in a variety of species there exists the possibility that sex differences in the morphology of the amygdala are related to sex differences in social behaviour.

As mentioned earlier, the possible involvement of neonatal androgens in the sexual differentiation of the limbic forebrain is as speculative as it is intriguing. Recent work on the development of the androgen receptor system, however, has added to the plausibilty of such an androgen-induced, organizational effect in the amygdala. Sheridan (1981), using autoradiograpghy with labelled androgens, has found direct evidence for androgen receptors in the amygdala of two-day old rats. Butte, Moore, and Kakihana (1980) have found that brains taken from one- and two-day old male rats contained about 10 times more testosterone than did brains taken from females of the same-age. Note that in Experiment 4 it was found that testosterone treatment on Day 1 and 2 of life masculinized the social play of female rats. Finally, Meaney, Poulin, and Stewart (Note 5) have found that the neonatal treatment of male pups with the anti-androgen Flutamide, blocked the masculinization of social play. Taken together with the results of Experiment 6 these findings suggest that one way this androgen hypothesis might be directly tested is through the use of androgen implants into the amygdala of Day 1

females. On the basis of the hypothesis presented here, these implants would be expected to masculinize social play behaviour. Such an effect would argue for a direct, androgen-induced organizational effect on the sexual differentiation of behaviour. Assuming that the androgenic exposure could be restricted to the amygdaloid area, this would also provide evidence for the proposed relationship between sex differences in amygdaloid morphology and sex differences in social play, thus also localizing the site of action.

On the basis of the work presented here and that of others, it is possible to dissociate the neuroendocrine influences on play-fighting from those on other forms of social behaviour. This dissociation rests largely on the finding that play-fighting, unlike other forms of social behaviour, is not influenced by circulating levels of hormones at the time the behaviour is observed. Joslyn (1973), for example, found that while juvenile female rhesus monkeys injected regularly with 2 mg of testosterone exhibited a dramatic increase in social dominance rank, eventually dominating their male peers, they showed no increase in the frequency with which they engaged in play-fighting. moreover, while the potential of an animal to engage in intraspecies aggression has been related to concurrent levels of androgens

(e.g., Beeman, 1947; Brain & Nowell, 1969; Rose, Rose, Holaday, & Bernstein, 1971), play-fighting occurs independent of circulating levels of androgenic hormones (Goy, 1970; Meaney & Stewart, Note 4.; Experiments 2 and 4). Meaney and Stewart (Note 3.) found that although testosterone treatment of juveniles resulted in precocious male copulatory behaviour (also see Baum, 1972), it did not affect play-fighting. Also, in some recent work Meaney, Stewart, and Beatty (Note 4.) found that while the neonatal exposure of male rats to corticosterone decreased the frequency with which they engaged in play-fighting, it did not affect the potential of the animals to exhibit either male or female copulatory behaviour. In addition, lesions of the medial preoptic area of the hypothalamus, an area whose integrity is essential for male sexual behaviour in many species, do not influence the play-fighting of either Norway rat pups (Leedy, Vela, Poplow, & Gerall, 1980) or juvenile rhesus monkeys (Goy, Kemnitz, Slimp, Irving, & Neff, Note 1.).

Thus, the factors that influence the occurrence of play-fighting can be dissociated from those that influence other forms of social behaviour. This is an important point when one considers the potential developmental consequence of social play, for it allows us to discount the possibility that a relationship between social play and, say, later success in agonistic encounters (see Taylor, 1980) is simply due to the fact that the two behaviours share the same

neuroendocrine mechanisms. Within this context, play-fighting can be considered as an independent behavioural system.

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REPRINT NO. 4 from *Animal Behaviour*, 29, 1, February, 1981

Anim. Behav., 1981, 29, 34–45

A DESCRIPTIVE STUDY OF SOCIAL DEVELOPMENT IN THE RAT (*RATTUS NORVEGICUS*)

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Abstract. The social interactions between juvenile rat pups (*Rattus norvegicus*) were observed daily between 21 and 55 days of age. The data were analysed for developmental changes, sex differences, play-partner preferences, and dominance relations. The results revealed clear patterns in the development of sexual and agonistic behaviours in the rat. This development refers to changes in either the manner in which animals interact or the behaviours involved in social play, but not to the topography of specific behaviours. Development toward the adult pattern of social behaviour was temporally associated with the period of sexual maturation (i.e. between days 36 and 50).

Within the social unit of the colony, the rat exhibits a complex and well-organized repertoire of social behaviour. Factors such as the age, sex, familiarity, and social rank of an animal and, in addition, the nature of the setting in which social interactions occur, influence the social behaviours exhibited by the animals. For example, while very little aggression is observed between two males placed in an unfamiliar setting, a great deal occurs if the setting is familiar to one of the animals, as in intruder-directed aggression (see Barnett 1958; Meaney & Stewart 1979). Thus, the normal adult social behaviour of the rat is characterized by differential responding to cues relating to other animals and to the environment. The ultimate social functioning of an animal, then, is dependent on its ability to exhibit the appropriate behaviours within the appropriate context.

While there exist several detailed, descriptive studies of these social skills in adult rats, the conditions necessary for their pre-adult development are far less well understood. One approach to this question has been to restrict or to eliminate the early social contact between animals for certain periods of time. The results of these social deprivation studies have established that in the rat, the juvenile period, between ages 21 to 55 days, is important for the development of normal adult social behaviour. When tested as adults, rats reared in social isolation during this period have been found to exhibit abnormal patterns of mating behaviours (Gerall *et al.* 1967; Hård & Larsson 1968; Gruendel & Arnold 1969), agonistic behaviours (Lore & Flannelly 1977), and affiliative behaviours (Meaney & Stewart 1979).

The results of these deprivation studies indicate that a certain amount of early social experience is necessary for the development of functional patterns of behaviour in adults and help to establish parameters for possible critical periods for this development. They do not, however, provide information about the nature of the early experience necessary for normal social development in any specific area, nor about the normal process of social development in the species (also see Bekoff 1976). Thus, while we may know that some form of early social experience is necessary for competency in some prescribed class of behaviours in the rat, we know little of the nature of that early experience, or of how that experience lends to subsequent competency. Indeed, although the rat has been a popular subject for deprivation studies, little is known of what the animals actually do during the period most commonly used for deprivation. In this paper we present the results of a descriptive study of the social interactions of juvenile rat pups.

Methods

The animals observed in this study were 24 hooded rats, 12 males and 12 females, of the Long-Evans strain. The animals were born in the animal colony at Concordia University to four dams that were obtained while pregnant from the Canadian Breeding Farms and Laboratories, St. Constant, Québec. Upon arrival at the animal colony the females were placed into observation units that served as housing throughout the study. The females were provided with an ample supply of nesting material (torn pieces of paper towelling and large wood chips) that was renewed every second day until the pups were 10 days of age. Each unit contained food and water that was provided ad libitum. The animals were

maintained on a 12L/12D schedule (lights on at 0800 hours).

At six days of age (the day of birth was considered as day 0) six pups, three males and three females, were selected from each litter on the basis of the distinctiveness of their markings. The remaining pups were removed. The markings of the six pups were recorded and later used for identification. No pups whose body weight was one standard deviation above or below the mean body weight for the litter were used in the study. Following weighing, the pups were returned to the nest. The total time of separation from the mother was about 15 min. Thereafter, the animals were not handled until the end of the study. Two litters were observed during the period of October to December 1978, and two during the period of February to April 1979.

The observation units were comprised of two areas: a larger area and an adjacent nesting area. The nesting area was 20 cm × 20 cm × 20 cm. The larger area was 60 cm long, 30 cm wide, and 45 cm high. The floors and walls of the nesting area were made of 1.25-cm plywood, while the top was made of darkly tinted Plexiglas. The side walls and the back of the larger area were also made of 1.25-cm plywood, the floor was made of 0.75-cm wire mesh, and the top and front were made of clear Plexiglas. An external feeder and water bottle were mounted on the back side of the larger area. A 5-×-5-cm grid permitted access to the feeder.

Observations began on day 21 and were conducted daily until day 55. All observations were conducted within the first 3 h of the dark cycle (a period of high activity in the rat). The observer sat about 1 m from the front of the observation units. During the dark cycle the room was illuminated by a single 25-W light, mounted about 5 m from the observation units.

Each day, each pup in each litter was observed for 30 observation sessions. Each session lasted 20 s. This period was chosen because it was sufficient in length to describe in detail the ongoing social behaviour of a pup and yet brief enough to allow for several observation sessions per animal per day. Within each litter the pups were observed sequentially until all six had been observed for one 20-s session each. The observer then changed to the second litter and repeated this procedure until all six pups in both of the litters had been observed for 30 sessions.

During each observation session the observer focused on one pup and recorded (1) any social behaviour exhibited by that pup and (2) the pup toward which the behaviour was directed or, in cases where the observed pup was the recipient of the act, the pup that directed the social act. Thus, all social behaviour that occurred within the 20-s session that involved the observed pup was scored. It was, then, possible for a behaviour to be scored more than once during a single observation session. Also, a behaviour was scored even if it occurred only in part during the 20-s session (e.g. ongoing behaviour at the onset of the 20-s session, behaviour that was terminated before the end of the session, etc.). Social interactions involving the mother were also scored, but were analysed separately. The following is a list of the behavioural categories used in this study.

Pouncing. This behaviour is most similar to what would be considered as attack behaviour in adult animals. One pup lunges at another with its forepaws extended outward. It is the forepaws that first make contact with the other animal. Pouncing was considered a play-initiation act since the animals exhibiting this behaviour invariably engaged in a play-bout so long as the recipient animal responded, and because it temporally preceded any other behaviour in the play sequence (also see Poole & Fish 1976).

Wrestling. Two animals roll and tumble with one another.

Boxing. Two animals stand upright facing one another and make pawing movements toward each other (the occurrence of this behaviour between adult rats has been referred to as 'upright defensive posture' by Blanchard et al. 1977).

Lateral display. One animal arches its back and, with all four limbs extended, directs its flank toward another animal (the occurrence of this behaviour between adult animals has been referred to as 'lateral attack behaviour' by Blanchard et al. 1977).

Neck Grooming. One animal vigorously grooms another, while firmly gripping the fur of that animal, usually around the region of the neck, with its forepaws (the occurrence of this behaviour between adult rats has been referred to as 'aggressive grooming' by Grant & Mackintosh 1963).

On-the-Back Posture. One animal lies on its back, fully exposing its ventral surface to another animal (the occurrence of this behaviour between adult animals has been referred to as

'submission posture' by Grant & Mackintosh 1963).

On-the-Back Posture/Kicking. This category involves the On-the-Back Posture as well as the animal kicking at the other animal.

On-Top Posture. One animal positioned over another animal with its forepaws placed on the second animal (the occurrence of this behaviour between adult animals has been referred to as 'dominance posture' by Barnett 1958).

Social Grooming. The mutual grooming of one animal by another.

Anogenital Sniffing. Sniffing directed at the anogenital region of another animal.

Chase. The pursuit of one animal by another.

Mounting. Included the full mounting pattern of one animal approaching another animal, placing its forepaws on that animal, and executing pelvic thrusts.

Lordosis. An animal presents its anogenital region elevated and accompanied by a downward arching of its back.

Grab/Social Groom. One animal grabs the fur of another animal and grooms that animal in the region of its neck. This behaviour could be distinguished from Neck Grooming by the fact that Neck Grooming was directed toward an animal that was lying on its back during the course of a play-bout, whereas Grab/Social Groom never occurred within the context of a play fight. Social Grooming was also far less intense than was Neck Grooming.

For Wrestling and Boxing the same bout was actually scored twice since both behaviours involved two animals concurrently performing the same behaviour (i.e. if animal A wrestled or boxed with animal B, then both animals were scored as having engaged in a bout of Wrestling or Boxing). For all other behaviours only one animal of a dyad could be engaged in a particular behaviour at any one time. The other animal was necessarily engaged in some other behaviour. In no cases would the same bout be scored in two or more observation sessions. If, for example, a bout of Boxing lasted long enough to be scored in the observation sessions of both the participants, it was ignored following the first scoring.

The behaviours of Pouncing, Wrestling, Boxing, Lateral Display, Neck Grooming, On-the-Back Posture, On-the-Back Posture/Kicking, and On-Top-Posture were considered as play fighting (also see Poole & Fish 1976). As with many other species, the play fighting of juvenile rats is not easily defined, although it can be distinguished from the aggressive encounters between adults. One distinctive feature of play fighting, for example, is the almost complete absence of the distress vocalizations that are prevalent in the aggressive encounters between adult rats (also see Calhoun 1962). Another distinctive feature is that, while an adult male rat which is dominated by another is most likely to flee or, if that alternative is blocked, to engage in appeasement behaviours (see Barnett 1958), a pup that has been 'dominated' by another during a play fight is likely immediately to initiate another bout of play fighting, frequently with the animal that has just 'dominated' it. While we do not wish to define play as being a class of behaviours independent of agonistic behaviours, the distinctions are important. The play fighting between pups is, perhaps, best considered as an agonistic encounter between immature animals.

Data Analysis

It should be made clear that, in this study, the sampling technique was chosen to provide data concerning the frequency with which individual animals engaged in particular behaviours rather than the frequency with which particular behaviours occurred. With the exception of Wrestling and Boxing (see Methods), however, these two frequencies are the same since with all other behaviours only the behaviour of one actor was scored. The frequency scores presented in Table I are, then, the frequency with which groups (i.e. males and females) of individual animals engaged in the various behaviours.

The frequency score for each behaviour for each pup was calculated over five-day intervals (age periods). There were seven of these five-day age periods between days 21 and 55.

The data for each behavioural category were examined in three ways. To investigate possible developmental trends the data were examined for total observed frequency over age. This was done by correlating (Spearman's r) the observed frequency of a behaviour with age, where age is represented by the seven age periods ($N = 7$). A high positive correlation indicated a developmental increase in the frequency of a behaviour, whereas a high negative correlation indicated a decrease in the frequency of a behaviour with age. It should be noted that a behaviour would correlate with age only if the frequency of that

behaviour consistently increased or decreased in successive age periods.

To investigate possible sex differences, the data for each behavioural category were examined by comparing the total observed frequency in each age period for male and female pups. The tests for sex differences were done using Mann–Whitney U tests ($N_1 = 12$, $N_2 = 12$ for all comparisons). In these tests the frequency of male-performed acts was compared to that of female-performed acts regardless of the sex of the partner. Tests were done separately for each age period.

To investigate possible sex-of-partner preferences the data were examined by comparing the relative frequency of male-performed and directed toward female (♂ → ♀), male-performed and directed toward male (♂ → ♂), female-performed and directed toward male (♀ → ♂), and female-performed and directed toward female (♀ → ♀) social acts for each behavioural category. The reason for using relative frequency rather than absolute frequency was that if one female of a group performed a specific behaviour there would be five other animals (excluding the mother) toward which the behaviour could have been directed. Of these five animals, three were males and only two were females. Thus chance alone would predict that 60% (three of five) of the female-performed behaviours would be ♀ → ♂ acts, and 40% (two of five) would be ♀ → ♀ acts. To correct for this inherent probability difference, the frequency of ♀ → ♀ and of ♂ → ♂ behaviours was multiplied by 0.6, and the frequency of ♀ → ♂ and of ♂ → ♀ behaviours was multiplied by 0.4. Sex-of-partner preference for male pups was determined by comparing the observed frequency of ♂ → ♂ acts and of ♂ → ♀ acts to the frequencies expected by chance. The sex-of-partner preference for female pups was determined by comparing the observed frequency of ♀ → ♀ acts and of ♀ → ♂ acts to the expected frequencies. A sex-of-partner preference was defined as a difference between the observed and the expected frequencies of same-sex-directed and opposite-sex-directed acts. Statistical comparisons were made using 2 × 2 contingency tables ($df = 1$ for all comparisons).

Results

The frequency score for male and female pups for each behavioural category and by age is presented in Table I. A summary of the sex differences in juvenile social behaviour is presented in Table II. The behaviours presented in the 'over all ages' column of Table II were analysed by collapsing the frequency score across all age periods. This was done because of the low frequency of these behaviours in one or more age periods. A summary of the sex-of-partner differences over the various age periods is presented in Table III. In this section the data for the individual behavioural categories are presented under the classes of mating behaviours and agonistic behaviours. In addition data are presented concerning pup–mother interactions.

Play Fighting

The relative frequency of Pouncing in each age period for each of the four dyads is presented in Fig. 1. Pouncing increased markedly between the period of days 21 to 25 and that of days 26 to 30, and remained relatively constant thereafter. The fact that the frequency of this play-initiation behaviour remained stable after day 26 suggests that a change in the frequency of any one behavioural component of play observed after 26 days of age cannot be attributed simply to a change in the frequency of play in general.

Developmental changes in the frequency of the various behavioural components of play fighting were also observed. Specifically, the frequency of Wrestling ($r = -0.99$, $P < 0.001$) and, to a lesser extent, of On-the-Back Posture/Kicking ($r = -0.59$, $P < 0.09$), decreased with age. In contrast, the frequency of Lateral Display ($r = 0.73$, $P < 0.04$), of Boxing ($r = 0.77$, $P < 0.04$), and of On-the-Back Posture ($r = 0.77$, $P < 0.04$), increased with age. Behaviours that, among adult animals, are directly related to the expression of dominance (i.e. On-Top Posture and Neck Grooming) showed no change in frequency over age.

There were also sex differences in play fighting (see Table II). Males engaged in more Pouncing (play initiation) behaviour than did females in all but the first age period. Males engaged in more On-Top Posture and Neck Grooming than did females in all but the final two age periods (see below). In the final age period, males engaged in more Lateral Display behaviour than did females.

During the three age periods between days 21 and 35, males directed more play fighting toward other males than toward females. This difference involved virtually all the behavioural components of play fighting (see Table III). During the period between days 36 and 40 this play-partner preference shifted from ♂ → ♂ to ♂ → ♀. As in the previous periods, this differ-

Table I. The Frequency Scores for Male and Female Pups for Each Behaviour in Each Age Period

Behaviour	Age period (days) and sex													
	21–25		26–30		31–35		36–40		41–45		46–50		51–55	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Pouncing	42	21	166	72	185	67	233	91	187	45	172	92	179	54
On-Top Posture	20	6	73	28	65	26	67	30	52	15	45	16	61	19
Wrestling	199	93	149	109	80	46	24	40	22	18	15	13	5	9
Boxing	40	16	60	33	54	40	57	50	41	27	97	100	88	45
On-the-Back Posture	6	0	12	5	18	11	11	16	14	12	14	13	24	13
On-the-Back/Kick	38	24	38	27	33	19	28	30	24	33	15	32	11	36
Neck Groom	15	7	34	5	31	14	37	10	26	5	21	3	26	4
Lateral Display	0	0	4	0	8	1	11	6	20	8	12	15	38	15
Chase	16	16	49	24	44	21	53	27	73	11	71	23	37	12
Social Groom	19	31	14	37	8	19	6	21	7	30	14	42	10	30
Anogenital Sniff	1	1	4	1	10	5	28	7	65	8	56	19	34	4
Mounting	0	0	0	0	6	0	10	7	70	0	136	0	53	1
Lordosis	0	0	0	0	0	0	0	0	0	41	0	64	0	24
Grab/Social Groom	2	0	1	1	17	2	14	3	14	2	6	0	2	1

Table II. Summary of Significant Sex Differences in Behaviour in Each Age Period

Days 21–25 Male > Female	Days 26–30 Male > Female	Days 31–35 Male > Female	Days 36–40 Male > Female	Days 41–45 Male > Female	Days 46–50 Male > Female	Days 51–55 Male > Female
Box*	Pounce**	Pounce**	Pounce**	Pounce**	Pounce*	Pounce**
Wrest*	OTP†	OTP†	OTP†	OTP†	Chase*	Chase*
OTP*	Chase*			Chase**	Mount**	Box**
				Mount**	A-G Sniff**	LD*
		Over All Ages		A-G Sniff**		Mount**
		Male > Female				A-G Sniff**
		Neck Groom				
		Female > Male		Female > Male	Female > Male	Female > Male
		Soc Groom†		Lordosis**	Lordosis**	Lordosis**

List of Abbreviations: Box: Boxing, Wrest: Wrestling, OTP: On-Top Posture, Pounce: Pouncing, Mount: Mounting, A-G Sniff: Anogenital Sniffing, LD: Lateral Display, Soc Groom: Social Grooming, OBP: On-the-Back Posture, OBP/K: On-the-Back/Kicking, G/Soc Groom: Grab and Social Groom.
*$P < 0.05$, †$P < 0.01$, **$P < 0.005$.

Table III. A Summary of the Male-Performed Behaviours for which there were Sex-of-Partner Differences in Each Age Period

Days 21–25 ♂→♂	Days 26–30 ♂→♂	Days 31–35 ♂→♂	Days 36–40 ♂→♂	Days 41–45 ♂→♂	Days 46–50 ♂→♂	Days 51–55 ♂→♂
Pounce*	Pounce**	Pounce**	OBP**	OBP**	OBP**	Pounce**
OTP*	OBP**	OBP/K**		Neck Groom†	Neck Groom*	OTP**
Neck Groom*	OBP/K**	LD*		LD*	LD†	Box**
Box (♂ + ♂)**	Neck Groom†			Box**		OBP**
	Box**					OBP/K**
						Neck Groom**
						LD**
♂→♀	♂→♀	♂→♀	♂→♀	♂→♀	♂→♀	♂→♀
	Soc Groom*	Soc Groom*	Pounce*	Chase**	Chase**	Chase**
		G/Soc Groom**	Chase†	A-G Sniff**	A-G Sniff**	A-G Sniff†
			Wrest (♂ + ♀)†	Mount**	Mount**	Mount**
			OBP/K*	G/Soc Groom†	G/Soc Groom†	
			A-G Sniff†			
			G/Soc Groom**			

*$P < 0.05$, †$P < 0.01$, **$P < 0.005$. For a list of abbreviations see Table II.

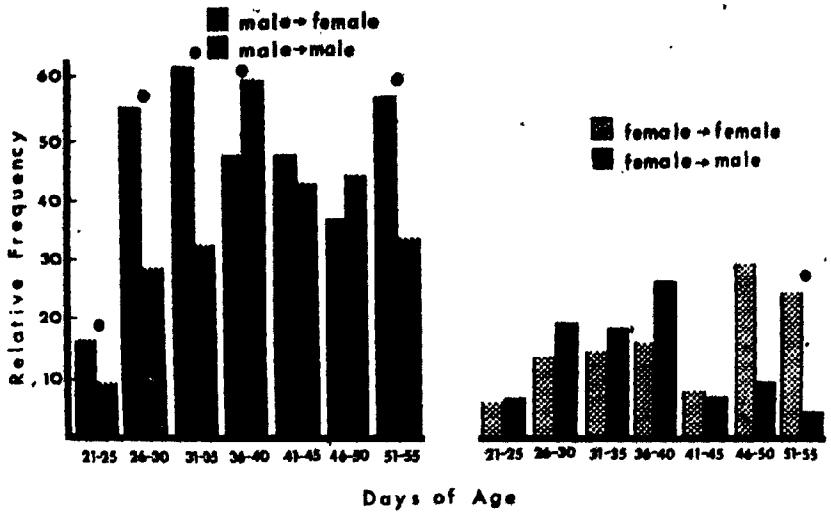

Fig. 1. The relative frequency of Pouncing (play initaition) in each age period for each of the four dyads. Significant sex-of-play-partner preferences are indicated by the closed circle.

ence involved most of the behavioural components of play fighting. In the two periods between days 41 and 50, there were no partner preferences for Pouncing behaviour, although there were differences in the behavioural components of play fighting. All of these behaviours, save for that of Wrestling, occurred most frequently in ♂ → ♂ encounters. In the final period between days 51 and 55, males once again directed more Pouncing toward males than toward females. Moreover, during this period all of the behavioural components of play fighting were observed more frequently in ♂ → ♂ bouts than in ♂ → ♀ bouts.

As previously mentioned, males engaged in more dominance-related behaviours than females did in all but the last two age periods. The absence of a significant difference in these later periods was due to the fact that in these periods the On-Top Posture score was accounted for almost entirely by only one male pup per litter, and in one case two males. In the earlier periods, On-Top Posturing was exhibited by all male pups and the scores were evenly distributed between them. Specifically, On-Top Posture was observed on more than three occasions in 10 of 12 males between days 26 and 30, in 8 of 12 between days 31 and 35, and in 8 of 12 between days 36 and 40. In contrast, during the periods between days 46 and 50 and days 51 and 55, only 4 of 12 males showed the On-Top Posture on more than three occasions. In addition, the rankings of the pups within each litter, based on the frequency of On-Top Posture, are virtually the same for these two final periods (see Table IV). Prior to day 46, no such consistency existed in the dominance relations. This consistency in the dominance rankings together with the change in the

Table IV. Dominance Rankings for Male Pups with Each Litter between Days 36 and 55*

Litter	Rank	Age period (days) 36–40	41–45	46–50	51–55
*1	1	♂1	♂1	♂2	♂1
	2	♂2	♂3	♂1	♂2
	3	♂3	♂2	♂3	♂3
2	1	♂2	♂1	♂3	♂3
	2	♂1	♂2	♂2	♂2
	3	♂3	♂3	♂1	♂1
3	1	♂2	♂2	♂1	♂1
	2	♂3	♂1	♂2	♂2
	3	♂1	♂3	♂3	♂3
4	1	♂2	♂3	♂3	♂3
	2	♂3	♂2	♂2	♂2
	3	♂1	♂1	♂1	♂1

*Rankings are based on the frequency of On-Top Posture.

distribution of the On-Top Posture scores suggests that a stable dominance hierarchy is formed in the play fighting of the older males.

Another observation was the emergence of a sex-dependent difference in response pattern during play fighting. The nature of this difference is shown in Fig. 2, in which the probability of a play-bout resulting in an On-Top/On-the-Back relation P(OT/OB) between two animals is plotted against age period. This measure was calculated by dividing the total number of play-bouts into the number of play-bouts that involved an OT/OB relation, for each age period. It can be seen that during the periods between days 26 and 40 the P(OT/OB) for male-initiated play-bouts is the same for both male and female partners. Thereafter, ♂ → ♂ play-bouts are about twice as likely to result in an OT/OB relation as are ♂ → ♀ play-bouts. There is no difference between the P(OT/OB) for ♂ → ♂ play-bouts and that for ♀ → ♀ play-bouts.

Mating Behaviour

In males, the onset of Mounting occurred between days 41 and 45, although Mounting was occasionally scored prior to this period. The frequency of male Mounting increased in the subsequent period, between days 46 and 50, and then decreased in the final period, between days 51 and 55. The onset of Mounting was associated with an increase in the frequency of both Anogenital Sniffing and Chase behaviours. The correlation between Chase and Mounting over the seven age periods ($r = 0.75$, $P < 0.03$) was significant, as was that between Anogenital Sniffing and Mounting ($r = 0.93$, $P < 0.002$).

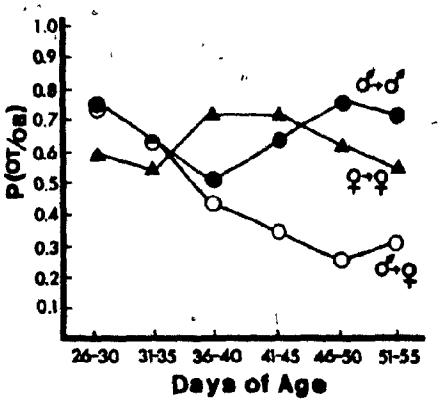

Fig. 2. The probability of a play-bout resulting in a On-Top/On-the-Back relation ($P(OT/OB)$) for ♂ → ♂ ♂ → ♀, and ♀ → ♀ dyads over age periods.

Each of these three behaviours was predominantly male-initiated.

A behaviour apparently unique to the juvenile period, Grab/Social Groom, was observed on several occasions. This behaviour is of interest for two reasons: it frequently elicited a lordotic response, and it was almost always a ♂ → ♀ behaviour (52 of 56 ♂ → occurrences were ♂ → ♀). Between days 46 to 50 this behaviour was rarely observed. It is interesting that this period coincided with a large increase in the frequency of Mounting behaviour.

Between days 36 and 40, males directed more Chase, Anogenital Sniffing, and Grab/Social Groom toward females than towards other males. In the subsequent periods these behaviours as well as that of Mounting, were predominantly ♂ → ♀. By days 51 to 55, then, males were directing more play-fighting toward males than toward females, and more mating-related behaviours toward females than toward males.

Lordosis was not observed in the female pups until day 42. During the period between days 41 and 45, 25% (3 of 12) of the females were observed showing the Lordosis posture. By day 50, 50% (6 of 12) had been observed showing Lordosis, and 58% (7 of 12) by day 55. The only female-predominant behaviour, other than Lordosis, was Social Grooming (see Table II).

Pup–Mother Interactions

During the periods between days 21 and 30, the most frequent pup–mother behaviour was that of Social Grooming. Female pups accounted for 66% (51 of 77) of these bouts of Social Grooming. Throughout the periods between days 31 and 50, males directed more Pouncing (66%, 84 of 126), Chase (74%, 62 of 84), Anogenital Sniffing (77%, 51 of 66), Grab/Social Groom (93%, 14 of 15), On-Top Posture (78%, 18 of 23), On-the-Back Posture (93%, 14 of 15), and Mounting (97%, 84 of 87) toward the mother than did the females. The females continued to direct more Social Grooming (76%, 37 of 49) toward the mother than did the males. These sex differences are consistent with those seen in pup–pup interactions. Pup–mother interactions virtually ceased to occur during the period between days 51 and 55.

On several occasions the mother was observed to initiate play-bouts with (i.e. to Pounce on) the pups. These mother–pup play-bouts did not appear to differ from pup–pup play-bouts. They occurred far less frequently, however, than did pup–pup play-bouts.

Discussion

We will discuss the behavioural development of the rat with respect to the classes of behaviour observed in this study. These classes are mating behaviour (Mounting, Lordosis, Anogenital Sniffing, and Grab/Social Groom) and play fighting (On-the-Back Posture/Kicking, Wrestling, Boxing, Lateral Display, Neck Grooming, On-the-Back Posture, On-Top Posture, and Pouncing). What is important here is not so much the specific ages at which certain behaviours emerge, but rather the patterns in the development of these behaviours. Indeed, the age of onset of these behaviours, particularly of sexual behaviours, may be expected to vary depending on factors such as the nature of the social unit in which the animals develop (see Christian 1971 for a review of the data from several species of rodents; Vandenbergh 1971 with house mice, *Mus musculus*).

For this reason it may be useful to consider, briefly, the nature of social groups used in the present study. Since the animals were maintained in intact litters until day 55, no contact with either non-siblings or with adults, other than the mother, was possible. Although this situation differs somewhat from that occurring in the wild state, the data of Calhoun (1962) suggest that the differences are not great. Calhoun reports that although wild rat pups between 35 and 56 days of age made extensive independent excursions from the nest site, 'close association with the mother, insofar as a place of harborage is concerned, was maintained until 2 months of age' (page 150). Thus, although the pups in this study were not afforded the diversity of social interactions provided in the wild state, it appears that, even under these conditions, the complete dispersion of the young does not occur before 60 days of age.

Mating Behaviour

Successful mating in the rat is the result of three phases: the attraction between conspecifics of the opposite sex, the initiation of copulation, and the successful execution and completion of copulation. Since the successful completion of one phase is necessary but not sufficient for success in the subsequent phase, then these phases are, to a degree, independent of one another. Among adult animals there exist males that show an attraction toward females but do not initiate copulation (e.g socially isolated males, Gerall et al. 1967, and perinatally anti-androgenized males, Stewart & Kaczender-Henfik 1971), as well as males that initiate but do not complete copulation (e.g. chronically subordinate males, Calhoun 1962). The selective effects of such abnormal conditions attest to the independence of the phases of successful mating. These same phases comprise stages in the development of sexual behaviour in the male rat. The present study shows that the independence of these mating phases is also indicated by their age of onset.

The onset of the sexual attraction of the male toward the female can be seen in the social interactions between the pups before the time of onset of Lordosis in the female (between days 41 and 45) and before the marked increase in Mounting in the male (between days 41 and 45). This is evident in the shift of play-partner preference of the males from ♂ → ♂ to ♂ → ♀ (see Table V) that occurred between days 36 and 40. The attraction of the males to the females during this period is also evident in the increase in ♂ → ♀ Anogenital Sniffing and in Grab/Social Groom (see Table V). Thus, the male pups in this study exhibited an attraction to same-aged females (between days 36 and 40) prior to the

Table V. Relative Frequency Score of Male Pouncing and Mating Behaviours in ♂ → ♂ and ♂ → ♀ Encounters over Age Periods and Dyad Composition

	Age period (days) and dyad composition									
	31–35		36–40		41–45		46–50		51–55	
Behaviours	♂ → ♂	♂ → ♀	♂ → ♂	♂ → ♀	♂ → ♂	♂ → ♀	♂ → ♂	♂ → ♀	♂ → ♂	♂ → ♀
Pouncing	63	32	48	62	48	43	36	45	58	33
Mounting	2	1	1	3	7	24	7	50	1	21
Chase	11	10	8	16	7	24	4	26	2	13
Anogenital Sniff	1	3	2	10	5	22	4	20	4	11
Grab/Social Groom	0	7	0	6	2	4	0	2	0	1

actual onset of copulatory behaviour (between days 41 and 45).

The onset of the sexual attraction of the males to females between days 36 and 40 resulted in a shift of virtually all social play behaviours to ♂→♀ encounters. At its onset, then, sexual attraction in the male resulted in an increase in ♂→♀ play and not in sexual behaviours. By day 51, however, males were once again directing more play-fighting toward males than toward females.

Although Mounting was observed prior to the period between days 41 and 45, it was not until this period that it occurred with notable frequency. The observable features of the Mounting behaviour (including pelvic thrusts) of puberal male pups were indistinguishable from those of adult males. The most noticeable change in the Mounting of immature male pups was the improvement in orientation. Between days 41 and 45 siblings were often mounted from the side or from the head; by day 51 these misdirected mounts were rarely seen (also see Miller et al. 1977 for a similar finding with golden hamsters; *Mesocricetus auratus*).

The periods between days 41 and 50 were also marked by a further increase in ♂→♀ Anogenital Sniffing and by the onset of Lordotic behaviour in the female pups. From these facts it might be argued that the onset of receptivity in the females is the critical event in the increase in the frequency of Mounting. Sachs & Meisel (1979), however, found that only 37% of 37-day-old male rats mounted when exposed to receptive females while 100% of 43-day-old males mounted the females. Thus, although the onset of mounting in males corresponded to that of lordosis in females, the two events do not appear to be causally related. Interestingly, Goldman & Swanson (1975) found that in golden hamsters the onset of male mounting actually preceded that of lordosis in the female by about a week.

Subsequent to the onset of Mounting there was a period, between days 46 and 50, during which the occurrence of Mounting was almost three times that seen in the later period between days 51 and 55. These differences in the frequency of male Mounting occur despite the fact that the availability of behaviourally receptive females (i.e. females that showed Lordosis in response to a Mount) was similar in the three age periods between days 41 and 55 (three females exhibited Lordosis between days 41 and 45, four between days 46 and 50, and three between days 51 and 55). This high frequency of Mounting between days 46 and 50 may have been due to the relative incompetency of the males. Larsson (1967) and Södersten et al. (1977) have found that the number of intromissions necessary for ejaculation by immature male rats decreased with age (but also Sachs & Meisel 1979). Moreover, Sachs & Meisel found that the penile reflexes necessary for the adult pattern of sexual behaviour did not develop in male rat pups until about day 48. The male pup between 40 and 50 days of age appears, then, to be less able to ejaculate than is an older animal. Stewart & Kaczender-Henrik (1971) found that perinatally antiandrogenized male rats which did not ejaculate mounted more frequently than did animals that did ejaculate. Thus, the decrease in the frequency of Mounting seen in the period between days 51 and 55 may have been due to the maturing capability of the male to ejaculate (i.e. fewer mounts and intromissions, leading to an ejaculation).

In summary, then, the development of sexual behaviour in the male rat can be traced in three stages:

(1) The onset of sexual attraction toward the female (between days 36 and 40).

(2) The onset of Mounting (between days 41 and 45).

(3) The progression toward adult sexual competency (from days 51 to 55 and beyond).

In the rat the onset of male sexual behaviour does not appear to be the result of any continuous process in development (also see Bekoff 1977). That is, the onset of Mounting is sudden and is not preceded by any pre-mounting play behaviour. It is, however, interesting to parallel the development of sexual behaviour with the activity of the testicular hormones and with the maturation of the structures involved in the penile reflexes. Knorr et al. (1970) and Sachs & Meisel (1979) have shown that there is an increase in the plasma levels of testosterone in male rat pups at about 35 days of age. This increase temporally corresponds to the age at which the male pups in this study first demonstrated a play-partner preference for female siblings. Although no further increase in testosterone levels was reported to have occurred until day 50 (well after the onset of Mounting), Sachs & Meisel have shown that penile erection was first seen to occur at about day 40 (the age at which mounting was observed to increase in both this study and that of Sachs & Meisel). In

addition, in the Sachs & Meisel study ejaculation was found to develop concurrently with the development of the necessary penile reflex (by about day 48). Miller et al. (1977) have suggested that, in the sexual maturation of the male golden hamster, increases in gonadal androgens serve not only to increase the frequency of Mounting, but also to improve its quality. Finally, Södersten et al. (1977) have presented evidence suggesting that in male rats the CNS sensitivity to androgens increases with age.

Thus, the period of the development of sexual behaviour in the male rat is characterized by increases in the circulating levels of testicular androgens, the maturation of the penile reflexes, and a possible increase in CNS sensitivity to androgens, as well as an increase in the number of hormone-secreting cells (Leydig cells) in the testes (Knorr et al. 1970). As Sachs & Meisel (1979) conclude, sexual behaviour in the male rat seems to develop concurrently with morphological maturation. The findings of Beach (1942) and of Södersten et al. (1977) that testosterone administered to pre-puberal male rats advanced the age of onset of mounting would seem to suggest further that it is these maturational events that underlie the development of sexual behaviour in the normal, socially reared male rat.

In the female pups the onset of Lordosis appeared between days 41 and 45. This finding is in general agreement with those (e.g. Kragt & Ganong 1968) that have shown that vaginal opening and the initiation of ovulation occur at about day 40 in the female rat. Although not recorded, ear wiggling frequently accompanied Lordosis in this early period. In the periods following the onset of Lordosis, the females showed a play-partner preference for females.

Unfortunately, in the present study we were unable to document the solicitation pattern of the female rat. A study of this behaviour would best be done using a larger enclosure (see McClintock & Adler 1978) and film analysis. Such an analysis might help to determine whether the female darting and hopping behaviours that appear to facilitate male sexual activity (Caggiula et al. 1976) show developmental trends different from that of Lordosis. The rather strict hormonal control of sexual behaviour in the female rat, however, might suggest otherwise.

Play Fighting and the Development of Agonistic Behaviours

Despite the differences between the play-fighting of juveniles and the agonistic encounters of adults, most of the behaviour patterns involved in aggression among rats first emerge within the context of play-fighting. Pouncing (attack), Boxing, On-Top Posture, On-the-Back Posture, and Neck Grooming are all observed in the play-fighting of pups as young as 21 days of age (also see Poole & Fish 1976). The age of onset of this play-fighting is about day 17 (Meaney & Stewart, unpublished data). Each behaviour listed above was, at its initial appearance, exhibited by the pups in the adult pattern. There was no observable evidence of any developmental change in the topography of these species-specific agonistic behaviours. There does not, then, seem to be any practice effect of play-fighting among rat pups in terms of perfecting the execution of specific behaviours. A similar result has been reported by Poole (1966) in the play-fighting among juvenile ferrets (*Putorius putorius furo*). In the ferret, five of seven behaviour patterns associated with attack and three of four behaviour patterns associated with defensive behaviour were present in the adult form in the play-fighting of juveniles. Interestingly, in both the ferret and the rat it was those behaviours associated with threat and attack (attempted biting) that developed later (see below).

Despite the lack of topographical changes in behaviour patterns associated with aggression, there were clear developmental changes in the play-fighting of rat pups. During the period of days 21 to 25, play-fights seemed to erupt spontaneously between any two animals in proximity. The bouts did not appear to be initiated by one of the animals. By days 26 to 30 most play-fights occurred as the result of one animal Pouncing (attacking) on another. Another significant early change in these play-fights was the decrease in frequency of Wrestling behaviour with age. By the period of days 36 to 40, Wrestling behaviour had decreased markedly, and it was virtually absent in the encounters of sexually mature animals. Thus, among younger pups (days 21 to 30) play-fights were physically contested, spontaneous events, whereas among older pups play-fights were initiated by one animal, and the animal that was pounced on was almost immediately dominated.

As the animals approached sexual maturity play-fighting was increasingly marked by the

presence of Lateral Display behaviour. In adult animals this behaviour has been shown to be an attempt by the attacking animal to bite the dorsal side of the defending animal (see Blanchard et al. 1977). Indeed, Blanchard et al. have referred to this behaviour as a 'lateral attack'. While this behaviour was frequently observed in sexually mature pups, no instances of actual biting were recorded in this study. By the time of sexual maturity, then, the pups exhibited the full repertoire of adult agonistic behaviours.

Although the play-fighting of juvenile pups was distinguishable from the agonistic encounters of adults, this distinction was easier to make in younger pups than in older ones. As male pups matured, their play-fighting came to approximate adult inter-male aggression. A number of changes attested to this development. One was the change in the behavioural components of play-fighting. Among the older pups the behaviours related to direct confrontation (i.e. Lateral Display and Boxing) were seen with increasing frequency. Another change was in the apparent function, or lack of function, of play-fighting. While there was no evidence of any dominance relations in the play-fighting of younger pups (21 to 40 days of age), there was evidence of such relations in the play fighting of older animals (45 to 55 days of age). Presumably, play fighting was serving a function among older animals that it did not among younger ones. Finally, there was the development of a differential pattern of play in ♂ → ♂ and ♂ → ♀ encounters. Evidence of this pattern was the difference in the probability of a ♂ → ♀ play-fight resulting in an On-Top/On-the-Back relation compared with a ♂ → ♂ play-fight. This difference was apparent only after day 45. All of these changes resulted in an increasing similarity between play-fighting and adult aggression. Moreover, Poole & Fish (1976) found that the degree of temporal organization among the play behaviours of rat pups increased with age. It seems as though these changes, at least in part, mark the onset of inter-male aggression. The evidence of dominance relations, in particular, seems to support this contention.

It is interesting to note that these changes occurred at about the time of sexual maturity. Indeed, in the mouse (*Mus musculus*) McKinney & Desjardins (1973) have reported that the onset of inter-male aggression occurred at the time of sexual maturation. Moreover, Johnson et al. (1972) have shown that in the rat the onset of predatory aggression (toward frogs, *Rana pipiens*), occurred at the time of sexual maturation.

General

A final point to be made here is that throughout the study there was no observable change in the topography of any of the behaviours recorded. At the point of onset all behaviours were exhibited in their adult form. This finding does not appear to be unique to the rat (see Bekoff 1977 for a review of relevant data concerning infra-primate, mammalian species). There appears to be little evidence that the ability to execute any specific behaviour is modified by early experience. Rather, it is the probability of a specific behaviour being executed within a particular context that is experientially modified (see Harlow 1969; Bekoff 1977). That is, the behaviour appears to become more appropriate to the salient stimuli in the environment, and especially to stimuli from other animals.

In the context of the present study it is difficult to state exactly what specific events contribute to this developmental change. A good example of such a change was observed in the social interactions in male-male and male-female dyads. Initially the male directed the same behaviours toward the female as it did toward other males (i.e. play-fighting). At the onset of sexual attraction, when the male started chasing and sniffing the female, there was a shift to a preference for females as play partners, but there was no evidence of explicit sexual behaviour. By the period between days 51 and 55, however, the male began to direct sexual behaviours toward females and to direct aggression-like behaviours toward males. It is, of course, this pattern that characterizes the adult male. While it is apparent that these changes are determined in part by hormonal and morphological development, it is important to note that animals deprived of any social experience have been observed to direct the same social acts toward both males and females (see Introduction). The crucial problem for the future will be to identify the processes whereby early experiences contribute to the development of this appropriateness in social behaviours.

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(*Received* 17 *September* 1979; *revised* 4 *February* 1980; *MS. number:* A2380)

HORMONES AND BEHAVIOR 15, 197–213 (1981)

Neonatal Androgens Influence the Social Play of Prepubescent Rats

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The results of six experiments designed to investigate the hormonal basis of the sex differences in the occurrence of social play in the rat are reported. From the time of weaning animals were housed in mixed-sex, peer groups of six, composed of some treated and some untreated animals. Observations were made of the animals in these groups each day between Days 26 and 40 of life in Experiments 1, 3–6 and between Days 31 and 40 in Experiment 2. In Experiment 1 it was found that males castrated on Day 1 of life engaged in less social play than did intact males, and did not differ from normal females. In Experiment 2, castration carried out at 23 days of age had no effects on the frequency with which males engaged in social play. In Experiment 3, it was found that neonatal ovariectomy had no effect on the frequency with which female pups engaged in social play. In Experiment 4, females treated on Days 1 and 2 of life with either 250 μg of testosterone propionate or 250 μg of dihydrotestosterone engaged in social play at rates comparable to those of normal males, whereas treatment with 5 μg of estradiol benzoate had no such effect. In Experiments 5 and 6 it was found that neither the reduction of testosterone-derived estradiol (by implants of the aromatization blocker, androst-1,4,6-triene-3,17-dione) nor that of testosterone-derived dihydrotestosterone (by implants of the 5α-reductase blocker, testosterone 17β-carboxylic acid) during the early neonatal period (Days 1 to 10 of life) changed the frequency of social play in intact males. The results of these experiments indicate that the sex difference in the social play of prepubescent rats is dependent on the neonatal exposure to testosterone or to its 5α-reduced metabolite, dihydrotestosterone. The reduction of testosterone to dihydrotestosterone, however, would not appear to be a necessary step.

The most predominant social behavior to be observed among prepubescent rats is that of social play or play-fighting. This behavior has been found to occur more frequently in male pups than in female pups (Meaney and Stewart, 1981; Olioff and Stewart, 1978; Poole and Fish, 1976). This form of social play, often referred to as rough-and-tumble play, has been reported to be male predominant in several other species including hamsters (Goldman and Swanson, 1975), rhesus monkeys (Goy and Goldfoot, 1974; Harlow, 1969), baboons (Owens, 1975), and humans (Blurton-Jones, 1976).

0018-506X/81/020197-17$01.00/0

Working with rhesus monkeys, Goy and Goldfoot (1974) found that females exposed to exogenous testosterone propionate (TP) during gestation engaged in rough-and-tumble play at rates comparable to those of their male peers. Similarly, Olioff and Stewart (1978) found that the administration of TP to female rats on postnatal Days 1 and 2 was sufficient to eliminate the sex difference in social play.

One problem of concern to those studying the role of testicular hormones in the determination of behavior is to specify which of testosterone (T) or its major metabolites is primarily responsible for any observed behavior difference between the sexes. This is a question of particular interest in view of the major role attributed to the metabolite 17β-estradiol (E_2) in the differentiation of the neural circuitry involved in gonadotropin release and in the defeminization and masculinization of sexual behavior patterns in many rodents. E_2 is known to be converted from T in many regions of the brain during the period of differentiation and to bind with high affinity to estrogen receptors within specific brain cells. It is this feature that has made it possible to begin to relate hormone action to changes in specific brain structures during this period. No such direct central nervous system action for T itself or for its 5α-reduced and nonaromatizable metabolite, dihydrotestosterone (DHT), has yet been determined, though it has been suspected. Thus while it is well recognized that DHT plays the major role in promoting the development of male genitalia and body type, the evidence that exists for direct androgenic action in the central nervous system during differentiation is considered equivocal. One reason for this is that until recently receptors for T or DHT were not known to be present in the brain of developing animals during critical periods for differentiation. The finding of androgen receptors in the limbic brain of 1-day-old rats, receptors that exhibit nuclear binding properties typical of androgen receptors in adult rats, is therefore of great interest (Fox, Vito, and Wieland, 1978; Lieberberg, Maclusky, Roy and McEwen, 1978; Vito, Wieland, and Fox, 1979). Furthermore, the report of 5α-reductase activity in the brain of neonatal rats adds to the possibility that androgen metabolites of T may play a role in the differentiation of neural tissue (see Martini, 1978). In this paper we report on the results of six experiments designed to investigate the influence of T and some of its metabolites on the social play of prepubescent rats.

The animals were studied during the prepubertal period from Days 26 to 40 of age, except in Experiment 2 in which the period studied was from Days 31 to 40 of age. Following weaning the animals used in any particular experiment were placed into mixed-sex groups of six, same-aged, animals for the duration of the experiment. This allowed the experimenters to make detailed observations of the social behavior of the pups in the groups throughout this prepubertal period. Each group within each experiment was composed of a similar number of treated and untreated ani-

mals. This provided each animal with rearing companions of both sexes including normal, untreated animals.

EXPERIMENT 1

In this first experiment the frequency of social play of intact male and female prepubescent rats was compared to that of male pups that had been castrated within 24 hr of birth. For while it had been found in an earlier experiment (Olioff and Stewart, 1978) that intact females exposed to TP injections on Days 1 and 2 of life engaged in more social play than did oil-treated females, the effects of removing the source of T in males during the critical period for the organizational effects of T had not been studied.

Methods

Subjects

The animals used in this experiment were 16 male and 8 female Long–Evans hooded rats. The animals were selected from among the offspring of eight dams that were obtained while pregnant from the Canadian Breeding Farms and Laboratories, St. Constant, Quebec. Within 24 hr following birth the animals were removed from their mothers, sexed, and randomly assigned to treatment groups. Twelve males were castrated and twelve were sham operated. Surgery was performed under hypothermia anesthesia. The animals were then assigned to six mothers. Each mother received 10 pups (the average litter size for this species) 4 of which were similarly treated males and 6 of which were same-aged females. The animals were then left undisturbed until weaning. The animals had continuous access to food (Purina Lab Chow) and water. A 12L/12D light schedule was maintained in the animal colony (lights off at 0900 hr).

On Day 23 the animals were separated from their mothers and housed in four groups of six animals each. By this time five castrated males, one intact male, and one female had died; therefore, three of the four groups were composed of two castrated males, two intact males, and two same-aged females, while the fourth was composed of three intact males, one castrated male, and two females making for a total of seven castrated males, nine intact males, and eight females. The animals were maintained in these groups throughout the experiment. The animals were marked for identification (colored felt-tipped pens) approximately every 5 days and were not otherwise handled. Although they were housed in a different room, the animals were maintained on the same feeding conditions and the same light schedule as in the animal colony.

Apparatus and Procedure

Each postweaning group was housed in a cage 51 × 33 × 26 cm, one wall of which was made of 1.25-cm plywood and had mounted on it a

wire-mesh feeder and two water bottles. The remaining sides were made of 0.6-cm wire mesh.

The animals were observed daily during the prepubertal period between Days 26 and 40. Each group of six animals was observed for 70 observation periods per day. Each lasted for 20 sec, and during that period the behavior of all six animals was scored. If, during the period an animal engaged in a play-bout it was given a score of "1." Animals that did not engage in a play-bout were given a score of "0." Thus, for any animal the possible range of scores for each day of observations was from 0 to 70. An animal that engaged in any of the behavioral components of a play-fight sequence (see below) was considered to have engaged in a play-bout. All observations were conducted between 1200 and 1600 hr.

Behavioral definition of social play. Social play in rats is comprised of several individual behavioral components. While these components resemble in some respect those of agonistic encounters in adult rats, there are important distinguishing features. For instance, the distress vocalizations that are common to the agonistic encounters of adult rats are rarely heard during social play (Calhoun, 1962; Meaney and Stewart, 1981). Another feature of social play is that, unlike adult agonistic encounters, roles are frequently reversed; an animal that is dominated for a brief period during a play-bout will often immediately pounce on the other animal and then dominate it (see Poole and Fish, 1976).

The following is a description of the behavioral components of a play-fighting sequence. *Pouncing:* One pup lunges at another with its forepaws extended outward. It is the forepaws that first make contact with the other animal. Pouncing is considered as a play-initiation act since animals exhibiting this behavior invariably engage in a play-bout so long as the recipient animal responds, and because it temporally precedes any other behavior in the play sequence. A complete play-fight sequence involves, in order, Pouncing, Wrestling, often Boxing and/or Lateral Display, and finally On-Top/On-the-Back Postures. In some bouts, however, animals withdraw prior to the On-Top/On-the-Back stage. (See Poole and Fish, 1976 for more information on behavioral transitions in play-fighting.) A play-fight sequence between pups 26 to 40 days of age usually lasts about 8 sec or less (Meaney and Stewart, in preparation). *Wrestling:* Two animals roll and tumble with one another. *Boxing:* Two animals standing upright facing one another and making pawing movements toward one another. *Lateral Display:* One animal arches its back and, with all four limbs extended, directs its flank toward another animal. *On-the-Back Posture:* One animal lies on its back fully exposing its ventral surface to another animal. *On-Top Posture:* One animal positioned over another animal with its forepaws placed on the other animal.

Data analysis. Total play scores were calculated for individual animals across all the observation days. The effects of the treatment conditions were studied by comparing the scores of animals in each of the three

conditions regardless of observation group using a Kruskal–Wallis H test. Post hoc, paired comparisons were made using Mann–Whitney U tests.

Results and Discussion

The results of Experiment 1 are summarized in Fig. 1. Intact males were observed to engage in social play more than either intact females or castrated males. Statistical analysis revealed a significant treatment effect ($H = 9.67, P < 0.01$). Post hoc analysis showed that intact males engaged in significantly more social play than did either castrated males ($U = 7$), $P < 0.005$) or intact females ($U = 8.5$, $P < 0.005$). The difference between the castrated males and the intact females was not significant. These results indicate that genetic male rats deprived of testicular hormones from birth show greatly reduced levels of social play in the prepubertal period.

EXPERIMENT 2

Since the castrated animals in the first experiment were deprived of testicular hormones from birth onward, it is not possible to specify the timing of the androgen effect. The results of Olioff and Stewart (1978) support the view that the androgen influence is limited to the neonatal period since females treated with TP, but which lacked, at least, the testicular source of androgens during the period of observation, did not differ from intact males in the frequency of social play. This suggests that there is no activational influence of circulating androgens on social play, and that the effect of testicular hormones is specific to the neonatal period.

Experiment 2 was designed to examine the possible effects of circulat-

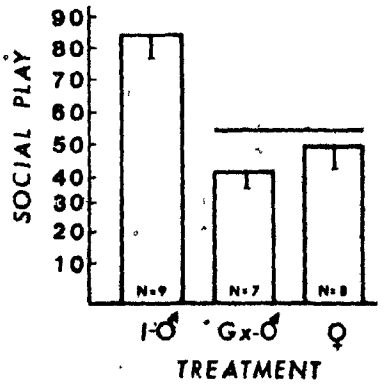

FIG. 1. Mean (±SE) number of 20-sec sessions in which intact males (*I*-♂), Day 1-castrated males (*Gx*-♂), and intact females (♀) were observed to be engaged in play-fighting.

ing androgens on the social play of male pups. In this experiment the frequency of social play of intact male and female pups was compared to that of males that were castrated on Day 23. Since the Day 23 castrates were without testes throughout the period of observation (Days 31 to 40), but not during the early neonatal period, it was possible to examine whether the presence of testicular hormones contributes to the expression of typical levels of social play seen in male pups.

Methods

Subjects

The animals used in this experiment were 16 male and 8 female Long-Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. On Day 23, 8 males were castrated and 8 were sham operated. Surgery was performed under Nembutal anesthesia. Following surgery the animals were placed into groups of six same-aged animals. There were four such groups each composed of two castrated males, two intact males, and two intact same-aged females.

Apparatus and Procedure

The apparatus and procedure were the same as in Experiment 1, with the exception that the period of observation was shortened to 10 days (Days 31 to 40). This was done to allow for recovery from surgery.

Data analysis. The data from this experiment were treated similarly to those from Experiment 1: Note, however, that there were 10 and not 15 days of observation in this experiment.

Results and Discussion

The results of Experiment 2 are summarized in Fig. 2. As can be seen both intact males and castrated males were observed to engage in more

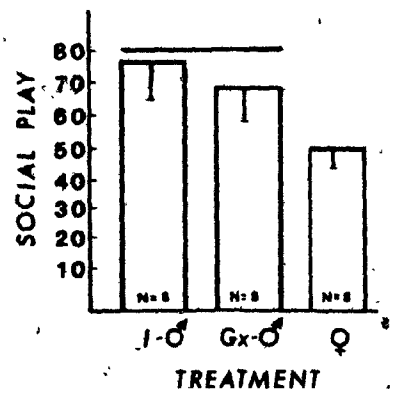

FIG. 2. Mean (±SE) number of 20-sec sessions in which intact males (*I-♂*) Day 23-castrated males (*Gx-♂*), and intact females (♀) were observed to be engaged in play-fighting.

social play than did normal females. The statistical analysis across all three groups revealed a moderate treatment effect ($H = 4.59$, $0.10 > P > 0.05$). The post hoc analysis showed that both the intact males ($U = 13.5$, $P < 0.05$) and the castrated males ($U = 17$, $P < 0.06$) engaged in more social play than did the females. There was no significant difference between the two male groups.

The fact that castration at 23 days of age did not reduce the frequency of social play in prepubescent male rats suggests that there is no activational influence of circulating androgens on social play. Together with the findings of Experiment 1 and those of Olioff and Stewart (1978) these results indicate that exposure to testicular hormones in the early neonatal period is crucial for the development of the sex difference in the social play of rat pups, but that the presence of testicular hormones at the time of testing does not contribute to the expression of the sex differences. This conclusion has received further support from the results of subsequent work. In one study (Beatty, Dodge, Traylor, and Meaney, 1981) it was found that castration on neither Day 10 nor 20 affected the frequency of play-fighting in male pups. In a second study we (Meaney and Stewart, in preparation) found that while daily injections of 200 μg of testosterone propionate to male pups between 26 and 40 days of age increased mounting behavior, it had no effect on the frequency of play-fighting.

EXPERIMENT 3

The results of Experiments 1 and 2 suggest that the sex difference in the social play of prepubertal rats is due, at least in part, to the influence of neonatal testicular androgens. It is possible, however, that this sex difference may also be influenced by a suppressive effect of ovarian hormones either at the time of testing or earlier. In Experiment 3 we examined the influence of ovarian secretions on the social play of prepubertal female rats. In this experiment the frequency of social play of intact male and female pups was compared to that of female pups that were ovariectomized on Day 1 of life.

Methods

Subjects

The animals used in this experiment were 16 female and 8 male Long–Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. Within 24 hr following birth the animals were removed from their mothers, sexed, and randomly assigned to treatment groups. Ten females were ovariectomized and ten were sham operated. Surgery was performed under hypothermia anesthesia. The animals were then reassigned to mothers as in Experiment 1.

On Day 23 the animals were separated into four groups of six animals. By this time two ovariectomized animals had died. Each group was comprised of two intact males, two intact females, and two ovariectomized females.

Apparatus and Procedure

The apparatus, procedure and analysis of the data were the same as in Experiment 1.

Results and Discussion

The results of Experiment 3 are summarized in Fig. 3. As can be seen the intact males were observed to engage in more social play than did either of the two female groups. The statistical analysis across all three groups revealed a significant treatment effect ($H = 6.76$, $P < 0.05$). The post hoc analysis showed that the intact males were observed to engage in significantly more social play than did either the intact females ($U = 10$, $P < 0.01$) or the ovariectomized females ($U = 16$, $P < 0.05$). The difference between the two female groups was not significant.

These results indicate that there is no detectable influence of ovarian hormones on the social play of prepubertal female rats. Goy (1970) has reported that ovariectomy of female rhesus monkeys does not alter the frequency with which they engage in social play. Thus, in both the rhesus and the rat sex differences do not appear to be due to any suppressive effects of ovarian hormones on the social play of females.

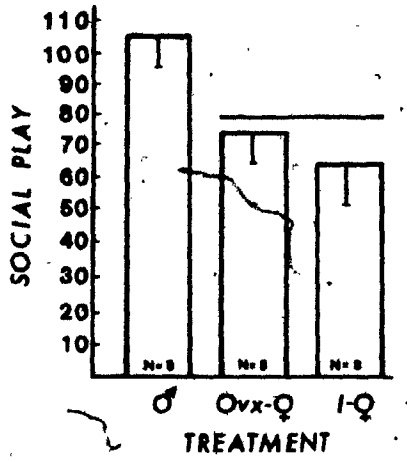

FIG. 3. Mean (±SE) number of 20-sec sessions in which intact males (*I*♂), Day 1-ovariectomized females (*Ovx*-♀), and intact females (*I*-♀) were observed to be engaged in play-fighting.

EXPERIMENT 4

The results of the first two experiments indicate that the sex difference in the social play of rat pups is dependent on the presence of testicular hormones during the early neonatal period. In this experiment we sought to examine whether T itself or one of its metabolites exerts this influence. It is known that within certain cells in the brain of the rat and of several other species, T can be converted either into E_2 through the aromatization pathway (Naftolin, Ryan, and Petro, 1972) or into DHT through the 5α-reductase pathway (Denef, Magnus, and McEwen, 1974; Martini, 1978). Moreover, there are receptor sites, especially within the limbic system, for E_2 (e.g., Stumpf, Sar, and Keefer, 1974), for DHT, and for T itself (Sar and Stumpf, 1974; Sheridan, 1979). It is conceivable then, that the testicular hormone effect described in Experiments 1 and 2 may be due to the action of T itself, to the action of T-derived E_2, or to the action of T-derived DHT.

In Experiment 4 the frequency of social play in genetic females treated neonatally (Days 1 and 2) with either TP, EB, or DHT was compared to that of normal male and female pups.

Methods

Subjects

The animals used in this experiment were 24 female and 12 male Long–Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. On both Days 1 and 2 of life 6 females were injected subcutaneously with 250 μg of TP, 6 with 250 μg of DHT, 6 with 5 μg of EB, and 6 with the oil vehicle alone. All steroids were in peanut oil solution and were delivered in 0.05-ml amounts. Collodion (Fisher Scientific Ltd.) was applied at the point of injection to prevent leakage. On Day 23 the animals were rehoused into six groups of six animals, each group containing 1 TP-treated female, 1 DHT-treated female, 1 EB-treated female, 1 oil-treated female, and 2 untreated, same aged males.

Apparatus and Procedure

The apparatus, procedure, and analysis of the data were the same as in Experiment 1.

Results and Discussion

The results of Experiment 4 are summarized in Fig. 4. As can be seen TP-treated females, DHT-treated females, and untreated males did not differ in the frequency with which they were observed to engage in social play, and all three groups were observed to engage in more social play than did both EB-treated females and oil-treated females. The statistical analysis revealed a significant treatment effect ($H_{adj} = 21.48$, $P < 0.005$).

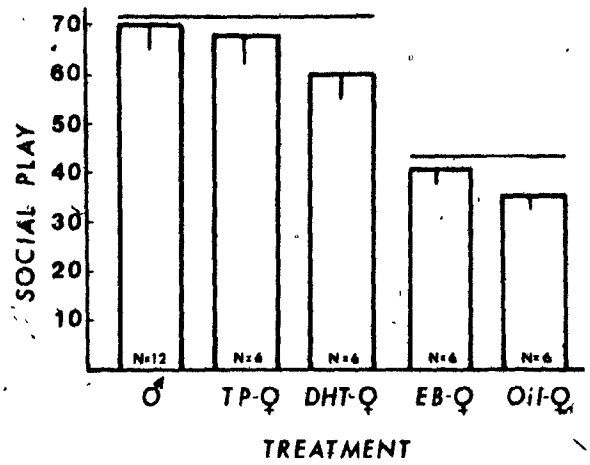

FIG. 4. Mean (±SE) number of 20-sec sessions in which intact males (♂), TP-treated females (*TP*-♀), DHT-treated females (*DHT*-♀), EB-treated females (*EB*-♀), and oil-treated females (*Oil*-♀) were observed to be engaged in play-fighting.

Post hoc analysis showed that there was no significant difference between the males and either the TP-treated females or the DHT-treated females. Both TP-treated females and DHT-treated females were observed to engage in significantly more social play than did either the EB-treated females ($U = 0$, $P < 0.001$ and $U = 5.5$, $P < 0.03$, respectively) or the oil-treated females ($U = 0$, $P < 0.001$ and $U = 1.5$, $P < 0.01$, respectively). There were no significant differences between the EB-treated females and the oil-treated females.

These results indicate that either T or its 5α-reduced metabolite, DHT, administered during the early neonatal period is able to increase the frequency of social play in prepubescent female rats and, in the doses used, to eliminate the sex difference. In contrast, EB, even in doses sufficiently large to defeminize both open-field and lordotic behavior (see Stewart, Vallentyne, and Meaney, 1979), has no such effect. This suggests that the influence of T on the social play of rats is a true androgen effect.

EXPERIMENT 5

The results of Experiment 4 suggest that the high levels of social play observed in male pups are due to the action of T or its DHT metabolite in the neonatal period, and that there is no effect of T-derived E_2. In the present study we tested this hypothesis using intact male rats. This was done by implanting newborn male pups with Silastic capsules containing either androst-1,4,6-triene-3,17-dione (ATD) or 4-androsten-3-one 17β-carboxylic acid (testosterone 17β-carboxylic acid or 17β-CA). ATD is an

aromatase inhibitor and is presumed to limit the conversion of T to E_2 (Leiberberg, Wallach, and McEwen, 1977). ATD has been shown to attenuate the E_2-mediated defeminizing effects on female sexual behavior (Booth, 1977; Clemens and Gladue, 1978; McEwen, Lieberberg, Chaptal, and Krey, 1977; Vreeburg, van der Vaart, and van der Schoot, 1977). In the present study the ATD capsules were left in the animals from Days 1 to 10. Thus, the endogenous levels of T-derived E_2 should have been substantially reduced during the period when E_2 normally acts to defeminize rat behavior. If T-derived E_2 were involved in the development of male-typical levels of social play in the male rat, then ATD would be expected to reduce the observed frequency of social play. In the first part of this experiment (5A) the frequency of social play in normal male and female rats was compared to that of males implanted with ATD during the early neonatal period.

Similarly, 17β-CA is a compound that inhibits the reduction of T into DHT (Kao & Weisz, 1979; Luttge, Jasper, Sheets, and Gray, 1978). In the second part of this experiment (5B) we implanted male pups with Silastic capsules containing 17β-CA from Days 1 to 10, thus reducing the endogenous levels of T-derived DHT. If the metabolism of T into DHT is necessary for the development of male-typical levels of social play, then animals implanted with 17β-CA during the neonatal period should differ from normal males in the frequency with which they engage in social play. In Experiment 5B we compared the frequency of social play in normal male and female pups to that of 17β-CA-treated males.

Methods

Subjects

The animals used in this experiment were 36 male and 18 female Long–Evans hooded rats. The animals were obtained and housed in the same way as in Experiment 1. Within 24 hr following birth three groups of males were given Silastic implants containing either ATD (n = 10), cholesterol (Chol; n = 18), or 17β-CA (n = 8). The implants were inserted under the skin using hypothermia as anesthesia and removed on Day 11 using ether anesthesia. The implants were made from Silastic tubing 0.058 mm i. d. and 0.077 mm o. d. The 13-mm-long implants were filled with 7 mm of steroid and were sealed at each end with 3 mm of Silastic adhesive. The implants were then soaked in absolute alcohol for 1 hr to clean them and to check for leakage. They were then kept in 1% bovine serum albumin in PBS for at least 24 hr before use (see McEwen *et al.*, 1977). In our laboratory we have used this procedure with ATD to prevent the defeminization of lordotic behavior in male rats (Stewart *et al.*, 1979). At 23 days of age the animals in Experiment 5A were placed into five groups of six, each group composed of 2 ATD-treated males, 2 Chol-treated

males, and 2 untreated, same-aged females. At the same age the animals in Experiment 5B were placed into four groups of six, each group composed of 2 17β-CA-treated males, 2 Chol-treated males, and 2 untreated, same-aged females.

Apparatus and Procedure

The apparatus, procedure, and analysis of data were the same as in Experiment 1.

Results and Discussion

The results of Experiment 5A are summarized in Fig. 5. As can be seen, both ATD-treated males and Chol-treated males were observed to engage in more social play than did the females. The statistical analysis of the data revealed a significant treatment effect ($H = 12.77$, $P < 0.005$). Post hoc analysis confirmed that both ATD-treated males ($U = 20$, $P < 0.01$) and Chol-treated males ($U = 19$, $P < 0.01$) had significantly higher social play scores than did the females. There was no significant difference between the two male groups. There was, then, no effect of ATD treatment between Days 0 and 10 on the frequency with which male pups engaged in social play. It might be argued that after the removal of the ATD implants on Day 10, the resulting normal levels of T-derived E_2 might be capable of masculinizing social play after Day 10. Recently, however, it has been found that the castration of male pups as early as Day 10 does not influence the frequency with which they engage in social play (see Beatty *et al.*, 1981). Thus, in males the masculinization of social play occurs before Day 10 and, with respect to the present experiment, before that time when the implants were removed. The results of this experi-

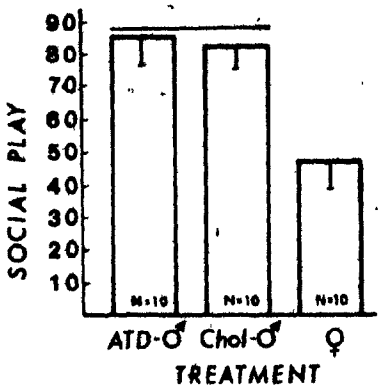

FIG. 5. Mean (±SE) number of 20-sec sessions in which cholesterol-treated males (*Chol-♂*), ATD-treated males (*ATD-♂*), and intact females (♀) were observed to be engaged in play-fighting.

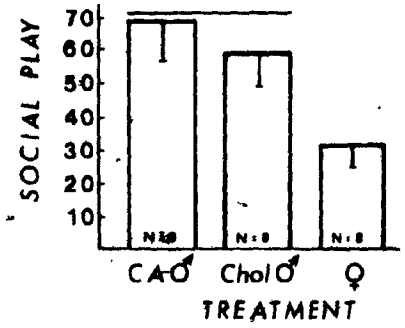

FIG. 6 Mean (±SE) number of 20-sec sessions in which 17β-CA-treated males (*CA-♂*), cholesterol-treated males (*Chol-♂*), and intact females (♀) were observed to be engaged in play-fighting.

ment, then, extend and confirm the findings of Experiment 4 that in the the rat E_2 in the immediate postnatal period neither promotes nor is necessary for the development of male-typical levels of social play.

The results of Experiment 5B are summarized in Fig. 6. As can be seen both 17β-CA-treated males and Chol-treated males were observed to engage in more social play than did the females. The statistical analysis of the data revealed a significant treatment effect ($H = 9.02$, $P < 0.02$). Post hoc analysis showed that both 17β-CA-treated males ($U = 2$, $P < 0.001$) and Chol-treated males ($U = 14.5$, $P < 0.04$) had significantly higher social play scores than did the females. There was no significant difference between the two male groups.

The results of Experiment 5B would seem to indicate that the metabolism of T into DHT is not a necessary step for the androgenic effect on social play. This conclusion must be somewhat tentative, however, in that we have no direct measure of the degree of inhibition produced by 17β-CA.

GENERAL DISCUSSION

Before beginning a discussion of these results it may be useful to consider briefly the nature of the sex difference in the play-fighting of prepubescent rats. The usual sequence involved in a play-fight between two young rats is that one animal approaches and pounces on another, wrestling and less often boxing ensues, and finally one animal emerges on top of the other (also see Poole and Fish, 1976). In animals younger than 40 to 45 days of age these bouts usually last 7–8 sec or less (Meaney and Stewart, in preparation; also see Poole and Fish, 1976). This sequence is the same for both male and female pups (Poole and Fish, 1976). One difference in the pattern between males and females is that females tend to withdraw sooner from play bouts then do males (Poole and Fish, 1976;

Meaney and Stewart, in preparation). Thus, Meaney and Stewart (1981) found that males engaged in more On-Top Posture than did females. The single feature that contributes most to the sex difference in play-fighting, however, is that of Pouncing or play-initiation; in addition males initiate play more often with males than with females (Meaney and Stewart, 1981). Thus while male rats are more playful than females (i.e., males initiate and become involved in more play-fights) the basic patterns of play-fighting of males and females are similar (see also Poole and Fish, 1976).

The results of these experiments support the view that the sex difference in the social play of prepubescent rats is an androgen-mediated effect, dependent on neonatal exposure to either T or DHT. The E_2 metabolite of T does not appear to be directly involved in the processes that result in the development of male-typical levels of social play.

The present experiments do not provide information about precisely how long into the neonatal period T or DHT may be effective in influencing social play, but the results of Experiment 4 do indicate that exposure to TP or to DHT on Days 1 and 2 of life is sufficient to masculinize female rats. In addition, the results of Experiments 2 and 4 demonstrate that circulating testicular androgens are not necessary for the expression of male-typical levels of social play. Neither males castrated at 23 days of age nor females given TP neonatally, both of which lacked testicular androgens at the time of observation, differed from intact males in the frequency with which they engaged in social play. Taken together, these findings indicate that, while testicular androgens are not directly responsible for the expression of male-typical levels of social play, their activity during the neonatal period contributes to the sexual differentiation of some CNS function that in turn mediates this behavioral sex difference.

It is possible that this androgen-related sexual differentiation is mediated by androgen-receptor proteins in the CNS, most of which bind with both T and DHT (Sar and Stumpf, 1974). Androgen receptors have been detected in the limbic brain of the rat by postnatal Day 1 (Fox *et al.*, 1978; Lieberberg *et al.*, 1978). Moreover, these neonatal androgen receptors exhibit properties typical of androgen receptors in adult rats; most notably they bind to DNA cellulose with the same affinity (Fox *et al.*, 1978; Lieberberg *et al.*, 1978). This characteristic is important because the intracellular effects and in particular the growth-related effects of steroid hormones are mediated by DNA (e.g., Salaman and Birkell, 1977). Thus, one possible explanation for the masculinizing influences of neonatal androgens on the social play of rats is that the exposure to androgens during the neonatal period promotes the formation of sex-specific neural circuitry and that this circuitry serves to mediate the sex difference in social play that is seen in prepubescent animals.

Recently we (Stewart *et al.*, 1979) have reported a role for the androgenic actions of T in neonatal rats in the establishment of the sex difference in open-field behavior seen in adult animals. The sex difference in open-field behavior, like that in social play, is not dependent on the presence of gonadal hormones at the time of testing (Bengelloun, Nelson, Zent, and Beatty, 1976; Blizard, Lippman, and Chen, 1975; Bronstein and Hirsch, 1974; Stewart and Cygan, 1980) although it is dependent on the presence of androgens in the neonatal period (Blizard and Denef, 1973; Gray, Lean, and Keynes, 1969; Pfaff and Zigmond, 1971; Stewart, Skvarenina, and Pottier, 1975). Female rats injected on Days 1 and 2 with DHT and males treated neonatally with ATD, when tested as adults, exhibited male-typical levels of open-field activity (Stewart *et al.*, 1979). Thus, both social play and open-field behavior can be masculinized through the neonatal exposure to the androgenic component of T. These findings, taken together with the evidence that the androgenic actions of T play a role in the perinatal period in the development of adequate male sexual behavior (Clemens, Gladue, and Coniglio, 1978; Davis, Chaptal, and McEwen, 1979; Nadler, 1969; Stewart and Kaczender-Henrik, 1971; Ward and Renz, 1972), strongly suggest that there is a role for neonatal androgen receptors in the development of male-typical behavior.

Finally, it is interesting to note that these androgenic effects on the behavior of the rat closely parallel those found in the rhesus monkey. Goy (1978) has found that prenatal exposure to either T or DHT acts to masculinize both prepubertal play and male copulatory behavior in female rhesuses. This suggests that the masculinization process in the rat is comparable to that of this primate species.

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Physiology & Behavior, Vol 26, pp 467–472 Pergamon Press and Brain Research Publ , 1981 Printed in the U S A

Sex-Dependent Effects of Amygdaloid Lesions on the Social Play of Prepubertal Rats[1]

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Received 29 August 1980

MEANEY, M. J., A. M. DODGE AND W. W. BEATTY. *Sex-dependent effects of amygdaloid lesions on the social play of prepubertal rats*. PHYSIOL. BEHAV. 26(3) 467–472, 1981.— Male and female prepubertal rats, like many primate species, differ in the frequency with which they engage in social play (play-fighting), males engage in more social play than females. This sex difference in the rat has been found to be under the control of gonadal hormones acting during the neonatal period. In the present study we examined the effects of lesions of the amygdala, a prominent neuroendocrine control area, on the play-fighting of male and female prepubertal rats. Bilateral, electrolytic lesions and sham control treatments were made at 21 or 22 days of age and the animals were then observed daily in intact groups between 26 and 40 days of age. Amygdaloid lesions suppressed social play in male rats to levels that were indistinguishable from those of control females. In contrast to males, amygdaloid lesions had no effect on the social play of females. Sex differences in the anatomy and physiology of the amygdala are discussed as a possible explanation of its apparent differential influence on the social play of male and female prepubertal rats.

Play Social development Amygdala Social behavior Sex differences

SEX differences have been described in the social behavior of several social-living mammalian species. These sex differences appear to be related to a sex-dependent division of labor with respect to reproductive functions, territorial defense, and the maintenance of a group structure through dominance relations. In several primate species, particularly the well-studied terrestrial monkeys (e.g., the rhesus, Japanese, and vervet monkeys, and several species of baboons) maternal care is performed primarily by the adult females, while territorial defense and within-group dominance relations appear to be the domain of the males [9, 12, 16]. Interestingly, there are also sex differences in the social behavior of the juveniles of these species. Sub-adult males engage in more play-fighting than do females (e.g., [16,24]), while sub-adult females engage in more social grooming, and, in at least one species, the vervet monkey, females engage in more play-mothering [17].

These patterns of sex differences in social behavior are not unique to primates, although they are less-well studied in other species than they are in primates. In rodents, for instance, among both the Norway rat [2,4] and the Roof rat [10] the maternal care of infants is performed almost exclusively by females, while the maintenance of both territorial integrity and of within-group dominance relations are primarily male activities. Among juvenile Norway rats, as well, there are sex differences in social behavior. Juvenile males engage in more play-fighting than do their female peers [21,25], while young females engage in more social grooming than do males [21]. Thus, both the juvenile and the adult behavioral sex differences in the Norway rat are similar to those in the above-mentioned primate species.

These sex differences in the early social behavior of immature animals have important implications for the study of the biosocial determinants of social behavior. What they suggest is that the potential opportunities for early learning may be sex dependent. This is of particular significance for play behavior considering the potential significance of this behavior in the development of adult social behavior. One question of interest, then, concerns the determinants of these sex differences in play behavior. The present study

[1]This work was supported by Grant HD 12620-01 from NICHHD to William W. Beatty. Michael J. Meaney was supported by a predoctoral fellowship from the Ministere de l'Education, Province de Quebec. Please direct correspondence to Michael Meaney, Department of Psychology, Concordia University, 1455 Boul. de Maisonneuve Ouest, Montreal H3G 1M8, Quebec, Canada.

TABLE 1

MEAN FREQUENCY FOR ON-TOP AND ON-BOTTOM BEHAVIORS AND MEAN BODY WEIGHTS ON DAY 25 (BW1) AND DAY 41 (BW2) MEASURE

Group	N	OT	OB	BW1	BW2
AMX Male	24	30 8	23.3	66 6	174 1 (b)
CONT Male	12	37.6	41 0 (a)	68.4	170.3 (b)
AMX Female	11	27 4	26.8	68 0	153.0
CONT Female	12	27 8	29 9	68.3	146.5

(a) CONT male>AMX male ($p<0$ 01)
(b) AMX male=CONT male>AMX female=CONT female ($p<0.0001$).

represents an initial attempt to identify the neural mechanisms involved in the social play of rat pups. The well-documented effects of amygdalectomy on the agonistic encounters of adult rats [5,11] suggested that this limbic structure may be involved in play-fighting, since the behavioral components of inter-specific aggression are morphologically similar to those of play-fighting. In this study, then, male and female pups were either amygdalectomized or sham operated. Surgery was performed on days 21 and 22. On day 25 the animals were placed into peer groups. In these groups the animals were observed daily for social play (see also [22]) during the prepubertal period between days 26 and 40.

METHOD

General

The animals used in this experiment were 38 male and 24 female Holtzman albino rats born in the animal colony at North Dakota State University. The animals were the offspring of 14 dams that were obtained while pregnant from the Holtzman Co., Madison, WI. Upon arrival at the animal colony the pregnant females were placed into 24×46×15 cm plastic maternity cages. The cages had a wire-mesh top with an overhead feeder and water bottle. Within 24 hr following birth the animals were removed from their mothers, sexed, and randomly assigned to litters of 10 animals (the average litter size for this species), 5 of each sex. The animals had continuous access to food and water. The animal colony was maintained on a 12L:12D light schedule (lights on at 0600 hr).

Surgery

Twenty-six males and 12 females were given bilateral electrolytic lesions under Chloropent anesthesia by passing a 1.5 mA current for 15 sec between No. 1 stainless steel insect pins that were insulated to the tip with Epoxylite and a rectal cathode. With the rat's head flat between bregma and lamda the coordinates were 2.0 mm posterior to bregma, 3.9 mm lateral to the midline, and 7.3 mm from the surface of the brain. Six males and 6 females received sham lesions in which the electrode tip was lowered 6.0 mm from the surface of the brain. Six males and 6 females received the Chloropent anesthesia only. All animals were between 21 and 22 days of age at the time of treatment. Recovery seemed to be complete 2–3 days after surgery. By this time the animals were playing vigorously, and, through casual observation, seemed to be normally active.

Body weights were taken just prior to the first day of observation (BW1) and at the time of histology (BW2). The results are presented in Table 1, where it can be seen that the only noticeable difference in body weights was between males and females at the time of histology. The amygdaloid lesions, then, seem to have had no effect on the overall growth of the animals.

Procedure

Following surgery the animals were placed into groups and housed in adjoining cages 41×61×38 cm made from wire mesh with a wooden frame for support. The groups were separated from each other by sheet metal dividers. The front of the cages was made from clear plastic. Six of the groups were comprised of eight animals each and 2 were comprised of 7 animals each. Each group contained some treated and some untreated animals and was balanced for sex. The animals were maintained in these groups throughout the experiment. The animals were marked on the tail (with colored felt-tipped pens) approximately every 5 days and were not otherwise handled

The animals were observed daily during the prepubertal period between days 26 and 40. Each group was observed for 105 observation periods per day. Each observation period lasted for 20 sec, and during that period the behavior of all the animals in the group was scored. If, during the period an animal engaged in one of the behaviors described below it was given a score of 1 for that category. Animals that did not engage in any play were simply given a score of 0. Thus, for any animal the possible range of scores for each of the behavioral categories for each day of observation was from 0 to 105. Although precise estimates of interrater reliabilities were not obtained, the three observers trained together in two previous studies using the same method. Further, they were blind to the lesion condition of the animals.

Behavioral Categories

Social play in rats is comprised of several individual behavioral components. While these components resemble in some respects those of agonistic encounters in adult rats, there are important distinguishing features. For instance, the distress vocalizations that are common to the agonistic encounters of adult rats are rarely heard during social play [4,21]. Another feature of social play is that, unlike adult agonistic encounters, roles are frequently reversed; an animal that is dominated for a brief period during a play-bout will often immediately pounce on the other animal and then dominate it [25].

The following is a list of the behavioral components of social play. *Pouncing*. One pup lunges at another with its forepaws extended outward. It is the forepaws that first make contact with the other animal. *Wrestling*. Two animals roll and tumble with one another. *Boxing*. Two animals standing upright facing one another and making pawing movements towards one another. *On-Bottom Posture*. One animal lies on its back fully exposing its ventral surface to another animal. *On-Top Posture*. One animal positioned over another animal with its forepaws placed on the other animal. *Play Bite*. One animal grips another animal with its teeth; this is actually an inhibited bite. Animals play biting another animal were never seen to inflict a wound.

In this study there was one behavioral category and three sub-categories. The main category was that of *social play* and this category included all of the above-described behav-

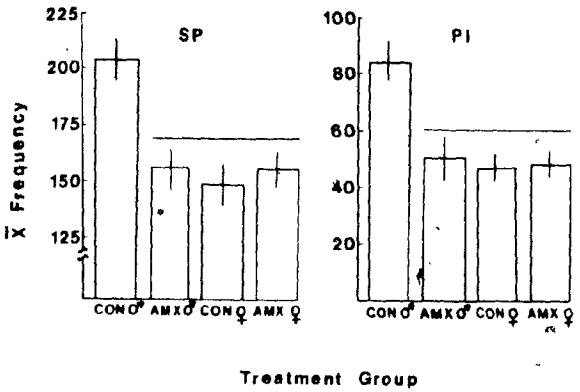

FIG 1 Mean (±SEM) frequency of social play (SP· left panel) and play initiation (PI) responses (right panel) Horizontal lines denote groups that were not statistically different

iors. An animal that engaged in any of these behaviors during an observation period was given a score of 1 for the social play category for that particular observation period. The first sub-category was that of *play initiation*. Pouncing was considered as the play initiation act (see also [21,25]). Thus, an animal that engaged in pouncing during an observation period was given a score of 1 for this sub-category as well as for the social play category. The on-top posture and the on-bottom posture were the remaining two sub-categories and were scored in the same way. It should be noted that the social play category subsumed the three sub-categories.

Histology

Following completion of the behavioral tests all rats with lesions and a representative sample of sham-operated animals were sacrificed under deep Chloropent anesthesia. After perfusion with physiological saline and 10% Formalin the brains were sectioned on a cryostat at 40 μ. Every fifth section through the lesion was saved and stained with thionin according to the procedures of Donovick [7]. Each lesion was reconstructed with the aid of a microprojector and categorized with respect to the anterior-posterior plane of maximum extent, presence of optic tract damage (absent, unilateral, or bilateral), and size (3=largest, 1=smallest) by an observer who was unaware of the rat's performance or sex. Nearly all of the lesions were extensive and bilaterally symmetrical. In three animals (2 males and 1 female) the lesions were asymmetrical and posterior to the amygdala (2 cases) or could not be detected (1 case). Behavioral data from these animals were discarded.

RESULTS

Behavioral Data

Social play, play initiation, on-top, and on-bottom scores were calcualted for individual animals across all observation days. The data for the four behavioral categories were analyzed separately. Since there were no differences between the sham-operated males and the anesthetic-only males on any of the behavioral measures, the data from these animals were pooled to form the control (CONT) male group. Likewise the data from the sham-operated and anesthetic-only females were pooled to form the control (CONT) female group. The data were analyzed using a two-way analysis of variance for treatment (lesion-sham)×sex (male-female). The results are summarized in Fig. 1 and Table 1.

Social play. As can be seen in Fig. 1, the control males engaged in more social play than did either the males with lesions of the amygdala (AMX), the AMX females, or the control females. Statistical analysis revealed a significant effect of Sex, $F(1,55)=8.96$, $p<0.005$, and a significant Sex×Treatment interaction, $F(1,55)=5.75$, $p<0.02$. Post hoc analysis showed that the CONT males engaged in significantly more social play than did the AMX males, $t=3.13$, $p<0.01$, the AMX females, $t=3.04$, $p<0.01$, and the CONT females, $t=3.57$, $p<0.01$. No other comparisons approached significance.

Play initiation, on-top, and on-bottom sub-categories. As can be seen in Fig. 1, the pattern of results for play initiation was similar to that for social play. The statistical analysis showed that there was a significant effect of Sex, $F(1,55)=13.55$, $p<0.001$, and a significant Sex×Treatment interaction, $F(1,55)=8.42$, $p<0.006$. Post hoc analysis revealed that CONT males initiated more social play than did the AMX males, $t=4.05$, $p<0.001$, the AMX females, $t=3.94$, $p<0.001$, and the CONT females, $t=4.41$, $p<0.001$. The means for the on-top and the on-bottom measures are presented in Table 1. Statistical analysis showed that there were no significant differences among the groups on the on-top measure. There was, however, a significant lesion effect, $F(1,58)=5.52$, $p<0.03$. Post hoc analysis revealed that the CONT males engaged in the on-bottom behavior more often than did the AMX males, $t=3.07$, $p<0.01$. The difference between the AMX females and the CONT females (see Table 1) did not approach significance.

Also presented in Table 1 are body weight data from weighings on day 25 (BW1) and day 41 (BW2). Statistical analysis of this data showed that there were no group differences for BW1, while there was, as to be expected, a significant sex difference, $F(1,58)=30.72$, $p<0.0001$, for BW2. There was no lesion effect on either BW1 or BW2.

Histological Findings

The lesions were large, bilaterally symmetrical, and centered in the medial part of the basolateral nuclei. Damage was greatest in the posterior part of the amygdala. Destruction was most severe in the basolateral nuclei which ware bilaterally involved in most cases. In the majority of cases there was bilateral damage to the cortical nuclei as well. The medial and posterior nuclei were damaged in some cases; often the invasion of these areas was unilateral. More posteriorly placed lesions invariably damaged the ventral hippocampus and the entorhinal cortex. The posterior portion of the caudate-putamen was damaged in some of the more dorsally placed lesions. Nine of the rats sustained unilateral damage to the optic tract and in 3 there was bilateral damage to the optic tract. There was no relationship between the extent of damage to the optic tract and any of the behavioral measures. Likewise neither lesion size nor location correlated with any of the behavioral indices. Overall the lesions in males and females were quite similar in terms of posterior location, degree of optic tract involvement, and size.

In Fig. 2 reconstructions of 4 lesions at the locus of the greatest extent are presented. Rat 2, a male, is typical of the more posteriorly placed lesions. Note the invasion of the

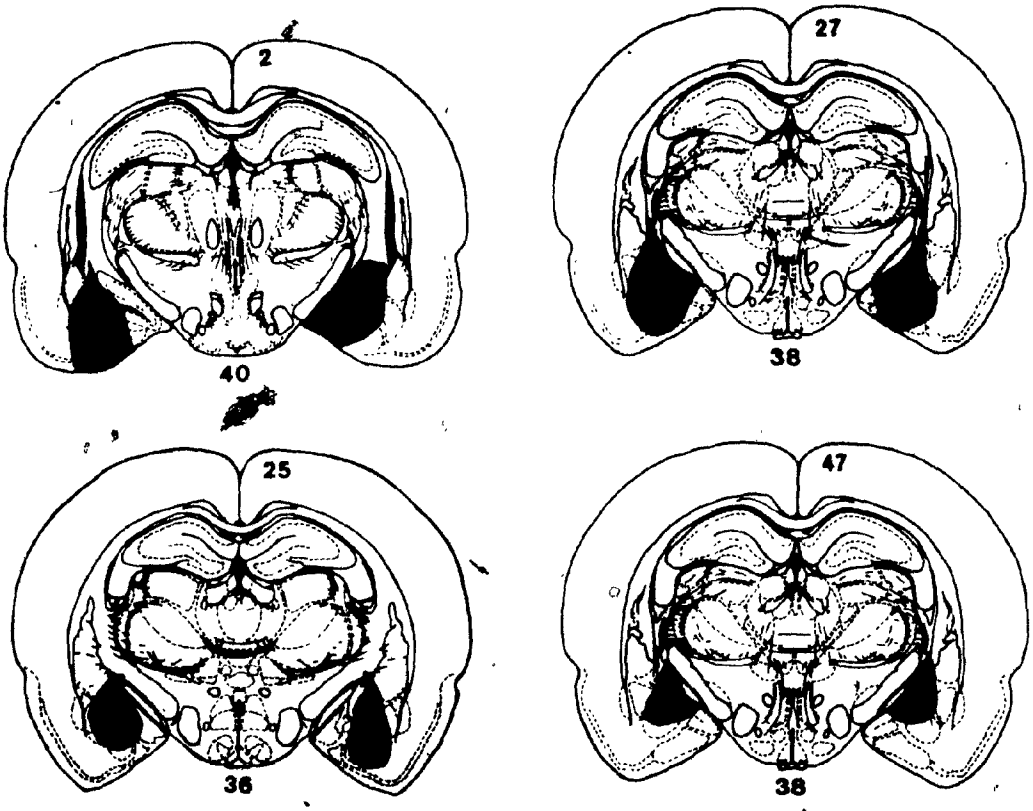

FIG 2. Reconstructions of 4 representative lesions at the anterior posterior plane of maximum extent. Animal numbers are on the upper right side of each section. Numbers below the sections are plate numbers from the Konig and Klippel [14] atlas

ventral hippocampus. Rat 25, also a male, illustrates the appearance of the more anteriorly located lesions. Rats 27, another male, and 47, a female illustrate the variation in the size of the lesions at the anterior-posterior location where the majority of the lesions were largest. Rat 47 sustained the most severe damage to the optic tracts of any subject in the study. Despite the presumed visual impairment, its play frequency (194) was well above the mean for AMX females. By contrast, the male (rat 27) sustained more extensive damage to the amygdala without involvement of the optic tracts and played comparatively little (play frequency=108).

DISCUSSION

The results of this study demonstrate a clear sex-dependent effect of amygdaloid lesions on the social play of prepubertal rats. AMX males engaged in less social play than did CONT males, whereas there was no such difference between AMX females and CONT females. The effect of the amygdaloid lesions, then, was to eliminate the sex difference in social play.

One possible explanation for these results is that the amygdalectomy in the male pups produced some testicular dysfunction that in turn suppressed the frequency of play-fighting in AMX males. Recently, however, it has been found that castration just prior to the period of observation used in this study (i.e. on Day 20 or 23) has no effect on the frequency with which male pups engage in play-fighting [3,22]. It is unlikely, then, that the effects of amygdalectomy on the play-fighting of male pups is mediated by any related gonadal dysfunction.

An alternative explanation, namely that the changes in play by male rats with amygdaloid lesions are secondary to alterations in adrenocortical function, is less easily evaluated. First, there are presently no data assessing the importance of circulating corticosteroid levels on play in juvenile rats although this issue is currently being investigated. Second, while elevations in corticosterone have been linked to the development of submissiveness in male mice subjected to defeat by trained fighters [23], the relevance of this observation is unclear since play-fighting and adult aggression differ in their behavioral detail [1] as well as in aspects of their hormonal regulation [3,22]. Further, the present findings that amygdalectomy reduced both play-fighting and the frequency of on-bottom responses are not easily integrated into a simple dominance-submissiveness hypothesis. And in adult male rats amygdalectomy causes rather modest changes in the temporal pattern of corticosterone levels in plasma following stress without altering the basal concentration of the hormone [6]. Based on these considerations we tentatively conclude that the sex-dependent effects of amygdaloid lesions on play probably are not the result of altered adrenocortical function, although the data required to address this question directly are not yet in hand.

While normal circulating levels of androgens at the time of observation do not appear to be necessary for the expres-

sion of male-typical levels of play-fighting by rats, the presence of androgens during the neonatal period is critical. Castration of males on Day 1 life results in a decrease in play-fighting to levels similar to those observed in normal females, but if males are not castrated until Day 10 or later, the frequency of their play-fighting is similar to that of intact males [3,22]. Moreover, it has been shown in both the rhesus monkey [13,14] and in the Norway rat [22], that the early (prenatal in the monkey and neonatal in the rat) administration of either testosterone or its 5-α-reduced metabolite, dihydrotestosterone, to females will increase play-fighting to levels comparable to those in normal males. In the male rhesus, as in the rat castration after this critical period (between birth and 3 months of age in the monkey) has no effect on play-fighting [13]. These results are suggestive of an early organizational effect on the neural mechanisms involved in social play. Moreover, this organizational effect seems to be a true androgen effect since, at least in the Norway rat, Day 1 estrogen treatment of females has no effect on their levels of social play [22] This latter finding is of special interest since so many of the well-documented organizational effects of testosterone, especially on rat sexual behavior, have been found to be mediated by testosterone-derived estrogen [19].

Within this context it is interesting to note that the amygdala is a prominent target area for steroid hormones Testosterone [26], dihydrotestosterone [18,26], and corticosterone [20,29], all of which have been found to influence the social play of prepubertal rats (Meaney, Stewart and Beatty, in preparation, see also [22]) when administered neonatally, are taken up by cells within various regions of the amygdala. The medial nucleus of the amygdala is one of the few known regions that contains receptors that will bind only with testosterone [27]. One, currently popular hypothesis is that steroid-promoted differences in brain structure mediate behavioral sex differences. With respect to the amygdala, Staudt and Dorner [28] have found a sex difference in nuclear cell size in the central and medial regions of the amygdala that is influenced by neonatal exposure to gonadal hormones. Moreover, Dyer, MacLeod, and Ellendorf [8] found that when they stimulated the corticomedial amygdala and recorded from the medial-preoptic and anterior regions of the hypothalamus (areas that receive projections from the corticomedial amygdala), many more of the cells were driven by amygdaloid stimulation in males and neonatally androgenized females than in females or in neonatally castrated males. These findings suggest that there are sex differences in the neural circuitry of the amygdala that are influenced by neonatal androgen activity. Perhaps the differences in the anatomy and physiology of the amygdala are, in part, responsible for the sex differences in the frequency of play-fighting in prepubertal rats

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