PERINATALE PROGRAMMERING VAN VOLWASSEN SEKSUEEL GEDRAG
EN PARTNER PREFERENTIE VAN DE RAT

PERINATAL ORGANIZATION OF ADULT SEXUAL BEHAVIOR
AND PARTNER PREFERENCE IN THE RAT

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof. Dr. C.J. Rijnvos
en volgens het besluit van het college van dekanen.
De openbare verdediging zal plaatsvinden op
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door

TEUNIS BRAND
geboren te Dordrecht

PROMOTIECOMMISSIE

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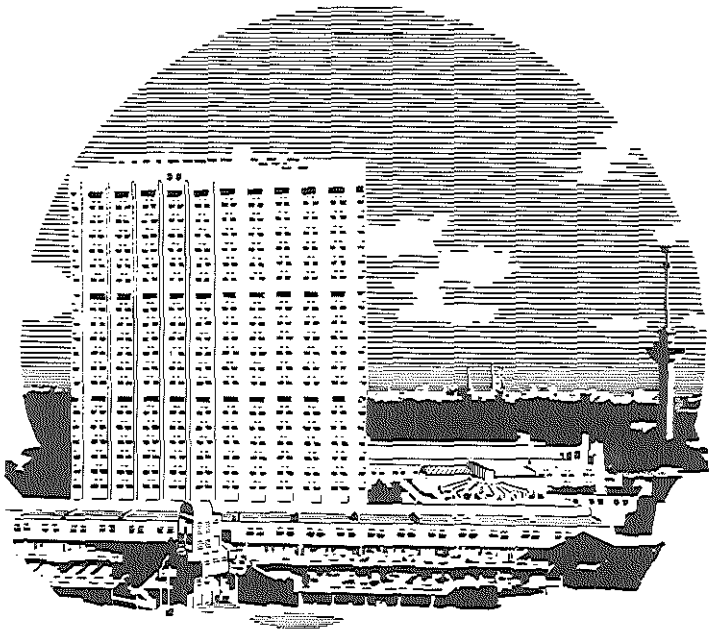

Dit proefschrift werd bewerkt binnen het Instituut Endocrinologie, Groei & Voortplanting, thans opgenomen in de Vakgroep Endocrinologie & Voortplanting van de Faculteit der Geneeskunde en Gezondheidswetenschappen, Erasmus Universiteit Rotterdam.
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Voor Ineke, Jan en Annemarie
Aan mijn vader
Ter nagedachtenis aan mijn moeder

INHOUD

Hoofdstuk 1

Inleiding

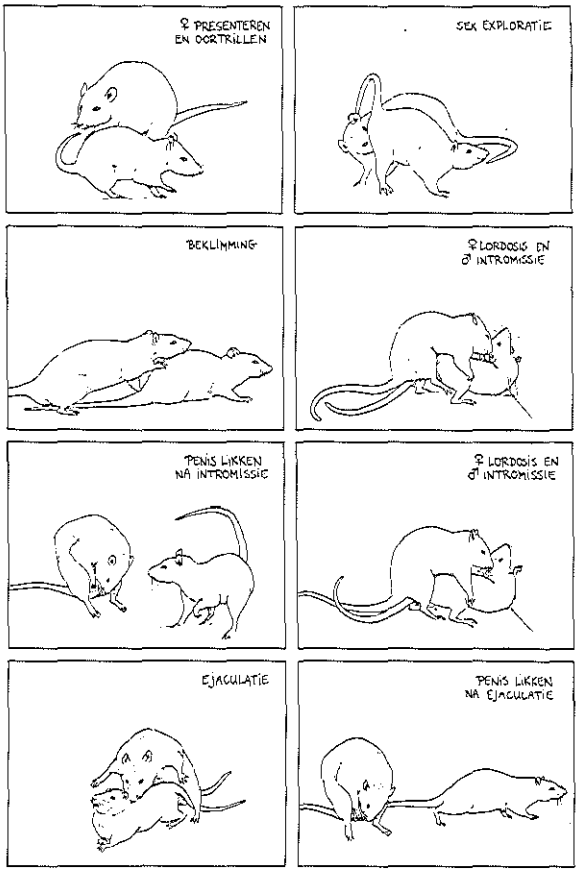
In dit hoofdstuk komen eerst de verschillende componenten van het seksueel gedrag van ratten aan de orde. Daarna wordt ingegaan op de stimulerende invloed van geslachtshormonen op het seksueel gedrag van het mannetje en van het vrouwtje. Vervolgens wordt, naar analogie van wat bekend is over de somatische seksuele differentiatie, de organiserende invloed van rond de geboorte in de rat aanwezige androgenen op het volwassen seksuele gedrag besproken. Daarna wordt besproken wat bekend is over activatie en organisatie van partner preferentie van de rat door androgenen en oestrogenen. Tot slot wordt kort ingegaan op de functionele relatie tussen hersengebieden en gedrag.

1.1 Componenten van seksueel gedrag

Het seksueel gedrag van ratten bestaat uit een aantal duidelijk van elkaar te onderscheiden componenten. Als reactie op haar uitnodigende gedragingen volgt het mannetje het bronstige vrouwtje en vertoont beklimmingen, intromissies en ejaculaties. Bij beklimmingen, die al dan niet met bekkenstoten gepaard gaan, benadert het mannetje het vrouwtje van achteren en beklimt haar waarbij hij zijn voorpootjes tegen haar flanken drukt. Een intromissie is een kortdurende (circa 200-400 ms; Sachs & Meisel, 1988), diepe bekkenstoot waarbij de penis in de vagina van het vrouwtje komt. Hierna springt het mannetje van het vrouwtje af. Na een aantal beklimmingen met intromissie volgt een wat langere beklimming met intromissie en ejaculatie. Hierbij richt het mannetje zich op van de rug van het vrouwtje en strekt de voorpootjes ietwat zijwaarts. Na een ejaculatie volgt een rustperiode, de zogenaamde refractaire periode, die bij mannelijke ratten gemiddeld 4 tot 5 minuten duurt. Dan kan een nieuwe copulatie-serie gestart worden.

Een bronstig vrouwtje zoekt de nabijheid van een mannetje. Als reactie op de beklimmingen van het mannetje vertoont ze een holle rug, lordose genoemd. Hierbij richt zij haar kop op, brengt haar perineum omhoog en beweegt haar staart opzij om zodoende intromissies en ejaculaties mogelijk te maken. Naast deze zogenaamde receptieve componenten van het paargedrag, heeft Beach (1976) de aandacht gevestigd op het bestaan van attractieve en

Verschillende gedragingen tijdens seks-test



Figuur 1-1 Verschillende gedragingen van een mannetjes- en vrouwtjesrat tijdens paring (Naar: Timmermans, 1978).

proceptieve componenten van het bronstgedrag van het vrouwtje. De attractiviteit van het wijfje is een abstract begrip en komt kort gezegd neer op de seksuele stimulus-waarde van het ene dier voor het andere dier, anders gezegd de aantrekkelijkheid van de een voor de ander. Proceptief gedrag bestaat uit gedragscomponenten, die verondersteld worden seksueel uitnodigend te zijn voor het mannetje om tot seksueel gedrag over te gaan. Hiertoe wordt 'darting' gerekend, waarbij het vrouwtje op karakteristieke wijze wegschiet om na enkele decimeters weer abrupt tot stilstand te komen. Vaak zal ze dan ook een bolle rug maken (presenteren), waarbij ze haar achterzijde naar het mannetje toewendt. Tijdens dit wegschieten en stilzitten valt het trillen van de oortjes op, 'ear-wiggling', dat eigenlijk berust op het snel bewegen van de kop (Vreeburg & Ooms, 1985). Ook maakt het vrouwtje sprongetjes waarbij ze maar weinig van plaats verandert, 'hopping'.

1.2 Activatie van seksueel gedrag

Bronstgedrag van vrouwtjes

Intacte vrouwelijke ratten hebben een ovariële cylcus van 4 of 5 dagen. Het optreden van bronstgedrag hangt ten nauwste samen met de ovariële cylcus. Slechts gedurende ongeveer 12 uur in de nacht voorafgaande aan de dag van vaginale oestrus zijn zij bereid met een mannetje te paren (Bermant & Davidson, 1974; Lodder, 1977). Op de overige dagen van de cyclus worden seksuele toenaderingspogingen van een mannetje geweerd. De hormonen uit de eierstokken spelen bij het tot uiting komen van bronstgedrag een belangrijke rol, want operatieve verwijdering laat de periodiek optredende bronst bij vrouwtjes verdwijnen. Substitutie met oestradiol is in staat het lordosegedrag en andere bronstige verschijnselen zoals 'ear wiggling' en presenteren op een dosisafhankelijke wijze te herstellen (Davidson, Rodgers, Smith & Bloch, 1968; de Jonge, Burger & van de Poll, 1986b). Wanneer ook progesteron wordt toegediend, faciliteert dit steroid het lordosegedrag, d.w.z. dat er vooraf minder oestradiol nodig is voor herstel van dit gedrag. Oestradiol en progesteron worden normaliter geproduceerd door de ovaria. Ook testosteron, met of zonder progesteron, kan lordosegedrag opwekken in geovariëctomeerde vrouwtjes (de Jonge & van de Poll, 1986; Leshner, 1978). Hierbij wordt testosteron vermoedelijk omgezet in oestradiol (Gladue, Dohanich & Clemens, 1978). Dihydrotestosteron is niet in staat lordosegedrag van vrouwtjes te activeren (van de Poll, van Zanten & de Jonge, 1986). Uit het bovenstaande wordt duidelijk, dat sommige hormonen, toegediend op volwassen leeftijd, in staat zijn seksueel gedrag te stimuleren. Daarom wordt dit de **activerende** werking van hormonen genoemd.
De wijze waarop steroïdhormonen in de hersenen werken is nog niet opgehelderd. Ongetwijfeld spelen ook neurotransmitters een rol. Dit zijn stoffen die in de zenuwspleet (synaps) vrijkomen en door binding aan een pre- of postsynaptische receptor effect sorteren, d.w.z. een zenuwprikkel doorgeven. Twee bekende neurotransmitters zijn gamma-aminoboterzuur (GABA) en acetylcholine (ACh). Onderzoek naar het effect op lordosegedrag van geovariëctomeerde vrouwelijke ratten, liet zien dat GABA-receptor-agonisten lordosegedrag, geïnduceerd door oestradiol-benzoaat (EB) en progesteron (P), kunnen inhiberen (Ågmo, Soria & Paredes, 1989). In een ander onderzoek werd lordosegedrag geremd door scopolamine, een acetylcholine-receptor antagonist (Menard & Dohanich, 1989).

Beklimgedrag van mannetjes

Wanneer mannelijke ratten samengebracht worden met een bronstig vrouwtje vertonen ze

spoedig beklimgedrag. De componenten van dit gedrag zijn reeds hiervoor beschreven. Al heel lang is bekend dat beklimgedrag na castratie afneemt en uiteindelijk stopt. Berthold (1849) was de eerste die de effecten van castratie wetenschappelijk heeft onderzocht en gepubliceerd. Hij maakte gebruik van hanen. Hij vond, dat na verwijdering van beide testes de hanekam in omvang afnam; ook kraaiden, vochten of copuleerden de hanen niet meer. Verwijdering van één testis veroorzaakte geen somatische- en gedragsveranderingen, evenmin wanneer beide testes verwijderd werden en er één werd teruggeplaatst in de buikholte. Door deze laatste ingreep werd de innervatie van de testis verbroken. Dit is van betekenis omdat men in die tijd tussen de testis en het centrale zenuwstelsel een zenuwverbinding veronderstelde, welke het effect van de testis bewerkstelligde. Uit zijn waarnemingen leidde Berthold af, dat de testis inwerkt op het bloed en dat dit inwerkt op (doelwit)organen elders in het lichaam. Dit kwam tot uitdrukking in de instandhouding van de kam en het hanegedrag. Veel later is gebleken dat deze aan het bloed afgegeven stof testosteron was.

Castratie heeft bij mannelijke ratten duidelijke effecten op het paargedrag. Binnen enkele uren na castratie blijkt de testosteron-concentratie tot (bijna) nul gedaald (Bermant & Davidson, 1974). Het seksueel gedrag is de eerste dagen echter nog volledig intact. In de daaropvolgende weken neemt het seksueel gedrag geleidelijk af. Eerst verdwijnt het ejaculatiegedrag en pas later de beklimmingen met intromissie. Beklimmingen met bekkenstoten blijven heel lang op een laag niveau bestaan. Het is opmerkelijk dat zelfs 10 weken na castratie bij sommige mannetjes nog ejaculaties kunnen voorkomen (Davidson, 1966a).

Substitutie met testosteron (vaak in veresterde vorm toegediend: testosteron-propionaat, TP) herstelt het copulatiegedrag tot het niveau van voor de castratie (Södersten, 1975; Damassa, Smith, Tennent & Davidson, 1977). Blokkade van de werking van TP met flutamide (4'-nitro-3'-trifluoromethyl-isobutyranilide), een anti-androgene stof die gedacht wordt de androgeenreceptor te blokkkeren, voorkomt het herstel van beklimgedrag in lang geleden gecastreerde ratten (Gladue & Clemens, 1980). Toediening van testosteron of oestradiol direct in het mediale preoptische gebied van de hersenen is eveneens in staat het seksueel gedrag te herstellen tot en met ejaculatiegedrag in veel, maar niet alle, langdurig gecastreerde mannetjes (Davidson, 1966b; Christensen & Clemens, 1974). In onderzoek van Baum en Vreeburg (1973) zijn aanwijzingen gevonden dat zowel 5α-dihydrotestosteron (DHT) als oestradiol (E_2) betrokken zijn bij de activatie van het mannelijke paargedrag. Aangenomen wordt dat E_2 voornamelijk in het CZS werkt en DHT voornamelijk op de in- en uitwendige geslachtsorganen. Blokkade van de omzetting van T in E_2 door de aromataseremmer 1,4,6-androstatriëen-3,17-dion (ATD), toegediend via injectie of via intracraniële implantatie, remde het beklim-

gedrag van met testosteron behandelde gecastreerde mannelijke ratten (Kaplan & McGinnis, 1989). Remmende effecten van ATD op paargedrag zijn ook gevonden in mannelijke hamsters (Steel & Hutchinson, 1988). In een ander onderzoek (Södersten & Gustafsson, 1980) werd het niet-aromatiseerbare androgeen methyltriënolon (R1881) toegediend aan gecastreerde mannetjes. Dit bleek in staat het seksueel gedrag te activeren. Mogelijk is androgeenwerking op zich soms voldoende om seksueel gedrag te activeren, of heeft R1881 ook een oestrogene werking op het CZS. DHT alleen is eveneens, zij het in mindere mate dan in combinatie met E_2, in staat gebleken mannelijk paargedrag te stimuleren in Wistar mannetjes (Baum & Vreeburg, 1973) en King-Holtzman mannetjes (Olsen, 1979).

Activatie "vrouwelijk" gedrag bij mannetjes

Mannelijke ratten vertonen, wanneer ze samengebracht worden met een bronstig vrouwtje, beklimgedrag en zelden lordosegedrag. Onder bepaalde omstandigheden zijn mannetjes bereid zich door een seksueel aktief mannetje te laten beklimmen en als reactie daarop lordose te vertonen. Beach (1945) beschrijft het gedrag van een onbehandelde mannelijke rat die ejaculaties vertoonde bij een bronstig vrouwtje en lordosegedrag bij een seksueel aktief mannetje. Na castratie verdwenen deze gedragingen. Na behandeling met TP kwam zowel het ejaculatie- als het lordosegedrag terug. Behandeling met EB, al dan niet gecombineerd met progesteron, had wel een activerend effect op het lordosegedrag, maar niet op het ejaculatiegedrag (Beach, 1945). Andere onderzoekers (van de Poll & van Dis, 1977) hebben zelfs hogere lordosequotiënten gerapporteerd in intacte Wistar mannetjes dan in gecastreerde Wistar mannetjes na injectie met een enkele dosis oestradiol (75 µg) gevolgd door progesteron (1 mg). Zowel in met oestradiol behandelde intacte, als in gecastreerde mannetjes bleek progesteron een faciliterend effect te hebben, zoals bekend is van vrouwtjes. Uit ander onderzoek is gebleken, dat in prepuberaal gecastreerde Wistar mannetjes zowel EB als TP een activerend effect heeft op het volwassen lordosegedrag (Södersten, 1975). Södersten, de Jong , Vreeburg en Baum (1974) vonden een stamverschil in het lordosegedrag van intacte mannelijke ratten: ongeveer 50% van intacte Deense Wistar mannetjes vertoonde lordose tegen 0% van de door Södersten gebruikte Nederlandse Wistar mannetjes.

Activatie "mannelijk" gedrag bij vrouwtjes

Volwassen, geovariëctomeerde vrouwtjes behandeld met testosteron, vertonen vaak beklimgedrag wanneer ze worden samengebracht met een bronstig vrouwtje (Beach, 1968; van de Poll, van der Zwan, van Oyen & Pater, 1982; Slob & van der Schoot, 1982; Slob & Vreeburg,

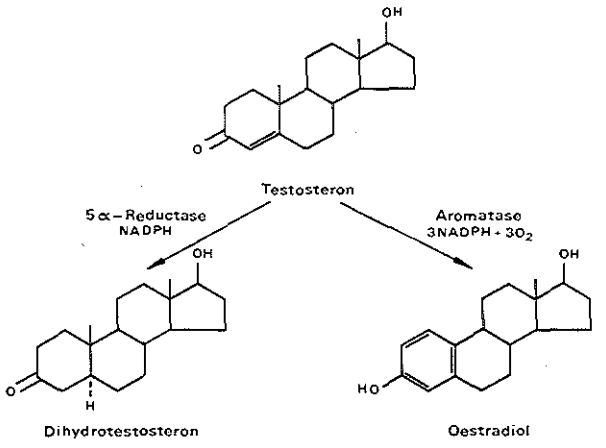
1985; de Jonge, Burger & van de Poll, 1986a). Het is echter reeds lang bekend, dat ook intacte vrouwtjes gedurende de gehele ovariële cyclus in ongeveer dezelfde mate beklimgedrag kunnen vertonen (Beach & Rasquin, 1942). Södersten (1972) vond de laagste beklimfrequentie in intacte vrouwtjes ratten gedurende de dag van de bronst. Na het verwijderen van de ovaria vond de laatste onderzoeker weinig beklimgedrag, terwijl de frequentie bij Beach en Rasquin ongewijzigd bleef. Naast T, is ook EB (Södersten, 1972), maar niet DHTP (van de Poll et al., 1986) in staat gebleken beklimgedrag in geovariëctomeerde vrouwtjes te activeren.

Samenvatting activatie

Wanneer een mannelijke en een bronstige vrouwelijke rat worden samengebracht vertoont het mannetje beklimgedrag (beklimmingen, intromissies, ejaculaties) en het vrouwtje proceptief (oortrillen, hopping en darting, presenteren) en receptief (lordose) gedrag. Beklimgedrag bij mannetjes stopt enige tijd na castratie en kan worden geactiveerd door testosteron. Testosteron wordt in de hersenen omgezet in oestradiol en dit wordt verondersteld voor de activatie noodzakelijk te zijn. In de geslachtsorganen wordt testosteron omgezet in dihydrotestosteron. Vooral DHT wordt gedacht de groei en ontwikkeling van de penis en accessoire geslachtsorganen te stimuleren. Bronstgedrag van vrouwelijke ratten stopt direct na ovariëctomie en kan worden geactiveerd door oestradiol, al dan niet gecombineerd met progesteron.

Omzetting van testosteron

De omzetting van testosteron in oestradiol en dihydrotestosteron wordt weergegeven in onderstaande figuur.



Figuur 1-2 Schematische weergave van de omzetting van testosteron in de 2 belangrijkste metabolieten. Dihydrotestosteron ontstaat uit testosteron door 5α-reductie; oestradiol door aromatisatie.

De metabolieten van testosteron spelen niet alleen een belangrijke rol bij de activatie van seksueel gedrag, maar ook bij de somatische seksuele differentiatie en de differentiatie van seksueel gedrag (zie verder).

1.3 Differentiatie van genitale structuren

Naast wat bekend is over de stimulerende werking van hormonen op seksueel gedrag in volwassenheid, bestaan er onderzoeksgegevens over de invloed van rond de geboorte van de jonge mannelijke rat in het bloed circulerende androgenen op de seksuele differentiatie. Deze worden hieronder besproken.

De normale seksuele differentiatie bestaat uit 3 opeenvolgende en met elkaar verband houdende processen. De seksuele differentiatie begint met het tot stand komen van het chromosomale geslacht bij de bevruchting. De bevruchte eicel bevat naast de 22 paar niet-geslachtsgebonden chromosomen (autosomen) 1 paar geslachtschromosomen. Deze laatste bepalen het geslacht, XX voor de vrouw en XY voor de man.

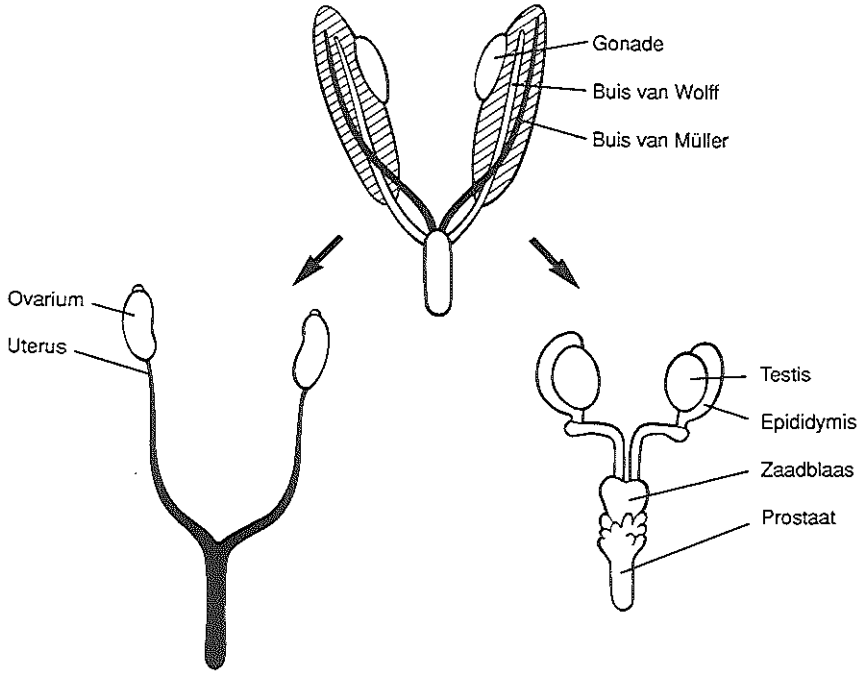
De tweede stap van seksuele differentiatie bestaat uit het vormen van het gonadale geslacht uit de ongedifferentiëerde gonade. Onderzoek heeft uitgewezen, dat een ongedifferentieerde gonade een ovarium wordt, tenzij een testis determinerend gen aanwezig is, Tdy genoemd in muizen en TDF in mensen. In een recent artikel wordt een overzicht gegeven van de kennis met betrekking tot dit gen (McLaren, 1991). Enige tijd is gedacht dat dit gen het Zfy gen was. Dit werd verworpen, omdat de homologe genen Zfy-1 en Zfy-2 niet tot expressie kwamen in de differentiërende embryonale testis van de muis (Koopman, Gubbay, Collignon & Lovell-Badge, 1989). Een betere kandidaat voor het testis determinerend gen lijkt het Sry gen (Sex determining region of the Y-chromosome) te zijn. Er vindt namelijk expressie van dit gen plaats tijdens de testis differentiatie in de muis (Koopman, Münsterberg, Capel, Vivian & Lovell-Badge, 1990) en er zijn mutaties gevonden in het SRY bij menselijke XY vrouwen (Berta, Hawkins, Sinclair, Taylor, Griffiths, Goodfellow & Fellous, 1990; Jäger, Anvret, Hall & Scherer, 1990). Recent volgde een overtuigend bewijs, doordat Sry geintroduceerd in het genoom van XX muizen in een aantal gevallen resulteerde in een fenotypisch mannelijke muis (Koopman, Gubbay, Vivian, Goodfellow & Lovell-Badge, 1991).

De derde stap bestaat uit de vorming van de inwendige geslachtsorganen uit de buizen van Wolff en de buizen van Müller en de vorming van de uitwendige genitalia uit de sinus urogenitalis. Elke foetus bezit in aanleg aan elke zijde naast de mesonephros (voornier) een buis van Wolff en een buis van Müller. Inzicht in de differentiatie van deze structuren is

voortgekomen uit werk van Jost (1953). Deze onderzoeker castreerde konijnefoeten vóór het tijdstip van seksuele differentiatie. Hieruit werden alleen diertjes geboren met vrouwelijke in- en uitwendige genitalia. Prenatale castratie en intraperitoneale implantatie van een kristal van TP liet dieren geboren worden met zowel vrouwelijke (=afkomstig van de buis van Müller), als mannelijke (=afkomstig van de buis van Wolff) inwendige genitale structuren (Jost, 1953; Jost, Vigier, Prépin & Perchellet, 1973). Dit en ander onderzoek, waarbij gebruik gemaakt is van anti-androgenen (Neumann, von Berwordt-Wallrabe, Elger, Steinbeck, Hahn & Kramer 1970; Neumann, 1990) hebben het proces van de somatische seksuele differentiatie opgehelderd, zoals hieronder samengevat.

Gedurende de differentiatie van het mannetje zorgt MIF (=Müller inhibitory factor; Miller, 1990), een glycoproteïne geproduceerd door de Sertoli cellen van de foetale testis, ervoor dat de buis van Müller in regressie gaat (Donahoe, Cate, MacLaughlin, Epstein, Fuller, Takahashi, Coughlin, Ninfa & Taylor, 1987). Testosteron, afkomstig van de interstitiële Leydig cellen van de testis, stabiliseert de buis van Wolff. In het volwassen dier zijn de epididymis, ductus deferens en de zaadblazen hiervan afkomstig. Het vrouwtje heeft geen testis en vormt derhalve geen MIF, waardoor de buis van Müller niet in regressie gaat. In het volwassen vrouwtje zijn de eileiders, de uterus en het bovenste een-derde deel van de vagina afkomstig van de buis van Müller. Een vrouwtje produceert geen testosteron, waardoor de buis van Wolff niet "gestabiliseerd" wordt en de embryonale aanleg dientengevolge te gronde gaat. Voor de indifferente sinus urogenitalis fungeert testosteron als prohormoon voor DHT (Wilson & Lasnitzki, 1971; Wilson, 1973). DHT wordt gevormd uit T door 5α-reductase (zie fig. 1-2). Het feit dat testosteron en dihydrotestosteron een verschillende rol spelen in de seksuele differentiatie is verder bevestigd door onderzoeken van mutaties bij mensen met een 5α-reductase-deficiëntie (Fisher, Kogut, Moore, Goebelsmann, Weitzman, Isaacs, Griffin & Wilson, 1978; Imperato-McGinley, Peterson & Gautier, 1984; Griffin & Wilson, 1989) en onderzoeken naar de effecten van specifieke 5α-reductase-remmers in ratte-embryos (Imperato-McGinley, Binienda, Arthur, Mininberg, Vaughan & Quimby, 1985; George & Peterson, 1988). Deficiëntie van 5α-reductase en dientengevolge van DHT leidt tot normaal geviriliseerde structuren, afkomstig van de buis van Wolff (epididymis, ductus deferens en zaadblazen), maar een 'vrouwelijke' sinus urogenitalis. Bij een gedeeltelijke 5α-reductase-deficiëntie is er vaak sprake van interseksualiteit waarbij de uitwendige genitalia niet duidelijk mannelijk of vrouwelijk zijn.

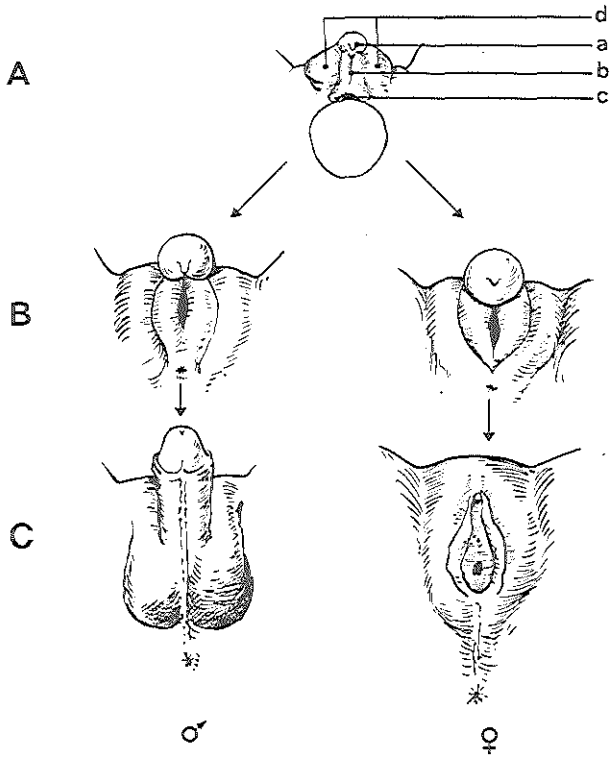
Naast penis en scrotum is bij de mens, en ook de rat, de prostaat afkomstig van de sinus urogenitalis. Onderzoek heeft laten zien dat DHT noodzakelijk is voor de differentiatie van de



Figuur 1-3 Schematische weergave van de seksuele differentiatie van de inwendige geslachtsorganen bij de rat. De ongediffentieerde gonade wordt testis of ovarium. Bij vrouwelijke individuen verdwijnt de aanleg van de buizen van Wolff en groeien de buizen van Müller uit tot de uterus, eileiders en het bovenste éénderde deel van de vagina. Bij mannelijke individuen verdijnt de aanleg van de buizen van Müller (door Müller Inhibitory Factor, MIF) en groeien de buizen van Wolff uit tot de epididymides, zaadleiders en zaadblazen (o.i.v. testosteron). De prostaat is afkomstig van de sinus urogenitalis.
(Overgenomen uit George en Wilson, 1988).

prostaat in de rat (George & Peterson, 1988). In een ander onderzoek bleek DHT echter ook in staat embryonale 'Wolffse' structuren bij ratten te behoeden voor regressie, waaruit de auteurs concluderen dat het receptor mechanisme niet specifiek is voor testosteron, dat normaliter deze structuren stabiliseert (Schultz & Wilson, 1974).

Samenvattend: een organisme wordt fenotypisch een vrouw tenzij androgenen en MIF, aanwezig gedurende een 'kritische' periode, bij de rat rond de geboorte, zorgen voor mannelijke in- en uitwendig genitalia. De inwendige genitalia worden bij de rat en ook de mens gevormd onder invloed van testosteron; de uitwendige onder invloed van DHT.



Figuur 1-4 Drie stadia in de ontwikkeling van de uitwendige geslachtsorganen bij de mens (een overeenkomstige ontwikkeling is ook gevonden bij de rat).

A. Aanvankelijk kan niet worden vastgesteld of het jongen of een meisje is: alle embryo's vertonen dezelfde aanblik van het onderbuiksgebied: een knobbeltje (a), waarachter een spleetje (b) en een dun stukje huid (c) vlak voor de aanleg van de staart gelegen. Het knobbeltje en spleetje zijn door een kussenachtige zwelling (d) van huid omgeven.

B. Jongens en meisjes kunnen hier van elkaar worden onderscheiden doordat bij jongens een grotere afstand is ontstaan tussen het spleetje en de vlak voor de staartaanleg doorgebroken anus. Het knobbeltje van A(a) is bij jongens meer naar voren gaan uitgroeien.

C. Het knobbeltje in tekening A is bij jongens uitgegroeid tot de penis, bij meisjes tot de clitoris. De kussenachtige zwelling (d in tekening A) is bij jongens scrotum geworden, bij meisjes zijn de labiae eruit ontwikkeld. Het spleetje heeft zich tot urine-opening ontwikkeld bij jongetjes en is op het uiteinde van de penis beland. Bij meisjes is het spleetje door een tussenschot in tweeën gedeeld: het voorste is urine-opening geworden, het achterste de schede-opening.

(Overgenomen uit: van der Schoot en Slob, 1990).

1.4 Differentiatie van seksueel gedrag.

Phoenix, Goy, Gerall en Young (1959) waren de eerste onderzoekers die systematisch onderzoek verrichtten naar volwassen seksueel gedrag van cavia's na prenatale blootstelling aan testosteron. Vrouwelijke cavia's, prenataal blootgesteld aan TP, hadden een vermannelijkt

genitaal, vertoonden in volwassenheid meer beklimgedrag na behandeling met testosteron en minder lordosegedrag na behandeling met oestradiol en progesteron dan controles. Uit dit experiment trokken de auteurs de conclusie dat prenataal aanwezig testosteron een **organiserende of differentiërende werking** heeft op de neurale weefsels die betrokken zijn bij het vertonen van volwassen hormoon geïnduceerd seksueel gedrag. Sinds die tijd zijn er zeer veel publikaties over de organisatie van seksueel gedrag verschenen. Hieruit en gebruikmakend van enkele overzichtsartikelen (Goy & Goldfoot, 1973; Baum, 1979; Goy & McEwen, 1980) komt voor de rat het volgende beeld naar voren. Wanneer de zich ontwikkelende foetus gedurende een gevoelige 'kritische' periode blootgesteld wordt aan androgenen dan treden twee processen op: enerzijds defeminisatie (ontvrouwelijking) van neurale weefsels die nodig zijn voor het vertonen van bronstgedrag; anderzijds masculinisatie (vermannelijking) van die neurale weefsels die gewoonlijk verantwoordelijk zijn voor de regulering van beklimgedrag.

Defeminiserende werking van testosteron

Wanneer vrouwelijke ratten neonataal (de eerste dagen na geboorte) blootgesteld worden aan TP vertonen ze minder lordosegedrag dan hun controles, wanneer ze in volwassenheid behandeld worden met oestradiol en progesteron (Barraclough & Gorski, 1962; Södersten, 1976). Wanneer mannelijke ratten neonataal worden gecastreerd en daarmee worden ontdaan van hun endogene testosteron-bron, vertonen ze meer lordosegedrag dan niet gecastreerde controle mannetjes, wanneer ze op volwassen leeftijd behandeld worden met oestradiol en progesteron (Beach, Noble & Orndoff, 1969). Toediening van TP aan neonataal gecastreerde mannetjes tussen dag 0 en 10, maar niet tussen dag 13 en 14, verminderd deze toename in vrouwelijke receptiviteit (Beach et al., 1969). De defeminiserende werking van testosteron doet zich niet alleen postnataal voor, waar de meeste onderzoeken naar zijn verricht, maar ook prenataal. De nakomelingen van vrouwtjes die voor de geboorte (dag 16-21 van de zwangerschap) behandeld werden met TP vertoonden in volwassenheid onder invloed van ovariële hormonen minder lordosegedrag dan hun controles (Ward & Renz, 1972). Wanneer vrouwelijke ratten prenataal (dag 10-22 van de zwangerschap) werden blootgesteld aan het antiandrogeen cyproteron-acetaat (CA) vertoonden ze in volwassenheid in een paartest minder lordoseresponsen op beklimmingen van een seksueel aktief mannetje dan controles wanneer ze behandeld waren met oestradiol en progesteron. Hetzelfde werd ook waargenomen bij vrouwelijke ratten die voor de geboorte waren blootgesteld geweest aan een ander antiandrogeen, flutamide (Gladue & Clemens, 1978; Gladue & Clemens, 1982). De vraag of in vrouwelijke ratten ook voor de geboorte testosteron aanwezig is kan positief worden

beantwoord (Baum, Brand, Ooms, Vreeburg & Slob, 1988; Baum, Woutersen & Slob, 1991; Corbier, Roffi, Rhoda & Kerdelhué, 1984; Roffi, Chami, Corbier & Edwards, 1987; Slob, Ooms & Vreeburg, 1978; 1980; Weisz & Ward, 1980). Er zijn derhalve aanwijzingen dat gewoonlijk ook vrouwelijke ratten enigszins worden gedefeminiseerd door hun eigen androgenen.

Masculinisatie door testosteron

Wanneer vrouwelijke ratten prenataal werden blootgesteld aan TP bleken ze in volwassenheid vermannelijkte in- en uitwendige genitalia te hebben en meer T-geïnduceerd beklimgedrag te vertonen (Gerall & Ward, 1966) en soms zelfs in staat te zijn ejaculatie-gedrag te vertonen (Ward, 1969). Baum (1979) bespreekt in een zeer lezenswaardig overzichtsartikel de resultaten van onderzoeken waarbij neonataal TP werd toegediend aan vrouwelijke ratten. Dit leverde in volwassenheid wat tegenstrijdige resultaten op. In een aantal studies was het volwassen beklimgedrag na T-behandeling verhoogd, in andere studies bleef het gelijk aan de controle behandeling. Baum (1979) leidt hieruit af dat neonataal androgeen geen grote rol lijkt te spelen bij de masculinisatie van vrouwelijke ratten. Wanneer mannelijke ratten neonataal worden gecastreerd vertonen ze in volwassenheid beduidend minder beklimgedrag, met name de ejaculaties ontbreken (zie b.v. van der Schoot, 1980). Dit is overigens niet zo verwonderlijk, aangezien de penis zich bij de rat na neonatale castratie niet volledig ontwikkelt. Goy en Goldfoot (1973) bespreken dit probleem in hun overzichtsartikel, waarbij ze, naar aanleiding van een uitspraak van Beach, vaststellen dat het zonder hamer (penis) niet goed mogelijk is te timmeren (copuleren).

> 'A problem that is shared to a degree by all researchers of the behavioral dimorphisms reviewed in this chapter is that many male-female behavioral differences might be caused not by different neural organizations or sensitivity but rather by the fact of differing male and female body structures. Beach has long argued that a female, without possessing a penis, cannot possibly display the same patterns of male copulation as the male. He has referred to this informally as "the you-can't-be-a-carpenter-without-a hammer-argument." (.....) Although the postulate is appreciated, sufficient evidence does exist to rule out both extreme positions of "all brain, no penis," as well a "all penis, no brain."
> (uit Goy & Goldfoot, 1973).

Een experiment beschreven door McDonald, Beyer, Newton, Brien, Baker, Tan, Sampson, Kitching, Greenhill en Pritchard (1970) bracht meer duidelijkheid in deze zaak. Het ejaculatie-gedrag van gecastreerde mannelijke ratten kon wel worden geactiveerd door injecties met TP, maar niet door injecties met DHTP. Bij autopsie bleek er geen verschil te bestaan in de gewichten van zaadblazen en prostaat van beide groepen. De auteurs concludeerden dat onvermogen van DHT om ejaculatie-gedrag te activeren mogelijk veroorzaakt werd door het feit dat DHT niet kan worden gearomatiseerd naar oestradiol. Dit leidde tot de aromatiserings-hypothese, dat oestrogenen in de hersenen, ontstaan uit testosteron, verantwoordelijk zijn voor de effecten van testosteron.

Onderzoekers die vrouwelijke ratten bloot stelden aan anti-androgenen vonden verminderd T-geïnduceerd beklimgedrag van deze vrouwtjes op volwassen leeftijd (Stewart, Pottier & Kaczender-Henrik, 1971, Ward & Renz, 1972; Clemens, Gladue & Coniglio, 1978). Ook hier werd verondersteld dat de in de vrouwelijke foetus aanwezige androgenen enigszins masculiniserend werkten op vrouwtjes. De oorsprong van deze androgenen bij vrouwtjes is niet geheel duidelijk. Als mogelijke bron voor androgenen zijn de bijnieren, de broertjes in dezelfde uterushoorn en de placenta voorgesteld (Vreebrug, Groeneveld, Post & Ooms, 1983). Ward en Renz (1972) stelden de bijnieren verantwoordelijk. Clemens et al. (1978; zie ook Clemens, 1974) veronderstelden diffusie via de amnionvliezen. Vrouwtjes die in utero tussen twee broertjes hadden gelegen vertoonden hogere beklimfrequenties dan vrouwtjes die tussen twee zusjes hadden gelegen. Clemens et al. (1978) en Tobet, Dunlap en Gerall (1982) vonden dat de anogenitale afstand, een androgeen-gevoelige maat, bij vrouwtjes die in utero tussen twee broertjes hadden gelegen groter was dan bij vrouwtjes tussen twee zusjes. Meisel en Ward (1981) meenden op grond van de bloedstroom in utero (Del Campo & Ginther, 1972) te mogen stellen dat caudale broertjes, aan de kant van de cervix, een masculiniserend effect zouden hebben. Vrouwtjes met één of meer broertjes caudaal vertoonden meer volwassen beklimgedrag onder invloed van testosteron dan vrouwtjes zonder een caudaal broertje. Richmond en Sachs (1984) vonden aanwijzingen dat de caudale ligging ook een significant effect had op de anogenitale afstand. In recent onderzoek uit ons eigen laboratorium (Houtsmuller & Slob, 1990) werd gevonden dat caudale broertjes een masculiniserend (meer vermannelijkend) en defeminiserend (meer ontvrouwelijkend) effect hadden op vrouwelijke ratten wanneer de zwangerschap ongestoord verliep: bij vrouwtjes met een broertje caudaal werd meer T-geïnduceerd beklimgedrag en minder E_2-geïnduceerd lordosegedrag gevonden dan bij vrouwtjes zonder caudaal broertje. Werd hemihysterectomie uitgevoerd tijdens de zwanger-schap, dan was dit effect verdwenen. In twee eerdere onderzoeken (Slob & van der Schoot,

1982; van de Poll et al., 1982) werd geen effect van foetale nestsamenstelling gevonden. Bij muizen zijn duidelijke effecten gevonden van prenatale ligging in de uterushoorn. In een recent artikel beschrijven vom Saal, Quadagno, Even, Keisler, Keisler en Khan (1990) welke effecten in de loop van de tijd gevonden zijn van intra-uteriene nestsamenstelling op een aantal gedragingen in volwassenheid. Hier beperk ik me tot de invloed op seksueel gedrag. Vrouwelijke muizen die intra-uterien tussen twee mannetjes hadden gelegen (2M-vrouwtjes) vertoonden op de leeftijd van 5 maanden significant hogere lordose-quotiënten na behandeling met oestradiol en progesteron, dan vrouwtjes die intra-uterien niet naast broertjes hadden gelegen (0M-vrouwtjes; Rines & vom Saal, 1984). Deze verschillen werden niet meer gezien toen de muizen 17 maanden oud waren. Op de leeftijd van 9 maanden vertoonde een hoger percentage 2M-vrouwtjes dan 0M-vrouwtjes testosteron-geïnduceerd beklimgedrag. Bij de oudere vrouwtjes (21 maanden oud) was dit effect afwezig. Andere onderzoekers (Gandelman & Kozak, 1988) vonden geen effect van intra-uteriene ligging op volwassen T-geïnduceerd beklimgedrag bij vrouwelijke muizen. Interessant is nog te vermelden dat vom Saal en medewerkers uit hun onderzoek concluderen dat bloed naar de uterus van de muis (vom Saal et al., 1990) en de rat (vom Saal, Dhar & Even, 1989) zowel van ovariële als cervicale kant wordt aangevoerd en ook weer daarnaar wordt afgevoerd; dit in tegenstelling tot het idee van Meisel en Ward (1981) die veronderstelden dat bij ratten het bloed stroomt van de cervicale naar de ovariële kant van de uterushoorn.

Rol van oestrogene metabolieten van testosteron

Oestradiol lijkt een belangrijke rol te spelen bij het activeren en organiseren van seksueel gedrag bij de rat. Zoals eerder beschreven, werd door McDonald et al. (1970) de aromatiseringshypothese opgesteld. Deze houdt in dat lokaal in de hersenen, door aromatisatie uit testosteron gevormde oestrogenen, verantwoordelijk zijn voor de werking van testosteron. In diverse hersendelen is aromatase aangetoond (McEwen, 1981) en zijn oestradiol-receptoren aangetroffen, zowel voor als na de geboorte (Kato, Atsumi & Inaba, 1974; Vito & Fox, 1979). Veel onderzoek is verricht om de rol van oestradiol voor de organisatie van seksueel gedrag te verduidelijken. Hiertoe is oestradiol toegediend gedurende de kritische periode. Tevens zijn aromatase remmers gebruikt, die de omzetting blokkeren van testosteron naar oestradiol, en zijn anti-oestrogenen toegediend.

Oestradiol toegediend aan vrouwelijke of mannelijke ratten tijdens een kritische periode leidde bijna zonder uitzondering tot defeminisatie van het volwassen gedrag (Baum, 1979). Perinatale toediening van oestradiol gaf minder eenduidige resultaten wat betreft de

masculinisatie (Baum, 1979). Neonatale toediening van de aromatase-remmer ATD aan mannelijke ratten verhoogde de bereidheid om lordosegedrag te vertonen in volwassenheid, zowel met als zonder oestradiol (E_2) en progesteron (P) (McEwen, Lieberburg, Chaptal & Krey, 1977; Vreeburg, van der Vaart & van der Schoot, 1977; Davis, Chaptal & McEwen, 1979; Fadem & Barfield, 1981). Prenatale blootstelling aan ATD leidde tot meer lordosegedrag van mannetjes dan behandeling met oplosmiddel na volwassen castratie en behandeling met oestradiol en progesteron (Clemens & Gladue, 1978; Whalen & Olsen, 1981; Whalen, Gladue & Olsen, 1986). Mannelijke ratten perinataal blootgesteld aan zogenaamde anti-oestrogenen, stoffen waarvan bekend is dat zij de werking van oestradiol tegengaan, waren minder gedefeminiseerd dan ratten die perinataal waren behandeld met oplosmiddel. Wanneer mannelijke ratten neonataal behandeld werden met het anti-oestrogeen MER-25 (Booth, 1977; Södersten, 1978) of CI-628 (McEwen et al., 1977) vertoonden ze op volwassen leeftijd, na castratie en toediening van oestradiol en progesteron, meer lordosegedrag dan controles. De masculinisatie bleek door aromatase-remmers moeilijker te beïnvloeden. Het beklimgedrag van mannelijke ratten, die prenataal behandeld waren met ATD vertoonden geen verandering na castratie op dag 35 en volwassen behandeling met TP (Whalen & Olsen, 1981). Evenmin werden effecten gevonden van neonatale ATD behandeling in gonadaal intacte mannetjes (Davis te al., 1979; Vreeburg et al., 1977). Het vermogen om ejaculatiegedrag te vertonen werd echter wel geblokkeerd door aan neonataal gecastreerde ratten binnen 24 uur na geboorte ADT (androst-4-een-3,6,17-trion), een andere aromatase-remmer, in combinatie met testosteron toe te dienen (Booth, 1978). Bij deze laatste mannetjes was de penisontwikkeling niet verstoord; de mannetjes vertoonden wel intromissies. Mannelijke ratten, neonataal behandeld met het anti-oestrogeen MER-25, vertoonden op volwassen leeftijd minder ejaculatie-gedrag dan controles (Booth, 1977; Södersten, 1978).

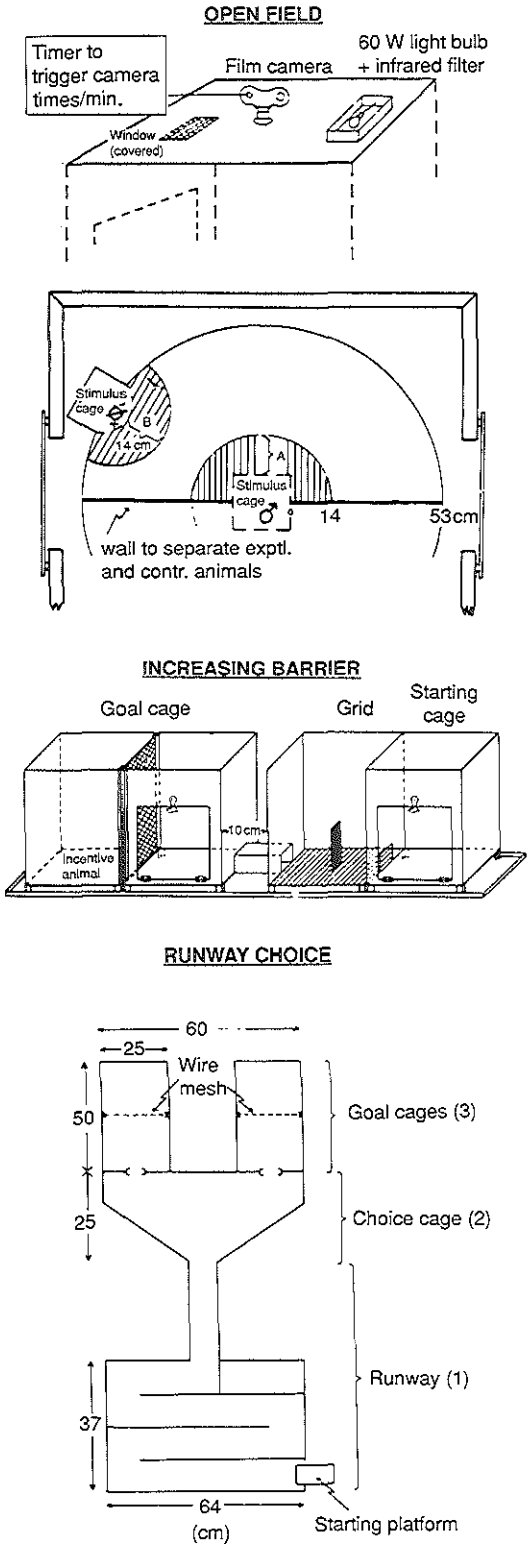
Samenvattend kan gezegd worden dat oestradiol, in het CZS gevormd uit testosteron, gedurende een kritische perinatale periode bij mannelijke ratten een defeminiserende en masculiniserende werking heeft op volwassen seksueel gedrag. Deze processen kunnen enigszins onafhankelijk van elkaar optreden. Er lijkt een analogie te bestaan tussen deze differentiatie van gedragingen en de ontwikkeling en het daaropvolgend verdwijnen of blijven bestaan van de buizen van Wolff en Müller bij de seksuele differentiatie van de inwendige genitalia. Er wordt wel verondersteld dat in de hersenen een analoge differentiatie optreedt waarvan echter de aard en lokalisatie nog niet geheel duidelijk is.

1.5 Partner preferentie van ratten

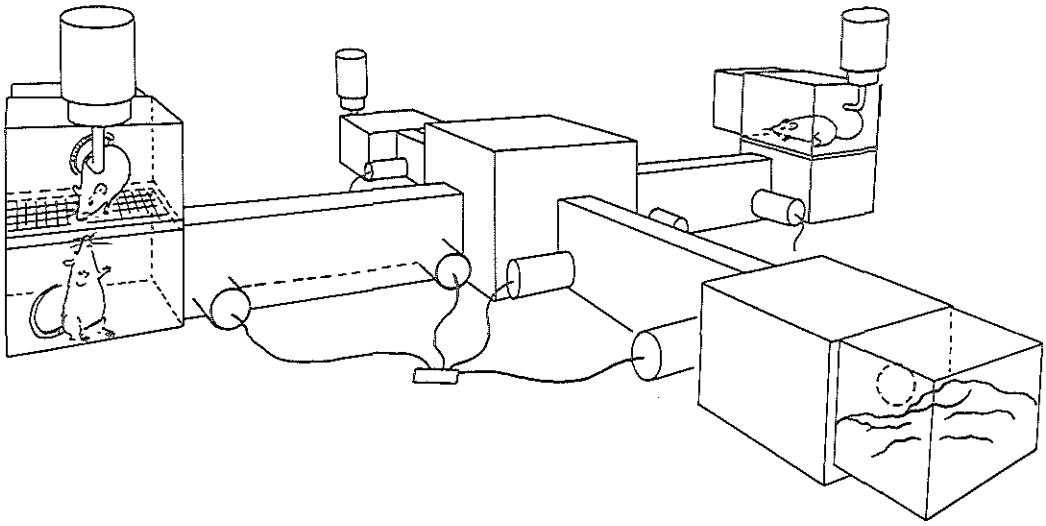
Beach (1976) heeft de aandacht gevestigd op pre-copulatoire gedragingen: gedragingen die aan de eigenlijke copulatie voorafgaan. Hieronder valt seksuele motivatie, de neiging van een dier om voorafgaand aan een seksuele interactie een ander dier op te zoeken en in de nabijheid daarvan te willen verkeren. Dit gedrag wordt partner-keuzegedrag of partner preferentie genoemd. Ook wordt wel de term seksuele oriëntatie gebruikt. In een overzichtsartikel gebruikt Adkins-Regan (1988) deze termen zonder onderscheid.
De halfcirkelvormige kooien die gebruikt worden voor het observeren van seksuele interacties zijn ongeschikt voor het bestuderen van partner preferentie. Voor het meten van partner-keuzegedrag bij ratten zijn diverse testopstellingen beschreven. Bijvoorbeeld een Y-vormig doolhof (Meyerson & Lindström, 1973; de Jonge & van de Poll, 1986), een kooi waarbij de stalen vloer tussen de startbox en de kooi met het stimulusdier onder spanning gezet kan worden (McDonald & Meyerson, 1973), kooien waarbij stimulusdiertjes zich in gazen kooitjes bevonden, geplaatst binnenin (Meyerson, Lindström, Nordström & Ågmo, 1973) en/ of langs de wand van de kooi (Eliasson & Meyerson, 1981; de Jonge & Meyerson, 1982), kooien waarbij de stimulusdieren zich in tuigjes bevonden (Webster, Williams & Dewsbury, 1982) en vrij recent een 'bilevel chamber', een testkamertje met een hoger en een lager deel (Mendelson & Pfaus, 1989).

In ons eigen laboratorium is uitgebreid ervaring opgedaan met een kruisvormige woonkooi (Merkx 1983, 1984a,b, Merkx, Slob & van der Werff ten Bosch, 1987, 1988a,b, 1989), een automatisch open veld (Slob, de Klerk & Brand 1987, Brand & Slob, 1988) en een 3-compartimenten-kooi (Slob et al., 1987; Broekman, de Bruin, Smeenk, Slob & van der Schoot, 1988; de Bruin, Broekman & van der Schoot, 1988).

Bij de meeste onderzoeken wordt een experimenteel dier de keuze geboden tussen een bronstig vrouwtje en een seksueel aktief mannetje of tussen een bronstig en een niet-bronstig vrouwtje, waarbij seksuele interactie tussen experimenteel en stimulusdier kan plaatsvinden of voorkomen wordt door metaalgaas. Hieronder volgt een beschrijving van wat bekend is in de literatuur aangaande de activatie en organisatie van (seksuele) partner preferentie bij ratten.



Figuur 1-5 Weergave van enkele proef-opstellingen die door Meyerson et al. (1973) werden gebruikt voor het bestuderen van seksuele motivatie. De bovenste figuur is een open veld met 2 metalen kooitjes voor stimulusdieren, één in het centrum van het open veld en één aan de rand. De middelste figuur is een apparaat waarbij de vloer tussen de start- en doelkooi (met stimulusdier) onder spanning gezet kan worden. De onderste figuur geeft een Y-vormig doolhof weer, met de mogelijkheid een stimulusdier te plaatsen aan het einde van elke arm. In de laatste 2 apparaten is seksueel contact mogelijk wanneer het deurtje van het stimuluskooitje wordt verwijderd. (Naar Meyerson et al., 1973).



Figuur 1-6 Schematische tekening van de kruiskooi zoals gebruikt door Merkx (1986). Duidelijk is te zien dat het loopdier door de bodem van gaas het stimulusdier kan zien, horen en ruiken, doch verder is lichamelijk contact slechts beperkt mogelijk. (Overgenomen uit Merkx, 1986).

Activatie van seksuele oriëntatie

Wanneer een vrouwtje bronstig is, zoekt zij actief de nabijheid op van een seksueel aktief mannetje (McClintock, 1984). In een keuzesituatie komt dit tot uitdrukking doordat zij meer tijd doorbrengt bij een sekueel aktief mannetje dan bij een bronstig vrouwtje. Na ovariëctomie verdwijnt deze preferentie. Eénmalige of langdurige toediening van oestradiol of testosteron aan geovariëctomeerde vrouwtjes laat deze voorkeur voor een seksueel aktief mannetje weer terugkomen (de Jonge, Eerland & van de Poll, 1986c; McDonald & Meyerson, 1973; Meyerson & Lindström, 1973; Meyerson et al., 1973; Slob et al., 1987). Geovariëctomeerde vrouwtjes behandeld met oestradiol-benzoaat brengen meer tijd door bij een seksueel aktief dan bij een seksueel inactief, gecastreerd mannetje (Edwards & Pfeifle, 1983). Toediening van dihydrotestosteron bleek, in tegenstelling tot testosteron of oestradiol, niet in staat preferentie voor een seksueel aktief mannetje (versus een geovariëctomeerd vrouwtje) te kunnen bewerkstelligen (McDonald & Meyerson, 1973). In een ander experiment vertoonden volwassen vrouwtjes behandeld met DHT geen interesse in en geen voorkeur voor stimulusdieren (een bronstig en een niet-bronstig vrouwtje) wanneer ze getest werden voor partner preferentie in een kruisvormige woonkooi (Merkx, 1983). Het niet aromatiseerbare androgeen methyltriënelon (R1881) was echter wel in staat preferentie voor een man versus een bronstig vrouwtje op te wekken (de Jonge, Kalverdijk & van de Poll, 1986d).

Bij dit soort onderzoek dient er rekening mee te worden gehouden dat copulatie-ervaring voor

of tijdens de test voor seksuele oriëntatie de preferentie kan beïnvloeden. In onderzoek verricht in ons laboratorium (Slob et al., 1987) werd duidelijk dat een 15 minuten durende paar-test met een seksueel aktieve man de preferentie van geovariëctomeerde en langdurig met TP behandelde vrouwtjes liet omslaan. Vooraf vertoonden deze vrouwtjes een geringe preferentie voor het mannetje, achteraf een duidelijke keuze voor het vrouwtje, zelfs wanneer tijdens de paar-test intromissies werden voorkómen door een pleister op de vagina van het vrouwtje. In een ander onderzoek vergeleken de Jonge, Burger, van Haaren, Overdijk en van de Poll (1987) de partner preferentie van seksueel ervaren versus seksueel onervaren geovariëctomeerde vrouwtjes na behandeling met olie of TP in volwassenheid. De seksuele ervaring bestond uit paar-testen met een bronstig vrouwtje. Onervaren vrouwtjes behandeld met olie vertoonden geen voorkeur; behandeld met TP vertoonden ze een voorkeur voor het mannetje. Ervaren vrouwtjes prefereerden het bronstige vrouwtje ongeacht olie- of TP-behandeling.
Gedurende de nacht van haar bronst brengen mannelijke ratten in een kruisvormige woonkooi (seksuele gedragsinteractie niet mogelijk) duidelijk veel meer tijd door in de nabijheid van het bronstige vrouwtje dan in de nabijheid van het niet-bronstige vrouwtje. Op de overige dagen van haar cyclus is dit niet het geval (Merkx, 1983). Castratie van mannetjes laat deze voorkeur na enkele dagen verdwijnen. Toediening van testosteron of oestradiol, maar niet dihydrotestosteron, laat de preferentie voor een bronstig vrouwtje weer terugkomen (Merkx, 1984a). In een automatisch open veld, waarbij seksuele interactie werd voorkomen door metaalgaas, activeerde testosteron de preferentie van gecastreerde mannelijke ratten voor een bronstig vrouwtje versus een niet-bronstig vrouwtje (Brand & Slob, 1988), alsmede de preferentie voor een bronstig vrouwtje versus een seksueel aktief mannetje (Hetta & Meyerson, 1978).
Samenvattend kan gezegd worden, dat de expressie van (seksuele) partner preferentie van mannelijke en vrouwelijke ratten op volwassen leeftijd geactiveerd wordt door geslachtshormonen. Hierbij speelt oestradiol, al dan niet afkomstig van testosteron, een belangrijke rol. Onderzoeken met niet aromatiseerbare androgenen, DHT en R1881, hebben tot nu toe tegenstrijdige resultaten opgeleverd. Opgedane copulatie-ervaring kan de preferentie in belangrijke mate beïnvloeden.

Organisatie van partner preferentie

In onderzoek naar partner preferentie van ratten is opmerkelijk weinig aandacht geschonken aan een mogelijke organiserende rol van perinatale androgenen. Hieronder worden de gepubliceerde onderzoeken besproken.

Betreffende vrouwelijke ratten zijn 2 studies bekend (de Jonge, Muntjewerff, Louwerse & van de Poll, 1988; Meyerson, Eliasson & Hetta, 1979). In het onderzoek van de Jonge et al. (1988) werden vrouwelijke jongen binnen 24 uur na geboorte geïnjecteerd met 1 mg TP of olie. Wanneer ze in volwassenheid werden geovariëctomeerd en behandeld met TP prefereerden neonataal geandrogeniseerde vrouwtjes een bronstig vrouwtje (versus een seksueel aktief mannetje). Neonataal met olie behandelde vrouwtjes hadden geen of een geringe voorkeur voor het stimulusmannetje. Meyerson et al. (1979) injecteerden vrouwelijke ratten op dag 5 na geboorte met 0.5 mg TP of olie. Neonataal geandrogeniseerde vrouwtjes vertoonden op volwassen leeftijd, na ovariëctomie en behandeling met EB of TP, een voorkeur voor een bronstig vrouwtje. Neonataal toegediend testosteron lijkt in staat partner keuze-gedrag van vrouwtjes te "organiseren".

Vier studies zijn bekend waarin gekeken is naar de effecten van neonataal aanwezige androgenen op volwassen partner keuze-gedrag van mannelijke ratten (zie tabel 2 van hoofdstuk 7.1). In deze experimenten werden mannelijke ratten tussen 0 en 36 uur na de geboorte gecastreerd. Op volwassen leeftijd werden ze getest voor hun partner preferentie, met en zonder substitutie met testosteron of oestradiol. Merkx (1984b) castreerde ratten binnen 24 uur na geboorte en behandelde de mannetjes met T, DHT of E_2 in volwassenheid. Mannetjes behandeld met T of E_2 prefereerden een bronstig vrouwtje (versus een niet bronstig vrouwtje). Mannetjes, behandeld met DHT, vertoonden geen voorkeur op volwassen leeftijd. Merkx concludeerde dat neonatale hormonen weinig effect lijken te hebben op volwassen "sociaal-seksueel preferentie gedrag". Eliasson en Meyerson (1981) vonden dat neonataal gecastreerde mannelijke ratten, getest op volwassen leeftijd zonder testosteron substitutie, lagere preferentie-scores hadden voor een bronstig vrouwtje versus een seksueel aktief mannetje, dan intacte controle mannetjes. In een eerdere studie (Meyerson et al., 1979), werden mannelijke ratten 24-36 uur na geboorte gecastreerd en op dag 2 en 4 na geboorte behandeld met TP of olie. Wanneer ze in volwassenheid behandeld werden met TP, prefereerden alle mannetjes een bronstig vrouwtje (versus een seksueel aktief mannetje). Bestond de behandeling op volwassen leeftijd uit EB, dan prefereerden de neonataal met TP behandelde mannetjes een bronstig vrouwtje en de neonataal met olie behandelde mannetjes een mannetje. Vega Matuszczyk, Fernandez-Guasti en Larsson (1988) castreerden mannetjes direct na geboorte per keizersnee of 4 uur later. Wanneer ze op volwassen leeftijd behandeld werden met T kozen beide groepen voor een bronstig vrouwtje.

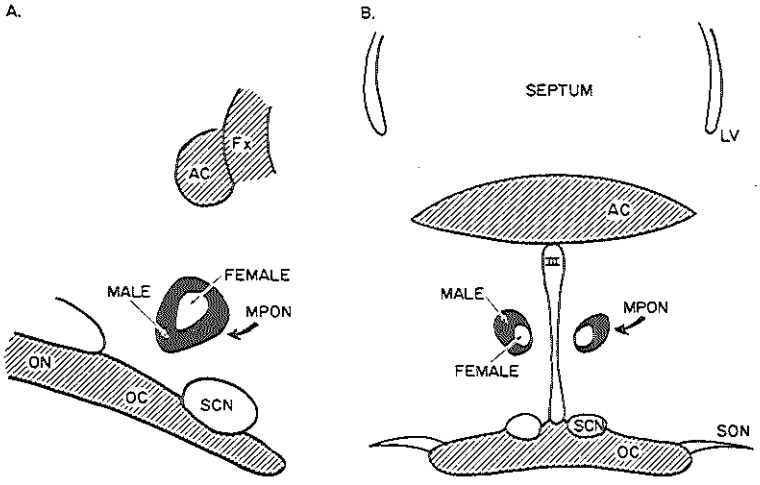
Een andere mogelijkheid om de effecten van neonataal aanwezige androgenen te beïnvloeden is door de omzetting van T naar E_2 te blokkeren door een aromatase remmer, bv. ATD. Davis

et al. (1979) hebben mannelijke ratten gedurende dag 2-10 blootgesteld aan ATD, maar vonden op volwassen leeftijd geen verandering in partner preferentie: alle dieren brachten meer tijd door bij een bronstig vrouwtje dan bij een mannetje.
Over het algemeen kan gesteld worden dat in de beschikbare literatuur weinig aanwijzingen gevonden worden, dat neonataal testosteron, al dan niet via de oestrogene metaboliet, volwassen seksuele oriëntatie van mannelijke ratten "organiseert". Neonatale behandeling met TP, maar niet DHT, is in staat gebleken volwassen seksuele oriëntatie van vrouwelijke ratten blijvend te beïnvloeden.

1.6 Hersenen en seksueel gedrag

Hieronder volgt een beknopt overzicht. In hun artikel vermelden de Jonge, Swaab, Ooms, Endert en van de Poll (1990) dat reeds lange tijd verondersteld wordt dat het mediale preoptische gebied (MPOA='medial preoptic area') betrokken is bij de regulatie van seksueel gedrag bij de rat. Lesies aangebracht in dit gebied lieten het seksueel gedrag, met name ejaculaties, verminderen. Electrische stimulatie van dit gebied werd door de rat als belonend ervaren. Raisman en Field (1971, 1973) gebruikten electronenmicroscopie om de synapsverbindingen van de area preoptica te analyseren. Zij ontdekten dat de dendrieten van vrouwelijke ratten meer synapsen hadden dan dendrieten van mannetjes. Neonatale castratie van mannelijke ratten werd gevolgd door een vrouwelijk aantal synapsen; neonatale toediening van TP aan vrouwtjes veroorzaakte een mannelijk aantal synapsen op de dendrieten. Gorski, Gordon, Shryne en Southam (1978) en Gorski, Harlan, Jacobson, Shryne en Southam (1980) waren in staat met behulp van lichtmicroscopie op een relatief eenvoudige manier het verschil in grootte tusen mannetjes en vrouwtjes aan te tonen van de door deze auteurs zo genoemde seksueel dimorfe kern van de preoptische area (SDN-POA). Het volume van deze kern, gelegen tussen commissura anterior en chiasma opticum aan weerszijden van de derde ventrikel, bleek bij mannelijke ratten circa vijf tot achtmaal zo groot te zijn als bij vrouwtjes.

Perinataal aanwezige androgenen bleken een "organiserend" effect op de grootte van de SDN-POA te hebben. Neonataal gecastreerde mannelijke ratten hadden een "vrouwelijk" volume op volwassen leeftijd. Neonatale behandeling met testosteron op dag 4 na geboorte leidde bij vrouwelijke ratten tot een SDN-POA met een "mannelijk" volume (Gorski et al., 1978). Hoewel op volwassen leeftijd aanwezige steroïden enig effect leken te hebben op de grootte en cytoarchitectuur van de SDN-POA, waren de verschillen minder uitgesproken dan die



Figuur 1-7 Schematische weergave van de area preoptica van de rat met daarin de localisatie van de seksueel dimorfe component van de mediale preotische nucleus (MPON), in het sagittale en coronale vlak. De kern van de vrouw is geheel binnen de omvang van de kern van de man getekend.
Afkortingen: AC, commisura anterior; Fx, fornix; LV, laterale ventrikel; OC, chiasma opticum; ON, nervus opticus; SCN, nucleus suprachiasmaticus; SON, nucleus supraopticus; III, derde ventrikel. Getekend bij vergroting van 37x. [Uit: Gorski et al. (1978). Deze kern wordt nu de seksueel dimorfe kern van de area preoptica genoemd (SDN-POA)].

veroorzaakt door perinatale manipulaties (Bloch & Gorski, 1988a,b). Een kern die veel gelijkenis vertoont met de SDN-POA bij de rat is ook gevonden bij andere diersoorten (zie b.v. Baum, 1987; de Jonge et al., 1990) en ook bij de mens (Swaab & Fliers, 1985).

De vraag of de SDN-POA functioneel van betekenis is voor de expressie van seksueel gedrag bij de rat is onderzocht door Arendash en Gorski (1983) en de Jonge, Louwerse, Ooms, Evers, Endert en van de Poll (1989) bij mannetjes en door door Turkenburg, Swaab, Endert, Louwerse en van de Poll (1988) bij vrouwtjes. Arendash en Gorski (1983) gebruikten seksueel ervaren mannetjes. Zij vonden minder hoge beklim-, intromissie- en ejaculatiefrequenties bij mannetjes waarbij met een stereotact een electrolytische lesie geplaatst was in het gebied juist dorsaal van de SDN dan bij mannetjes met een schijnlesie. Er werden geen lagere beklimfrequenties gevonden wanneer de lesie zich in de eigenlijke SDN bevond. De Jonge et al. (1989) gebruikten seksueel onervaren mannelijke ratten en vonden een remmend effect op het beklimgedrag van de dieren als de SDN geledeerd was. In de eerste test was de ejaculatie-latentie van geledeerde dieren significant langer dan de ejaculatie-latentie van onjuist of niet-geledeerde dieren. Na herhaald testen verdween dit effect. Wanneer vervolgens een minder bronstig vrouwtje (LQ=50) werd gebruikt en de mannetjes niet van tevoren werden geadapteerd aan de testomstandigheden, d.w.z. wanneer onder suboptimale condities werd getest, was de

ejaculatie-latentie van geledeerde dieren langer dan van onjuist of niet-geledeerde dieren. De basale testosteron waarden van deze geledeerde en niet-geledeerde dieren waren niet verschillend van schijn-geledeerde dieren (de Jonge et al., 1989).
In de studie van Turkenburg et al. (1988) werd bij volwassen seksueel ervaren geovariëctomeerde en met testosteron behandelde vrouwtjes een remmend effect gevonden van één- of tweezijdige lesie van de SDN. Vrouwtjes met een lesie in de SDN-POA beklommen minder frequent dan controles. Er werd geen effect gevonden op het receptief en proceptief gedrag en de seksuele oriëntatie van vrouwelijke ratten.

Uit het bovenstaande kan geconcludeerd worden dat de SDN-POA functioneel betrokken lijkt te zijn bij het beklimgedrag van mannelijke en vrouwelijke ratten. Lesies in de SDN-POA zijn overigens niet in staat gebleken alle seksuele gedragingen te elimineren. De SDN-POA lijkt weinig betrokken te zijn bij de expressie van bronst en sekuele oriëntatie van vrouwelijke ratten.

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Hoofdstuk 2

Vraagstellingen van het huidige onderzoek

In dit proefschrift is geprobeerd antwoorden te vinden op de volgende vragen:

1 Is volwassen door testosteron-geïnduceerd beklimgedrag van vrouwelijke ratten op enigerlei wijze geprogrammeerd door perinataal circulerend testosteron (T)?
2 Speelt de prenatale ligging ten opzichte van broertjes hierbij een rol?
3 Wordt de volwassen partner preferentie van vrouwelijke ratten geprogrammeerd door perinataal aanwezig T?
4 Wordt de volwassen partner preferentie van mannelijke ratten geprogrammeerd door perinataal T en/of de dihydrotestosteron (DHT) en oestradiol (E_2)?
5 Welke effecten hebben 8-OH-DPAT en yohimbine (bekende ratte-"aphrodisiaca") op partner preferentie en seksueel gedrag van mannelijke ratten?

Voor de beantwoording van de vraag of perinatale androgenen volwassen beklimgedrag programmeren werden vrouwtjesratten pre- en/of neonataal blootgesteld aan anti-androgenen, stoffen die de werking van testosteron (T) tegengaan. Op volwassen leeftijd werden de inmiddels geovariëctomeerde vrouwtjes getest voor T-geïnduceerd beklimgedrag en voor lordose gedrag na behandeling met E_2, al dan niet gecombineerd met progesteron (P).
Voor de beantwoording van de vraag of broertjes een rol spelen bij de programmering van beklimgedrag van vrouwtjesratten, werd voor de zwangerschap één uterushoorn verwijderd en/of tijdens de zwangerschap de nestgrootte gemanipuleerd. Dit werd gedaan om de kans op een nest bestaande uit allemaal vrouwtjes te vergroten. De prenatale ligging werd bekend door van (zwangere) vrouwtjes met één uterushoorn tijdens de bevalling het geslacht te noteren van de achtereenvolgende jongen. Op volwassen leeftijd werden de vrouwtjes geovariëctomeerd en onderzocht op T-geïnduceerd beklimgedrag. De experimenten die betrekking hebben op de vragen 1 en 2 zijn beschreven in hoofdstuk 5.
De rol van perinatale androgenen in de programmering van volwassen partner preferentie van vrouwtjes werd onderzocht door vrouwtjes prenataal bloot te stellen aan anti-androgenen, anandron of flutamide, of neonataal te behandelen met T, DHT of olie. Op volwassen leeftijd werden de vrouwtjes geovariëctomeerd en na behandeling met T, DHT of een combinatie van DHT en E_2 onderzocht op hun partner preferentie met als keuze: een bronstig vrouwtje versus

een niet-bronstig vrouwtje of een bronstig vrouwtje versus een seksueel aktief mannetje. Dit experiment is beschreven in hoofdstuk 6.

De rol van perinatale androgenen voor de programmering van volwassen partner preferentie van mannelijke ratten werd onderzocht in 4 experimenten. In één experiment werden mannelijke ratten neonataal gecastreerd, vervolgens behandeld met T, DHT of olie en in volwassenheid onderzocht op hun partner preferentie, op dezelfde wijze als de neonataal met T of DHT behandelde vrouwtjes. Om de programmerende betekenis van E_2 na te gaan werden in twee andere experimenten mannelijke ratten perinataal blootgesteld aan een aromatase-remmer, ATD of atamestan, stoffen die de omzetting van T naar E_2 blokkeren. Op volwassen leeftijd werden ze getest voor partner preferentie (keuze: een bronstig vrouwtje en een seksueel aktief mannetje). Tevens werd het seksueel gedrag van deze gonadaal intacte mannetjes onderzocht, al dan niet na een injectie met een seksueel gedrag-stimulerende stof (8-OH-DPAT of yohimbine). Deze experimenten staan beschreven in hoofdstuk 7.

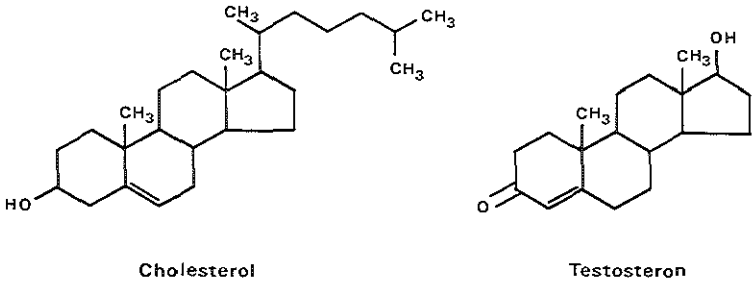
Hoofdstuk 3

Gebruikte stoffen en hun werking

In dit hoofdstuk worden de verschillende stoffen besproken, die gebruikt zijn in de in dit proefschrift beschreven onderzoeken. Allereerst testosteron en daarmee samenhangend de gebruikte anti-androgenen (cyproteron-acetaat, anandron en flutamide) en aromatase-remmers (ATD en atamestan). Tenslotte komen seksueel gedrag stimulerende stoffen (yohimbine en 8-OH-DPAT) aan de orde.

3.1 Testosteron

Gezien de belangrijke rol die testosteron en de metabolieten daarvan spelen in de in dit proefschrift beschreven onderzoeken wordt er een aparte paragraaf aan gewijd. Testosteron, een hormoon met een steroidstructuur, is in mannelijke zoogdieren een zeer belangrijk androgeen. Het wordt bij mens en dier voornamelijk geproduceerd in de testis door de Leydig cellen. Steroïdhormonen worden gemaakt uit cholesterol.



Cholesterol Testosteron

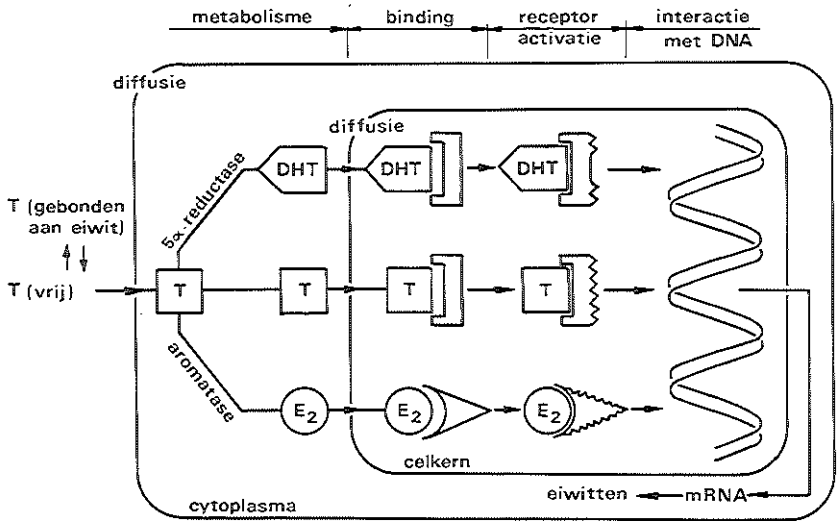
Figuur 3-1 De structuurformules van cholesterol en testosteron. Let op de structuurverwantschap na verwijdering van de zijketen van cholesterol.

De steroïdogenese omvat globaal 2 stappen: het enzymatisch verwijderen van de zijketen van cholesterol in de mitochondria, waardoor pregnenolon gevormd wordt. Dit wordt gevolgd door verdere omzetting in het gladde endoplasmatische reticulum naar testosteron en andere steroïden.

3.2 Werkingsmechanisme

Testosteron is lipofiel en hydrofoob. Transport in het bloed vindt voor het grootste gedeelte plaats gebonden aan drager-eiwitten: sexhormoon-bindend globuline (SHBG) of albumine (Griffin en Wilson, 1989). Alleen ongebonden, "vrij" testosteron is in staat de cel binnen te gaan. Verondersteld wordt dat dit gebeurt via passieve diffusie. Binnen de cel kan testosteron gemetaboliseerd worden. De twee belangrijkste metabolieten zijn 5α-dihydrotestosteron (DHT) en 17ß-oestradiol (E_2). DHT ontstaat door 5α-reductaseactiviteit uit testosteron; voor het ontstaan van oestradiol is het enzym aromatase nodig.

Eenmaal binnen de cel worden T, DHT en E_2 gebonden aan een specifieke receptor. Voor T en DHT is dit de androgeen-receptor, voor E_2 de oestradiol-receptor. Het steroid-receptor-complex wordt zodanig getransformeerd dat binding met DNA mogelijk wordt. Deze binding leidt ertoe dat transcriptie van DNA mogelijk wordt waardoor mRNA gevormd wordt. Dit mRNA wordt, na afsplitsing van kleine stukjes mRNA, een proces 'splicing' genoemd, vertaald in eiwit (translatie) op de ribosomen. Een vraag is lange tijd geweest waar de receptor zich in de cel bevindt. Vele jaren werd er van uitgegaan dat de receptor zich in het cytoplasma bevond en na binding met het hormoon geactiveerd werd om vervolgens getransporteerd te worden naar de kern. Hier zou dan binding plaatsvinden aan DNA. Voor de binding van glucocorticoiden aan de glucocorticoid receptor geldt dit model nog steeds (Wikström et al., 1987). Onderzoek heeft echter aangetoond dat voor andere steroïden dit model mogelijk



Figuur 3-2 Schematische weergave van het werkingsmechanisme van testosteron (zie tekst voor details).

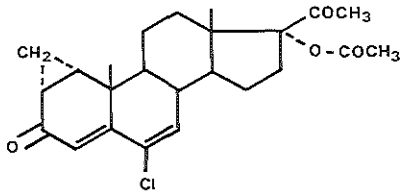
genuanceerd dient te worden. Onderzoek met antilichamen heeft laten zien dat de progesteron- en oestrogeen- receptoren zich vrijwel uitsluitend in de kern bevinden (Gasc et al., 1984, King & Greene, 1984). Recent onderzoek met antilichamen naar de lokalisatie van de androgeen-receptoren suggereert dat deze zich eveneens voornamelijk in de kern bevinden, hoewel de vorming plaatsvindt in het cytoplasma. De vroegere bevinding, waarbij androgeen-receptoren voornamelijk in het cytoplasma werden aangetroffen, berust mogelijk op een artefact. Verondersteld wordt dat tijdens de extractie van celkernen androgeen-receptoren in de cytoplasma-fractie terechtkomen, welke zich *in vivo* in de celkern bevonden (Husmann, 1990; Rommerts, 1990). Figuur 3-2 is een schematische weergave van de huidige kennis.

3.3 Beïnvloeding van de werking van testosteron

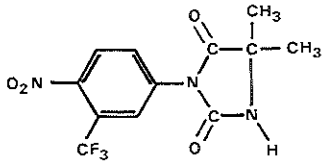
De werking van testosteron is op diverse manieren te remmen: door de synthese tegen te gaan, door remming van het metabolisme in de cel, door competitie voor de androgeen-receptor en door remming van de eiwitsynthese. In het huidige onderzoek is gebruik gemaakt van zogenaamde anti-androgenen, stoffen die zich binden aan de androgeen-receptor, en van aromatase-remmers, stoffen die de omzetting van testosteron in oestradiol remmen. Deze zullen hieronder besproken worden.

3.3.1 Anti-androgenen

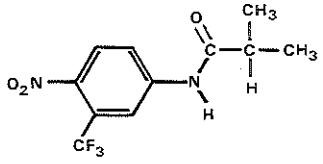
Er is gebruik gemaakt van 3 anti-androgenen: cyproteron-acetaat (1,2α-methyleen-6-chloor-4,6-pregnadiëen-17α-ol-3,20-dion-17α-acetaat), flutamide (SCH 13521; 4'-nitro-3'-trifluoromethyl-isobutyranilide) en anandron [RU 23908; 5,5-dimethyl-3-(4-nitro-3'-trifluoromethyl-isobutyranilide)].
De gebruikte anti-androgenen vertonen onderling verschillen. Cyproteron-acetaat is een steroïdaal anti-androgeen. Daarnaast bezit het ook progestagene eigenschappen. Flutamide en anandron zijn zogenaamde 'pure' anti-androgenen. Zij bezitten een niet-steroïdale structuur. Flutamide wordt in het lichaam snel gemetaboliseerd tot hydroxy-flutamide, de actieve metaboliet (Neri, 1976). Verondersteld wordt dat anandron niet eerst gemetaboliseerd behoeft te worden (Moguilewsky et al., 1986). Anti-androgenen zijn in staat om androgeen-afhankelijke processen te remmen. Cyproteron-acetaat zowel als flutamide, gedurende maximaal 12 weken toegediend, hebben een gewichtsverminderend effect op de prostaat en zaadblazen van intacte volwassen mannetjes ratten, alsmede bij gecastreerde en met DHT of TP gesubstitueerde



Cyproteron acetaat



Anandron



Flutamide

Figuur 3-3 Structuurformules van de anti-androgenen cyproteron-acetaat, anandron en flutamide.

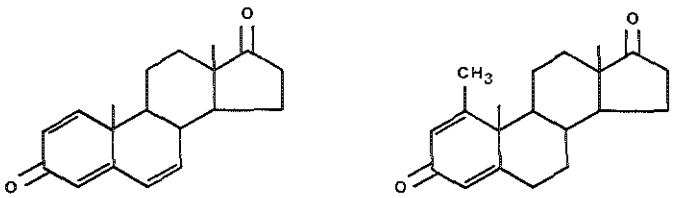
mannetjesratten (El Etreby et al., 1987; Neri et al., 1972; Peets et al., 1974; Poyet en Labrie, 1985). Volwassen mannelijke ratten, gedurende 5 maanden behandeld met anandron, vertoonden een geringe reductie in het gewicht van de zaadblazen, maar geen reductie in het gewicht van de prostaat (Labrie et al., 1983). Deze auteurs suggereren dat dit ook de beperking is van langdurig gebruik van pure anti-androgenen. Deze stoffen zouden namelijk ook de remmende feedback werking van androgenen op het hypothalame-hypofysaire niveau neutraliseren. Dit zou daarom leiden tot een verhoging van de gonadotropine en androgeensecretie. In later onderzoek is dit bevestigd, voor zowel acute als chronische toediening van anandron (Raynaud et al., 1984). Na 14 dagen toediening van anandron (10 mg/dag) aan volwassen mannelijke ratten waren de testosteron-bloedconcentraties zelfs 17 maal zo hoog als bij controles. Neumann (1985) beschrijft een experiment, waarin al 24 uur na start van behandeling met flutamide een significante verhoging werd gezien in de bloedconcentraties van T, LH en FSH van volwassen mannelijke ratten. Dit effect wordt niet waargenomen na toediening van cyproteron-acetaat. Bij de volwassen man is ook verhoging van T beschreven na flutamide en een daling bij cyproteron-acetaat toediening (Knuth et al., 1984). Anti-androgenen worden bij de mens toegepast bij prostaatkanker (Raynaud et al., 1984; de Voogt et al., 1986), bestrijding van acne (Neumann, 1985) en bij man-naar-vrouw transseksuelen (Asscheman,

1986; van Kemenade et al., 1989). Cyproteron-acetaat (Androcur), anandron en flutamide (Eulexin) zijn in Nederland als geneesmiddel geregistreerd.
Het werkingsmechanisme van deze androgeen-receptorblokkers is niet geheel duidelijk. Verondersteld is dat de binding van anti-androgeen aan de receptor voorkomt dat het anti-androgeen-receptor-complex vanuit het cytoplasma de kern in kan. Aangezien volgens de nieuwste theorie de receptor zich in de kern bevindt, dient dit idee herzien te worden. Algemeen wordt aangenomen dat het uiteindelijke effect van antagonisten of anti-hormonen is, dat er een inactief receptor-anti-hormoon complex gevormd wordt, zoals beschreven voor de progesteron antagonist RU486, dan wel dat het getransformeerde anti-hormoon-receptor complex niet in staat is DNA aan te zetten tot transcriptie en vorming van mRNA (Williams, 1990).

3.3.2 Aromatase-remmers

In de in dit proefschrift beschreven experimenten (hoofdstuk 7.2 en 7.3) zijn twee aromatase-remmers gebruikt, ATD; (1,4,6-androstatriëen-3,17-dion) en atamestan (1-methyl-1,4-androstadiëen-3,17-dion).

Deze stoffen worden competitieve, irreversibele aromatase-remmers genoemd. Allereerst



Figuur 3-4
Structuurformules van de aromatase-remmers ATD en atamestan.

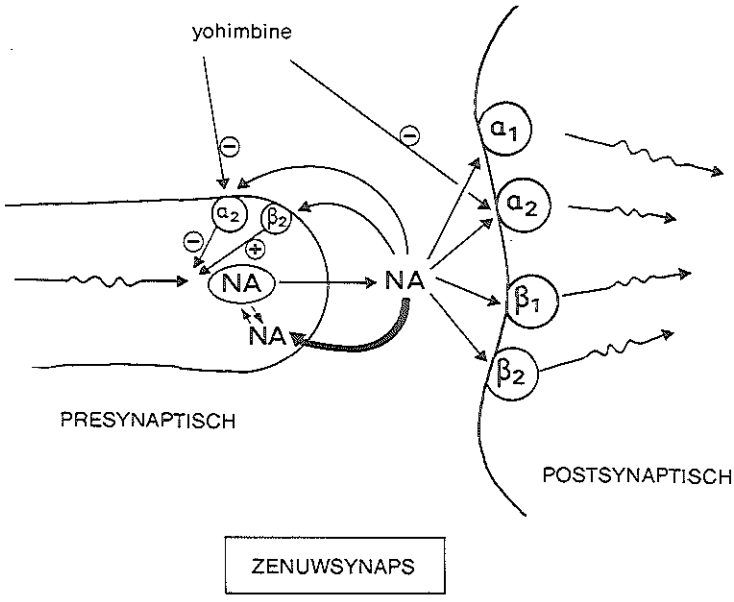
gaan ze met testosteron competitie aan voor de binding aan het enzym aromatase. Zijn ze eenmaal aan aromatase gebonden, dan zijn ze er niet meer af te krijgen en voorkomen daarmee de aromatisatie van testosteron in oestradiol (Covey & Hood, 1981; Henderson et al., 1986; Schwartel et al., 1973). Onderzoek heeft getoond dat remming van aromatase bij Java apen (Macaca fascicularis) behandeld met een combinatie van androsteendion en atamestan resulteert in lagere oestradiol spiegels in het perifere bloed dan bij controles die alleen met een androgeen (androsteendion) waren behandeld (Habenicht et al., 1987).

3.4 Seksueel gedrag-stimulerende stoffen

In een aantal onderzoeken (hoofdstuk 7.2 en 7.3) zijn stoffen gebruikt die mogelijk het seksuele gedrag van ratten kunnen stimuleren, yohimbine en 8-OH-DPAT.

3.4.1 Yohimbine

Yohimbine is een alkaloid afkomstig van de schors van de West Afrikaanse Yohimbéhé boom, in het Latijn Corynanthe yohimbe of ook wel Pausinystalia yohimbe genoemd van de familie der Rubiaceae (Clark et al., 1984; Taberner, 1987). Yohimbine werkt voornamelijk als een antagonist van adrenerge α_2-receptoren. Voor prikkelgeleiding in het sympathische zenuwstelsel is noradrenaline een bekende neurotransmitter. Stimulatie van de $\alpha_2$adrenerge receptor remt de noradrenaline afgifte in de synapsspleet en veroorzaakt een afname in sympathische outflow van het centraal zenuwstelsel. Yohimbine blokkeert de α_2-receptor waardoor de afgifte van noradrenaline in de synapsspleet blijft doorgaan (zie figuur 3-5).

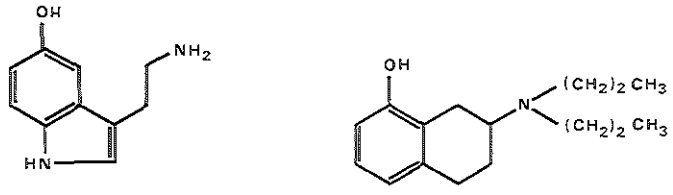


Figuur 3-5 Schematische voorstelling van de werking van yohimbine op de prikkeloverdracht in een synaps. NA= noradrenaline, de neurotransmitter welke vrijkomt in de zenuwspleet en postsynaptische receptoren (α1, α2, β1, β2) prikkelt. Met de prikkeling van de presynaptische receptoren zorgt NA er zelf voor dat het niet blijft afgegeven worden. Yohimbine blokkeert de α2-receptor waardoor afgifte van NA in de synapssleet blijft doorgaan. NA speelt een rol bij vasoconstrictie (Uit: Slob et al., 1987).

In 1984 publiceerden Clark, Smith en Davidson een opzienbarend artikel in Science. Zij vonden dat yohimbine (1) de 'arousal' doet toenemen in seksueel ervaren ratten, (2) het copulatiegedrag (inclusief ejaculatie) stimuleert in seksueel onervaren ratten en (3) het seksueel gedrag induceert van aanvankelijk seksueel inactieve ratten. Later rapporteerden dezelfde auteurs dat bij getrainde mannelijke ratten yohimbine zorgt voor een opvallende reductie van een aantal gedragsparameters: een reductie van de latentietijd tot de 1e ejaculatie, een verkorting van het postejaculatoire interval en een verlaging van het aantal intromissies tot de eerste ejaculatie (Clark et al., 1985). Ook bij langdurig (tot 91 dagen) gecastreerde mannelijke ratten bleek yohimbine een stimulerend effect te hebben op copulatie activiteit (Clark, Smith & Davidson, 1985). In 1987 verschenen twee uitgebreide studies waarin de effecten van yohimbine vergeleken werden met die van twee andere α_2-receptor antagonisten, idazoxan en imiloxan (Smith et al., 1987a,b). Yohombine deed het percentage ejaculerende dieren toenemen; het aantal intromissies tot de eerste ejaculatie veranderde niet. Yohimbine bleek ook in staat het seksueel gedrag van 14 maanden oude mannelijke ratten te stimuleren, echter niet tot het niveau van 2 maanden oude met yohimbine behandelde ratten, maar wel tot dat van 2 maanden oude onbehandelde ratten (Smith & Davidson, 1990).

3.4.2 8-OH-DPAT

8-Hydroxy-2-(di-n-propylamino) tetraline (8-OH-DPAT) is een selectieve, aan serotonine (5HT) verwante, $5HT_{1A}$-receptor agonist (Kwong et al., 1986), die geen stimulerende effecten uitoefent op dopaminerge en noradrenerge receptoren (Arvidsson et al, 1981).



Figuur 3-6 Structuurformules van serotonine en 8-OH-DPAT. Duidelijk is de structuurverwantschap tussen deze 2 stoffen te zien.

Er zijn meerdere onderzoeken verricht naar het effect van deze stof op seksueel gedrag van ratten. Bij intacte mannelijke ratten is 8-OH-DPAT in staat het aantal intromissies en de tijd tot de eerste ejaculatie te reduceren (Ahlenius et al., 1981; Morali & Larsson, 1984; Ahlenius & Larsson, 1984; 1988; Mendelson & Gorzalka, 1986; Schnur et al., 1989; Mos, 1990). In een aantal onderzoeken werd na behandeling met 8-OH-DPAT een verkorting van het post-

ejaculatoire-interval (PEI) gevonden (Ahlenius et al., 1981; Schnur et al., 1989). Ejaculaties kunnen na 8-OH-DPAT behandeling optreden bij de eerste intromissie (Ahlenius et al., 1981; Morali & Larsson, 1984). Tevens is gevonden dat 8-OH-DPAT stimulerend werkt in gecastreerde ratten, die al dan niet gesubstitueerd werden met TP (Ahlenius et al., 1981). Niet alleen toediening via een s.c. of i.p. injectie, maar ook intrathecale toediening in de lumbosacrale regio leidde bij mannetjes ratten tot vermindering van het aantal intromissies en de tijd tot eerste ejaculatie (Lee et al, 1990). Daarentegen had serotonine, toegediend in het mediale preoptische gebied, een remmende werking op het seksueel gedrag van mannetjes (Verma et al., 1989), mogelijk omdat $5HT_{1A}$ receptoren niet selectief worden geactiveerd.
Bij vrouwtjes werd het door oestradiol-geïnduceerde lordosegedrag door DPAT geremd waarbij de werking dosisafhankelijk was (Mendelson & Gorzalka, 1986; Fernandez-Guasti et al., 1987). Door T geïnduceerd beklimgedrag wordt door DPAT behandeling in mindere (Mendelson en Gorzalka, 1986) of meerdere mate (Haensel et al., 1991) gestimuleerd.

3.5 Literatuur

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Hoofdstuk 4

Proefdieren, materialen en methoden

4.1 Dieren

Voor de experimenten beschreven in dit proefschrift werden Wistar albino ratten gebruikt (HSD-CPB: WU; Harlan, Zeist, Nederland; Wistar Zentral Institut für Versuchtierzucht, Hannover, FRG). In één experiment (zie hoofdstuk 5.2) werd gebruik gemaakt van Sprague Dawley albino ratten, die rechtstreeks uit de USA geïmporteerd waren (Harlan Sprague Dawley Inc., Indianapolis, IN, USA). De dieren waren in groepjes van 2-4 gehuisvest in macrolon kooien met water en voer *ad libitum* beschikbaar. In de dierenstallen heerste een omgekeerd dag/nacht ritme met alleen kunstlicht gedurende 14 uur per dag: van 17.30 uur tot 07.30 uur. De temperatuur in de stallen was tussen de 22 en 24 °C.

4.2 Perinatale behandelingen

In dit proefschrift is de werking van testosteron of de oestradiol-metaboliet daarvan rond de geboorte op drie manieren gemanipuleerd. (I) Vrouwtjesratten werden perinataal blootgesteld aan een anti-androgeen: cyproteron-acetaat, anandron of flutamide (zie hoofdstuk 3, 5.1, 5.2 en 6). (II) Mannetjesratten werden perinataal behandeld met een aromatase-remmer: ATD of neonataal met atamestan (zie hoofdstuk 3, 7.2 en 7.3). (III) Mannetjesratten werden neonataal gecastreerd, gevolgd door substitutie met testosteron-propionaat (TP), dihydrotestosteron-propionaat (DHTP) of olijfolie (zie hoofdstuk 7.1). In hetzelfde experiment werden ook vrouwtjes neonataal geïnjecteerd TP, DHTP of olie (zie hoofdstuk 6).

4.2.1 Neonatale castratie

Voor het verrichten van neonatale castratie (zie hoofdstuk 7.1) werden mannetjes eerst circa 5 minuten verdoofd in ijs (ze zijn neonataal namelijk nog niet in staat hun eigen lichaams-temperatuur constant te houden). Daarna werden buikhuid en -spierlaag geopend via een mediane incisie met een klein schaartje zoals gebruikt in de oogheelkunde. Vervolgens werden de testes opgezocht en verwijderd. De huid werd hierna gesloten met dunne zijde. Na

opwarmen in een bakje, geplaatst in een grotere bak met warm water onder een bureaulamp, werden de ratten teruggeplaatst bij hun moeder.

4.2.2 Perinatale toediening van stoffen

Twee manieren werden gebruikt om foeten prenataal aan stoffen bloot te stellen. (I) Door de moeder gedurende de tweede helft van de zwangerschap (dag 11-22) dagelijks s.c. te injecteren met de stof opgelost in een kleine hoeveelheid, meestal 0.1 ml, oplosmiddel. Op deze wijze werden foeten blootgesteld aan cyproteron-acetaat, anandron, flutamide of ATD. (II) Door bij de moeder op dag 11 van de zwangerschap een siliconerubberen buisje in te brengen, onderhuids in de nek en dit direct na de bevalling weer te verwijderen. Dit is in één experiment toegepast (zie hoofdstuk 7.2), waarbij een 19 cm lang implantaat werd gebruikt (binnendiameter 1.5 mm; buitendiameter 2.1 mm). Dit werd in een lus onderhuids geplaatst. Voor neonatale toediening van stoffen zijn eveneens 2 manieren toegepast. (I) Door pasgeboren dieren, beginnend 5 tot 9 uur na geboorte, om de andere dag s.c. te injecteren met 0.05 ml oplosmiddel, welke afhankelijk van het experiment TP, DHTP, olie of flutamide bevatte. (II) Door tussen 5 en 9 uur na geboorte een 5 mm lang siliconerubberen buisje (binnendiameter 1.5 mm, buitendiameter 2.1 mm) s.c. te implanteren. Deze manier werd toegepast bij de behandeling van mannetjes met ATD of atamestan. De implantaatjes werden verwijderd na 10 of 21 dagen.

4.3 Behandeling op volwassen leeftijd

4.3.1 Ovariëctomie

Bij alle experimentele vrouwtjes werden, voorafgaande aan de gedragtests, de ovaria verwijderd, om geen storende invloed te ondervinden van hun eigen cyclus. Hiertoe werd het vrouwtje allereerst verdoofd met ether. Daarna werd een laterale incisie in de flank gemaakt en het ovarium met een pincet opgezocht. De toe- en afvoerende bloedvaten werden afgeklemd met een arterieklem en afgebonden met zijde. Hierna werd het ovarium verwijderd. Tot slot werd de spierlaag gesloten met zijde en de huid met agrafes (krammetjes). Daarna werd ook het andere ovarium op dezelfde manier verwijderd. Na afloop werd het dier in een schone bak gelegd om bij te komen.

4.3.2 Castratie

Slechts in één experiment werden mannetjes op volwassen leeftijd gecastreerd, voorafgaande aan de gedragtests (zie hoofdstuk 7.1). Het mannetje werd verdoofd met ether. Daarna werden buikhuid en -spierlaag geopend via een mediane incisie rostraal van de basis van de penis en de testes opgezocht. Het gubernaculum werd doorgeknipt. De ductus deferens alsmede de toe- en afvoerende bloedvaten werden afgebonden met zijde en de testis en epididymis verwijderd. Ditzelfde gebeurde met de contralaterale testis en epididymis. De spierlaag werd gesloten met zijde en de huid met agrafes. Tot slot werd het mannetje in een schone bak gelegd om bij te komen.

4.3.3 Hormoontoediening

Vrouwtjes werden vóór het testen van door testosteron geïnduceerd beklimgedrag (hoofdstuk 5.1 en 5.2) geïmplanteerd met een siliconerubberen buisje gevuld met kristallijn testosteron. Meestal werden implantaatjes gebruikt met een lengte van 2 cm, soms ook met een lengte van 0.5 of 1.0 cm, met een binnendiameter van 1.5 mm en een buitendiameter van 2.1 mm. In twee experimenten (hoofdstuk 6 en 7.1) is gebruik gemaakt van implantaatjes gevuld met dihydrotestosteron of oestradiol voor de activatie van keuze- en beklimgedrag van mannetjes en vrouwtjes.
Lordose-gedrag van vrouwtjes werd in één experiment (hoofdstuk 5.2) getest onder invloed van het aanwezige T-implantaat al dan niet gevolgd door een injectie met progesteron (0.5 mg) 4-6 uur voor de test. In een ander experiment (hoofdstuk 5.2) werd lordosegedrag bestudeerd na 3 dagelijkse EB-injecties en na een extra EB-injectie gevolgd door een injectie met 0.5 mg progesteron. In opeenvolgende weken bevatte een EB-injectie respectievelijk 0.4 µg, 0.8 µg dan wel 1.6 µg EB. Lordosegedrag van mannetjes (hoofdstuk 7.2 en 7.3) werd soms getest zonder volwassen behandeling, soms na injectie met 0.5 mg progesteron 4-7 uur voorafgaande aan de test.
Steroiden bestemd voor injectie werden steeds opgelost in olijfolie.

4.3.4 Toediening van seksueel gedrag-stimulerende stoffen

Op de werking van deze stoffen is reeds in het vorige hoofdstuk ingegaan. Yohimbine en 8-OH-DPAT werden in deze serie proeven toegediend aan intacte mannetjes, waarvan een aantal

als gevolg van een perinatale behandeling duidelijk verminderd ejaculatiegedrag vertoonde (hoofdstuk 7.2 en 7.3). Het was de bedoeling na te gaan of deze stoffen in staat waren deze vermindering op te heffen.

4.4 Stimulusdieren

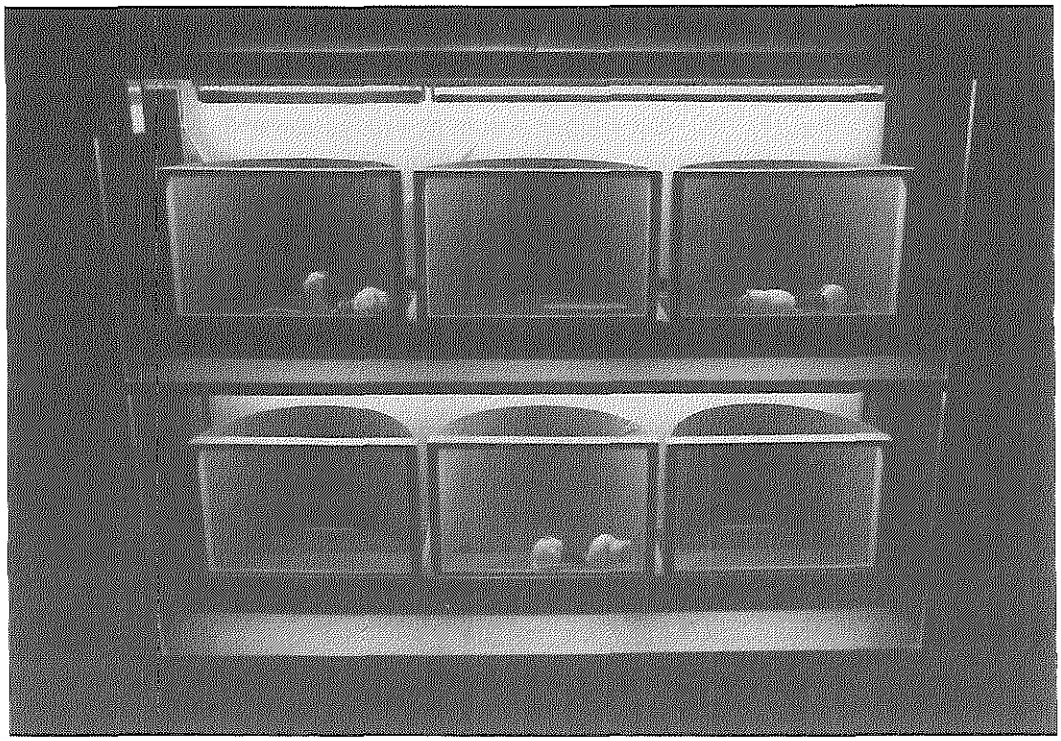
Als stimulusdieren werden veelal (F1) nakomelingen gebruikt van twee ingeteelde Wistar stammen (R x U). Als stimulusdieren fungeerden een seksueel aktief mannetje en een geovariëctomeerd vrouwtje, al dan niet bronstig gemaakt met oestradiol-benzoaat (30 µg 24-48 uur voor test) en progesteron (2.5 mg 4-7 uur voor test).

4.5 Gedragstests

De meeste tests duurden 15 minuten en werden uitgevoerd gedurende de eerste helft van de donkerperiode in zwakverlichte, enigszins geluidsgeïsoleerde ruimtes met achtergrondruis veroorzaakt door de air conditioning (56 dB) of door een ruisbron en een ventilator (64 dB); (Slob et al., 1987). Dit laatste was alleen het geval bij de keuze test in het automatische open veld (zie hoofdstuk 6).

4.5.1 Seksuele paartest met bronstig vrouwtje

Deze testen werden uitgevoerd in halfcirkelvormige kooien (62 x 40 x 36 cm), geplaatst op een stelling onder rood TL-licht aangevuld met indirect wit licht afkomstig van 2 bureaulampen, die langs de wanden van de testruimte waren geplaatst (zie figuur 4-1).
De experimentele dieren kregen de gelegenheid gedurende 5 minuten aan de kooien te wennen (adaptatie). Daarna werd er een bronstig vrouwtje bijgeplaatst en begon de observatie. Waren de experimentele dieren vrouwtjes, dan werden beklimmingen met bekkenstoten en intromissie-achtige gedragingen geregistreerd. Werden mannetjes getest, dan werden beklimmingen met bekkenstoten, intromissies en ejaculaties geregistreerd.



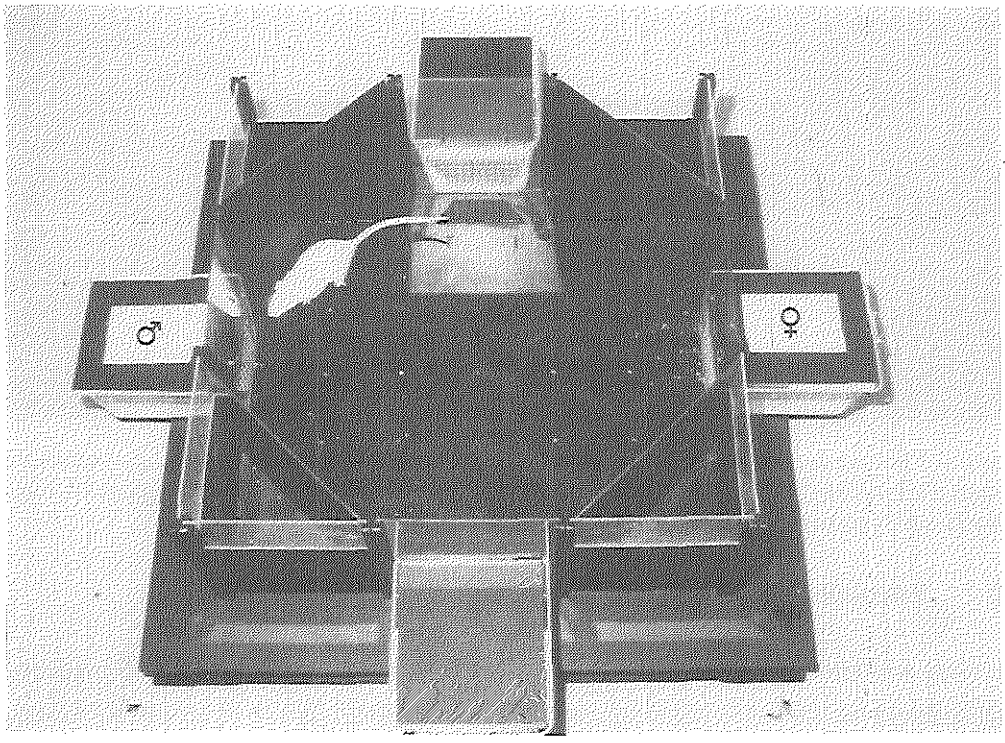
Figuur 4-1 Foto van de testopstelling voor seksuele paartests met een bronstig vrouwtje of een seksueel aktief mannetje. De halfcirkelvormoge kooien ontvangen rood of indirect wit licht afkomstig van TL-buizen. Als voorbeeld zijn 3 rattenpaartjes in de kooien geplaatst.

4.5.2 Seksuele paartest met een seksueel aktief mannetje

Deze tests werden eveneens in de halfcirkelvormige kooien (zie figuur 4-1) uitgevoerd en waren bedoeld om het lordosegedrag van het experimentele dier te observeren. Voorafgaand aan een dergelijke test werd het seksueel aktieve mannetje gedurende 5 minuten geadapteerd aan de kooi. Daarna werd een bronstig vrouwtje in de kooi geplaatst. Na de eerste intromissie van de man werd dit vrouwtje uit de kooi gehaald en werd het experimentele dier in de kooi gezet. Er werd geobserveerd of het experimentele dier lordose vertoonde als reactie op de beklimmingen (waaronder ook intromissies en ejaculaties gerekend worden) van het mannetje. De test werd gestopt als het mannetje het experimentele dier 10 maal beklommen had. Werd dit aantal niet gehaald dan werd de test na 10 minuten gestopt. Alleen voor dieren die 3 of meer beklimmingen ontvingen werd een lordose-quotiënt berekend. Dit is het aantal lordoses van het experimentele dier gedeeld door het totale aantal ontvangen beklimmingen (inclusief intromissies en ejaculaties) vermenigvuldigd met 100.

4.5.3 Partner keuze test in een automatisch open veld

Dit automatische open veld (zie figuur 4-2) is oorspronkelijk beschreven door Tanger, Vanwersch en Wolthuis (1978). Het is door ons zo gewijzigd dat er thans sprake is van een octagonaal open veld (75 x 75 x 30 cm), gemaakt van zwart perspex (Slob, de Klerk & Brand, 1987).



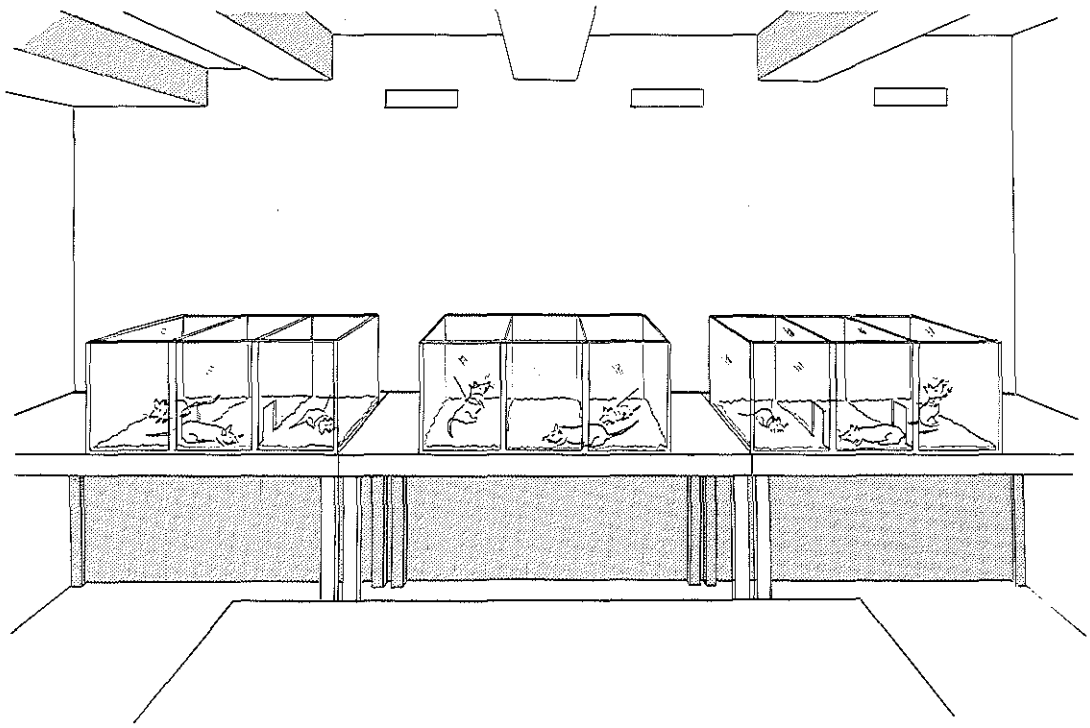
Figuur 4-2 Foto van het automatisch open veld. Twee kooitjes bevatten stimulusdieren, in dit geval een bronstig vrouwtje en een seksueel aktief mannetje. Duidelijk is te zien dat seksuele interactie wordt voorkomen door metaalgaas. De andere 2 kooitjes bevatten geen stimulusdieren.

Vier doorzichtige perspex kooitjes (25 x 20 x 13 cm) zijn bevestigd aan de zijkanten van het open veld. Een rechthoekig metalen gaas (15 x 17 cm) in elk kooitje zorgt ervoor dat de dieren elkaar wel kunnen horen, zien en ruiken, maar geen seksuele interacties met elkaar kunnen aangaan. Om preferentie te testen werd een stimulusdier geplaatst in elk van twee tegenover elkaar liggende kooitjes: een bronstig vrouwtje en een seksueel aktief mannetje of een bronstig vrouwtje en een niet-bronstig geovariëctomeerd vrouwtje.
Het open veld werd indirect verlicht met rood licht afkomstig van 4 TL-buizen van 40 W elk,

geplaatst langs de wanden van de testruimte. Een zogenaamde "witte" achtergrondruis van 64 dB werd door luidsprekers in de testruimte gebracht. Met behulp van een televisiecamera, 2 m boven het midden van het open veld, en electronische apparatuur werd de positie van het wit van de rat in het open veld automatisch geregistreerd en opgeslagen op een floppy disk van een personal computer.

Aan het eind van een 15 minuten test werd de registratie gestopt, het experimentele dier eruit gehaald, de vloer van de kooi gereiningd en de vier zijkooitjes kloksgewijs geroteerd. Dan werd het volgende dier in het centrum van het open veld geplaatst, waarna vrijwel direkt de registratie opnieuw werd gestart. Aan het begin en aan het eind van een testdag werd de bronst van het stimulusvrouwtje geverifieerd met een seksueel aktieve man.

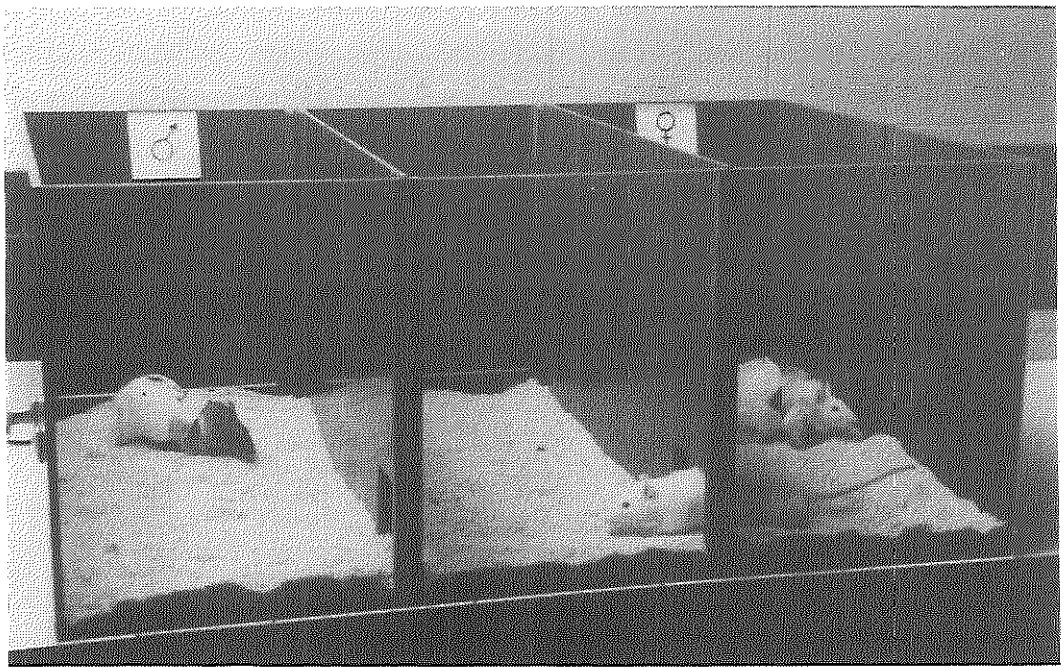
Als maat voor de preferentie werd voor ieder dier per test een preferentiescore berekend door de tijd doorgebracht in een vierkant van 25 bij 25 cm voor het kooitje van ene stimulusdier af te trekkken van de tijd doorgebracht in een vierkant van 25 bij 25 cm voor het kooitje van het andere stimulusdier. Deze methode werd overgenomen van Edwards en Pfeifle (1983).



Figuur 4-3 Impressie van de testopstelling van de 3-compartimenten-kooien.

4.5.4 Partner keuzetest in 3-compartimenten-kooi

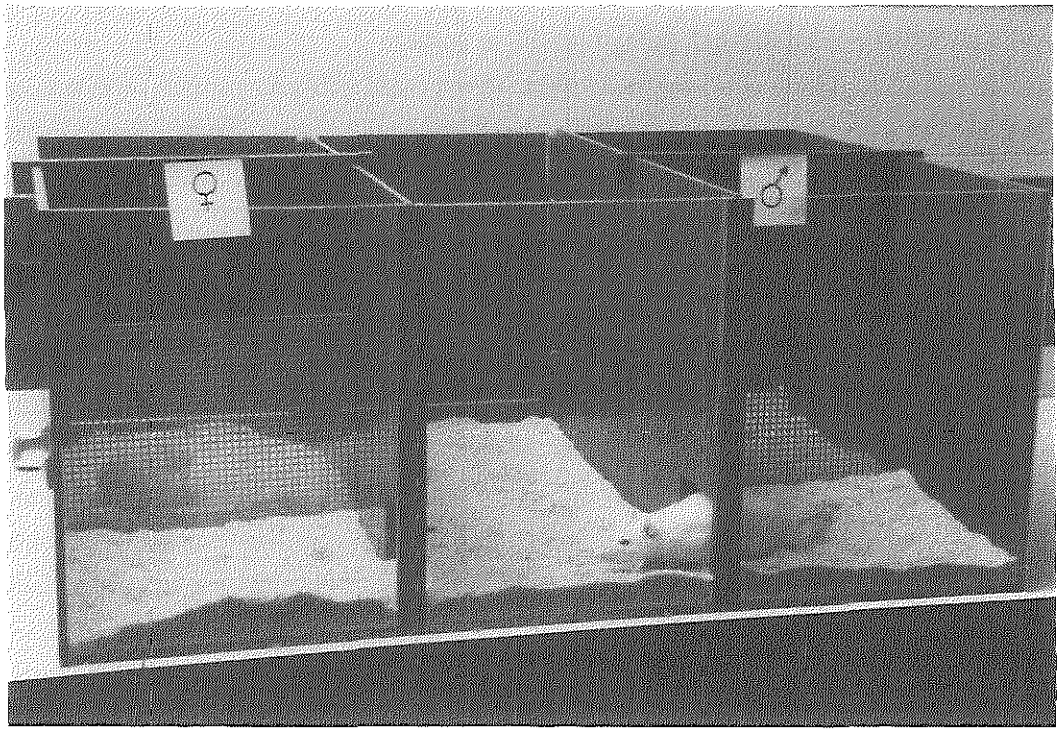
Hiervoor werd gebruik gemaakt van een aantal 3-compartimenten-kooien.(zie figuur 4-3 en 4-4).
De kooien zijn gemaakt van grijs perspex met een doorzichtige perspex voorkant (wandhoogte 40 cm). De kooi heeft 3 compartimenten van 60 x 30 x 40 cm elk. In de 2 scheidingswanden bevindt zich een kleine opening (13 x 12 cm) bij de voorruit. Deze doorgangen kunnen worden gesloten met een schuifdeurtje.



Figuur 4-4 Foto van een 3-compartimenten-kooi. De zij-compartimenten bevatten de stimulusdieren (een bronstig vrouwtje en een seksueel aktief mannetje), die een zeemleren tuigje dragen dat met een staaldraadje bevestigd is aan een railtje achterin het compartiment. Hierdoor is de actieradius van het stimulusdier beperkt tot ongeveer de helft van het compartiment. Het experimentele dier kan zelf bepalen waar hij/zij gaat zitten en welke seksuele interacties worden uitgevoerd of toegelaten.

In de zijcompartimenten konden stimulusdieren worden geplaatst. Deze dieren droegen een zeemleren harnasje dat door een roestvrij stalen draad met een paperclip bevestigd was aan een railtje aan de achterwand van het compartiment. Deze constructie zorgde ervoor dat de stimulusdieren tot ongeveer halverwege het zijcompartiment konden komen.

In de dagen voorafgaande aan de eerste test werden de stimulusdieren circa 1 uur in de kooi geplaatst voor gewenning aan de testomstandigheden. Hierbij waren de schuifdeurtjes gesloten. Direct voorafgaande aan een test werden de stimulus en experimentele dieren gedurende 15 minuten in de kooi geplaatst om aan de testomstandigheden te wennen. Aan het begin van een test werden de schuifdeurtjes geopend en kon het experimentele dier vrijelijk rondlopen. De tijd doorgebracht in elk compartiment alsmede alle seksuele gedragingen (met elke partner) werden geregistreerd.
Gedurende een aantal tests is halverwege de zijcompartimenten een grijs perspex schot geplaatst bestaande uit metaalgaas.



Figuur 4-5 Foto van een 3-compartimenten-kooi, nu met schuiven van perspex en metaalgaas halverwege de zij-compartimenten om seksuele interactie te voorkomen. De stimulusdieren lopen vrij rond achter het metaalgaas. Zie verder figuur 4-4.

Hiermee werd seksuele interactie tussen stimulus en experimentele dier verhinderd. Wel was het mogelijk voor het experimentele dier het stimulusdier te zien, te horen en te ruiken.

Om partnervoorkeur te kwantificeren werd, in navolging van Edwards en Pfeifle (1983), voor elk dier per test een preferentiescore berekend waarbij de tijd doorgebracht in het ene compartiment afgetrokken werd van de tijd doorgebracht in het andere compartiment.

4.6 Bloedafname en hormoonbepaling

Om de concentratie van testosteron in het serum van mannelijke of vrouwelijke ratten te bepalen werd bloed afgenomen. Hiervoor werd het dier licht verdoofd met ether. De narcose werd onderhouden door een glazen Erlenmeyer onder de neus te plaatsen, waarin zich met ether doordrenkte watten bevonden. Met een glazen capillair werd de plexus orbitalis van het oog aangeprikt en bloed afgenomen. Na verwijdering van de capillair werd het oog nog gedurende circa één minuut met een steriel gaasje afgedrukt om het bloeden te stoppen. Daarna werd het dier in een schone bak gelegd om bij te komen.
Voor de bepaling van de concentatie van testosteron werd gebruikt gemaakt van een in ons laboratorium gebruikelijke radioimmunoassay (RIA; zie b.v. Baum, Brand, Ooms, Vreeburg & Slob, 1988). De interassay and intraassay coëfficiënten van variatie waren respectievelijk 13,4% en 5,6%.

4.7 Statistische analyse

De data werden onderworpen aan één-, twee- of driewegvariantie analyse (ANOVA; Perlman, 1986). Voor vervolganalyse werd gebruik gemaakt van de 'honestly significant difference' (HSD) methode of de 'least significant difference' methode (LSD; Kirk, 1968). Analyse van het aantal dieren dat een bepaald gedrag vertoonde werd uitgevoerd met chi-kwadraat-analyse (Perlman, 1986) voor onafhankelijkre groepen of met de Binomiaal test voor afhankelijke groepen (Siegel, 1956).

4.8 Literatuur

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Hoofdstuk 5

Perinatale androgenen en volwassen seksueel gedrag van vrouwelijke ratten

5.1 Androgens and the propensity for adult mounting behavior in the female Wistar rat.

T. Brand, E.J. Houtsmuller & A.K. Slob.
In: J. Balthazart (Ed). Hormones, Brain and Behaviour in Vertebrates. 1. Sexual Differentiation, Neuroanatomical Aspects, Neurotransmitters and Neuropeptides. Comp. Physiol. Basel, Karger, 1990, vol 8, pp 15-29.

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Androgens and the Propensity for Adult Mounting Behavior in the Female Wistar Rat

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It has been suggested that the propensity for testosterone (T)-induced mounting behavior in adult female rats is 'organized' by androgens, presumably T, during prenatal development. This supposition was derived from studies in which the action of T or its metabolites was suppressed prenatally by antiandrogens. As adults, such females displayed less T-induced mounting behavior when paired with estrous female partners [1–3].

The origin of androgens in female fetuses is still a matter of debate. Various sources have been proposed: the adrenals of the mother [2], adjacent male fetuses through amniotic diffusion [4], male fetuses located caudally in the same uterine horn through venous drainage into the arterial supply [5], or the placenta [6, 7].

To shed further light on the possible source as well as on the biological significance of prenatal androgens for adult female mounting behavior, two series of experiments have been carried out. In the first series, the importance of the position of female rats relative to male siblings in utero was assessed. In the second series the role of prenatally circulating androgens per se was studied.

Methods

Albino Wistar rats (HSD-CPB:WU, Harlan, Zeist, The Netherlands; Wistar Zentral Institut für Versuchtierzucht, Hannover, FRG) were housed 2–4 to a cage, of same sex and treatment. Water and food were available ad libitum. Lights in the animal rooms were on for 14 h/day, between 5.30 p.m. and 7.30 a.m., and temperature ranged from 22 to 24 °C.

Operations were usually carried out under ether anesthesia. At hemihysterectomy, the left or right uterine horn was removed through a midline abdominal incision; both ovaries were left in situ. Reduction of the number of pups per uterine horn, by bipolar high frequency cauterization, was done under anesthesia with avertine (25 mg/ml/100 g b.w.).

Females were time-mated (day of mating = day 0 of pregnancy) and parturition occurred 22 days later. With watched deliveries one observer monitored parturient females at 5- to 10-min intervals. Newborn pups were taken from the mother right after birth. If necessary, the umbilical cord was cut, pups were cleaned, sexed when possible on the basis of genital appearance and marked individually by toe clipping. Approximately 12 h after birth, anogenital distance and body weights were recorded. Cesarian section was carried out on day 22 of pregnancy: the mother was lightly anesthetized with ether and killed by cervical dislocation. The abdominal wall and the uterine horn(s) were quickly opened through a midline incision. The pups were taken out and laid out one by one according to uterine position. After cleaning, the pups were treated and marked individually as described above and cross-fostered to untreated mothers which had recently given birth, usually the day before. Mortality with cesarian section was very low: in a total of 17 litters only 16 pups died within a few days (all deaths occurred in the 5 prenatally nCA-treated litters).

Following the manipulations neonatally, pups were weighed weekly, weaned at approximately 21 days of age and housed 3 or 4 to a cage. In adulthood, all females were ovariectomized prior to T administration.

Prenatal treatment consisted of daily subcutaneous injections from days 11 through 22 of pregnancy. T in adulthood was administered either in crystalline form in Silastic implants (i.d. 1.5 mm, o.d. 2.1 mm, SR-3; Talas, The Netherlands), or as daily testosterone propionate (TP) injections (s.c. 250 μg/0.1 ml).

Experimental females were behaviorally tested in a semi-circular arena with a hormonally primed ovariectomized stimulus female (20 or 30 μg estradiol benzoate (EB) 24–48 h and 2.5 mg progesterone (P) 4–6 h before testing). Testing was carried out usually during the dark period in red light (sometimes combined with some dim indirect white light), 15 min per test. Various behaviors were scored but for the analysis of the data only mounts with pelvic thrusts and intromission-like mounts were included.

On various occasions, the adult females were lightly anesthetized with ether and about 2 ml of blood was collected from the orbital venous plexus for later T determination.

Data were subjected to one- or two-way analyses of variance [8, 9]. Significant overall effects ($p < 0.05$) were further analyzed with the HSD procedure [10]. A comprehensive overview of the various experimental designs is given in tables 1 and 2.

Results

Prenatal Brothers and Adult Female Mounting

In 3 of 4 experiments (1, 2, 4a), carried out consecutively, one uterine horn was removed either about 3 weeks before impregnation (exp. 1, 2, 4a) or on day 12 (range 10–13) of pregnancy (exp. 4b, 4d) (table 1). This and also the reduction in the number of pups per uterine horn was done in an effort to

Table 1. Overview of the various experimental designs to study the importance of the in utero position of female rats relative to male siblings for adult T-induced mounting behavior

Exp. no.	Manipulation relative to pregnancy		Birth	Liters n	Total female young	Female offspring used: classification relative to males						Age at:			Mounting behavior tested relative to start T treatment, days
						contiguity				caudal ♂		ovex. weeks	test onset weeks	T-treatment manner	
	before	during				♀F♀	♀F♂[1]	♂F♀[2]	♂F♂	yes	no				
1	hemi-hysterex.	–	watched delivery	10	37	13	10	9	5	23	14				
2	hemi-hysterex.	day 9: reduction litter size to 3	watched delivery	17	23	7	8	5	3	10	13	17	22	T; Silastic (2 cm SR-3) implant	−1, 11, 27, 61, 87
3	–	day 11: reduction to 1 fetus per horn	sponta-neous	8	10					–	10				
4a	hemi-hysterex.	–	watched delivery	6	14	8	3	2	1	6	8				
b	–	day 12: hemi-hysterectomy	watched delivery	6	12	2	4	3	3	8	4				
c	–	–	cesarian section	3	14	5	3	4	2	12	2	7.5	9	TP s.c. (250 µg/day)	−2, 7, 14,
d	–	day 12: hemi-hysterectomy	cesarian section	6	21	9	5	4	3	13	8				

[1] Adjacent male rostrally.
[2] Adjacent male caudally.

obtain all-female litters. These procedures resulted in a total of 15 female pups that did not have brothers in the same uterine horn during the second half of their prenatal life.

The results of the first 3 experiments are depicted in figures 1 and 2. Plasma levels of T about 3 weeks following the onset of T treatment (either injections or 2-cm Silastic implants) ranged between 2 and 6 ng/ml.

In figure 1a and b the females are arranged according to the adjacent male contiguity in utero ('contiguity classification' [4, 11]). Prior to T treatment the females showed no or very low levels of mounting behavior. T treatment caused a very significant increase in female mounting.

In experiment 1 (fig. 1a), females from different uterine positions showed similar mount frequencies (groups: $F(3/33) = 0.42$, NS). There was a significant effect of testing ($F(3/99) = 7.22$, $p < 0.0001$). Further analysis showed that the mount frequency during the 2nd test with T (T-test) (day 27) was higher ($p < 0.05$) than the other 3 T-tests. There was no significant groups $\times$ test interaction ($F(9/99) = 1.18$, NS).

In experiment 2 (fig. 1b) there was an overall effect of tests ($F(3/57) = 13.8$, $p < 0.0001$) and a weak groups $\times$ tests interaction ($F(9/57) = 1.84$, $p = 0.08$). Further analysis showed that mount frequencies were highest during T-test 4 (87 days), different ($p < 0.05$) from T-tests 1 and 3 (days 11 and 61, respectively). The 1st T-test (day 11) differed ($p < 0.05$) from the 2nd (day 27). Furthermore, it seemed that females positioned prenatally with an adjacent caudal male displayed highest mount frequencies (4 T-tests combined: $\bar{x} = 28.2 \pm 2.8$ SEM), significantly different from females prenatally with an adjacent male rostrally (i.e. ovarian side) ($\bar{x} = 21.5 \pm 2.1$). Females prenatally surrounded by 2 sisters or by 2 brothers showed overall intermediate mount frequencies (24.7 ± 2.2 and 24.9 ± 3.8, respectively).

The females in experiment 3 (fig. 1c) also showed an effect of testing ($F(3/27) = 4.71$, $p = 0.009$). HSD testing revealed that mounting during the 1st T-test (day 11) was significantly lower than during the following tests, which did not differ significantly.

Comparison of the T-induced mount frequencies of females of experiment 3 and females prenatally between 2 sisters of experiments 1 and 2 revealed an effect of tests ($F(3/81) = 6.68$, $p < 0.0001$) and a group $\times$ test interaction ($F(6/81) = 2.79$, $p < 0.02$). Further HSD analysis only showed lower mounting of females of experiment 3 during their 1st T-test (day 11). All other differences did not reach statistical significance ($\alpha = 0.05$, HSD = 12.6).

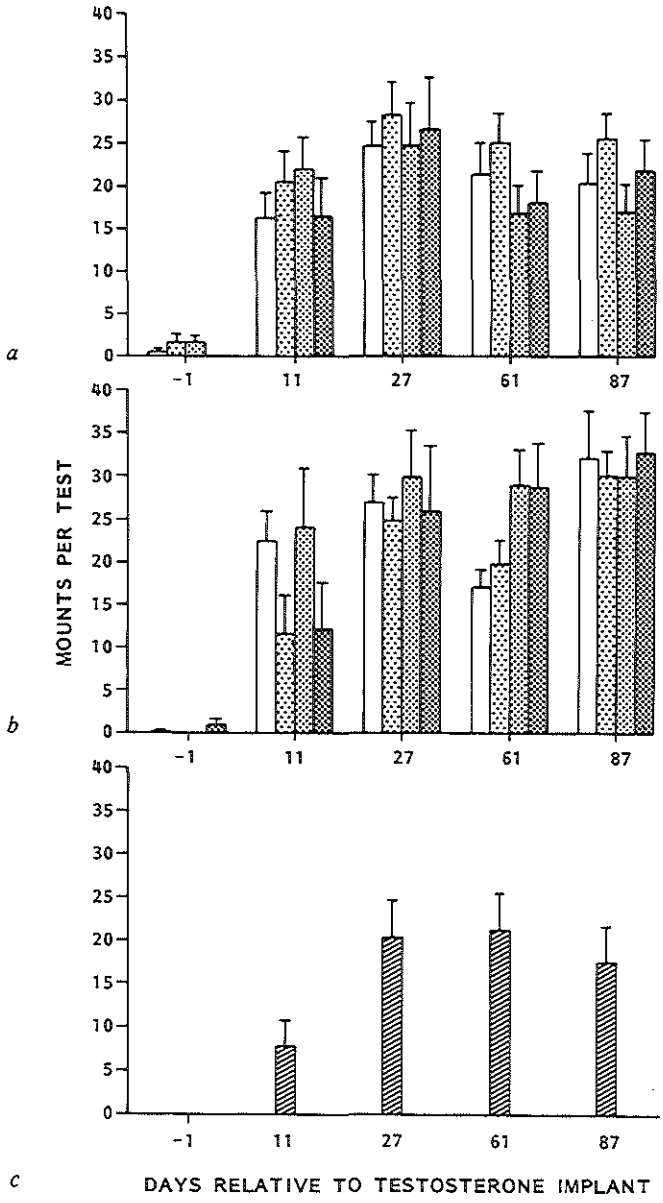

Fig. 1. Frequency (mean ± SE) of mounting behavior before and after T administration (2-cm Silastic implant) of adult female rats during 15-min tests. Females are classified according to 'adjacent male contiguity' in utero: open bars, ♀F♀; light dots, ♀F (rostral) ♂; middle density dots (caudal) ♂F♀; dark dots, ♂F♂; hatched bars (single) F. Details of the experiments 1 *(a)*, 2 *(b)* and 3 *(c)* are given in table 1.

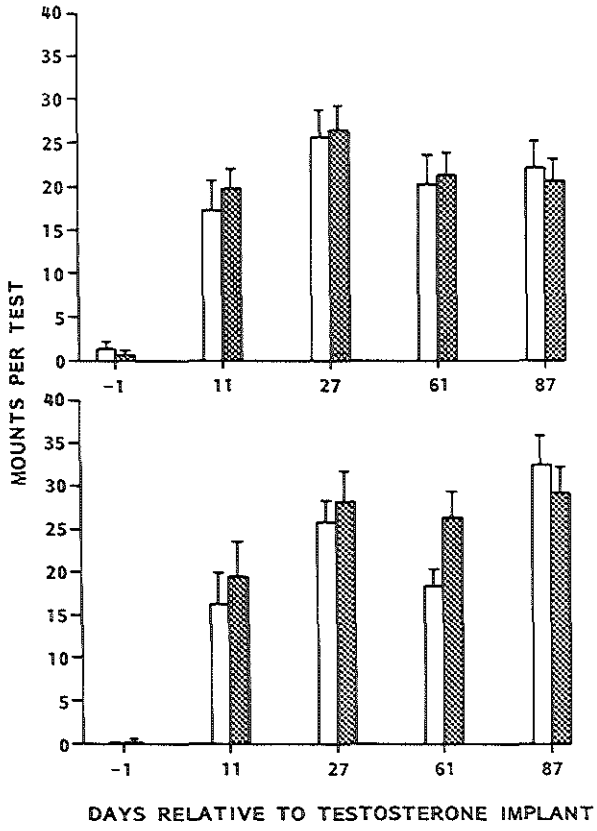

Fig. 2. Frequency (mean ± SE) of T-induced mounting behavior of adult female rats during 15-min tests. Females are grouped according to 'caudal male classification' in utero: open bars, no caudal male(s); shaded bars, 1 or > caudal male(s). For details of experiments 1 *(a)* and 2 *(b)*, see table 1.

In figure 2 the females are grouped according to the presence or absence of males located caudally in utero ('caudal male classification' [5]).

In experiment 1 (fig. 2a) there was only an effect of testing ($F(3/105) = 7.1$, $p < 0.0001$). Further analysis indicated that mounting during the 2nd T-test was highest ($\alpha = 0.01$, HSD = 4.5). In experiment 2 (fig. 2b) there was also a significant effect of testing ($F(3/63) = 13.6$, $p < 0.0001$), 1st T-test with lowest frequencies. There was a trend in groups × test interaction ($F(3/63) = 2.18$, $p = 0.099$).

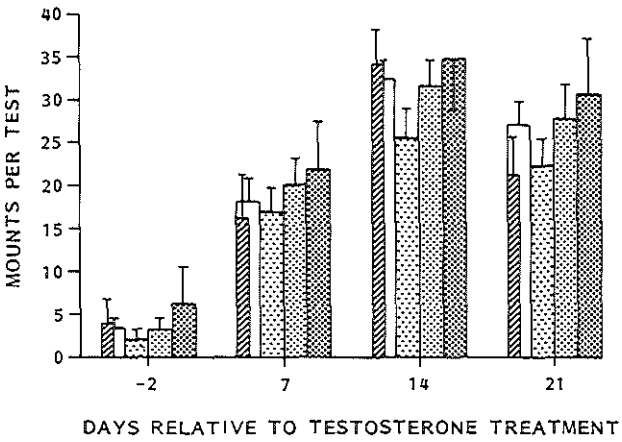

Fig. 3. Frequency (mean ± SE) of TP (250 µg/day)-induced mounting behavior of adult female rats during 15-min tests. Females are classified according to the 'adjacent male contiguity' in utero: open bars, ♀F♀; light dots, ♀F (rostral) ♂; middle intensity dots (caudal) ♂F♀; dark dots, ♂F♂. Note mean mount frequencies of females (n = 5) that had *no* male siblings in utero (narrow hatched bars).

In experiment 4, before the start of T injections, the females were tested 3 times on 3 consecutive days. Analyses were done with individual mean mount frequencies over these 3 tests. Data were analyzed with ANOVA, 4-way factorial design, for repeated measures with between subjects factors: hemihysterectomy before or during pregnancy, uterine position, and watched delivery or cesarian section, and within subjects factor tests.

When females were arranged according to 'male contiguity classification' (see fig. 3), statistical analyses only showed significant overall effects of tests ($F(3/135) = 101.2, p < 0.0001$). It is interesting to note that the 5 females which had no brothers prenatally showed high levels of mounting behavior (small hatched bars in figure 3).

When the data of the females grouped according to the 'caudal male classification' (see fig. 4) were analyzed, there was a highly significant effect of testing ($F(3/159) = 94.0$, $p < 0.0001$), an effect of uterine position ($F(1/53) = 4.46$, $p < 0.04$), and a significant uterine position × hemihysterectomy interaction ($F(1/53) = 4.68$, $p < 0.04$). Further analysis revealed that in the no-hysterectomy group there was a significant effect of caudal male(s) ($F(1/216) = 11.66$, $p = 0.002$), whereas in the hemihysterectomy-during-pregnancy group no such effect was found ($F(1/31) = 0.07$, NS).

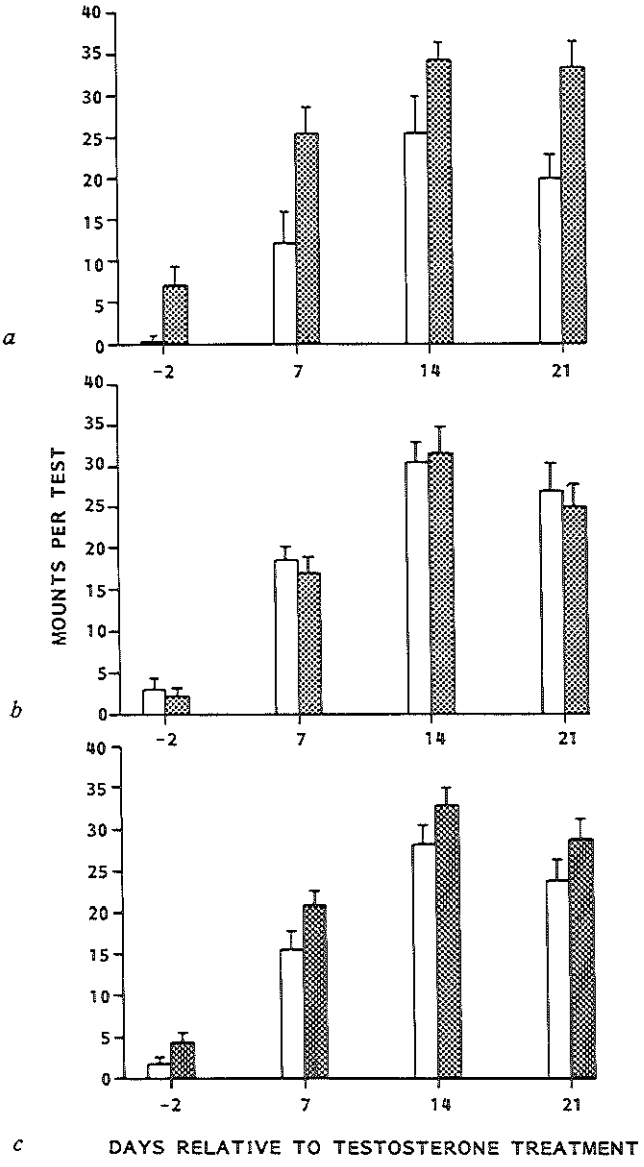

Fig. 4. Frequency (mean ± SE) of TP-induced mounting behavior of adult female rats during 15-min tests. Females are grouped according to the 'caudal male classification' in utero: open bars, no caudal male(s); shaded bars, 1 or > caudal male(s). For experimental details see table 1, exp. 4. *a* No hemihysterectomy during pregnancy (exp. 4a and c); *b* hemihysterectomy during pregnancy (exp. 4b and d); *c* both groups combined.

Prenatal Antiandrogens and Adult Female Mounting (table 2)

The four experiments (5–8) were carried out consecutively. Three different antiandrogens were administered to pregnant rats: two nonsteroidal compounds anandron (AN) and flutamide (FL), and one steroidal compound: cyproterone acetate (CA). The CA pups had to be delivered by cesarian section and were cross-fostered. AN and FL did not interfere with delivery and lactation. At birth, the anogenital appearance of the antiandrogen exposed pups was such that they all looked like females. This indicated that the antiandrogens had reached the fetuses and blocked normal genital masculinization of the males. At weaning, animals could be sexed by visual inspection of the genitalia.

The results of experiments 5 and 6 are depicted in figure 5. In both experiments ANOVA revealed only a significant effect of T-tests (AN: $F(2/61) = 7.1$, $p < 0.0001$; FL: $F(2/54) = 12.8$, $p < 0.0001$). Further HSD analysis indicated lower mount frequencies during the 1st test than during tests 2 and 3, the latter two did not differ. There was no significant difference

Table 2. Overview of the experimental designs to study the effects of prenatal exposure to antiandrogens on T-induced mounting behavior of adult female rats

Exp. no.	Treatment during pregnancy (days 11–12)		Birth	Litters, n	Female offspr. used, n	Age (weeks) at: ovex.	T-treatment onset	T-treatment manner	Mounting behavior tested relative to start of T-treatm., days
5	AN mg/kg/day	0	spont.	4	21	7	10	Silastic implant (2 cm SR-3)	22, 29, 36
		35		4	12				
6	FL mg/day	0	spont.	4	10	4.5	17		35, 42, 49
		5		3	10				
		10		3	10				
7	FL mg/day	0	spont.	2	8	14	18.5	Silastic implants (0.5, 1, 2 cm SR-3)	−8, −3, 0, 7 12, 14, 21, 26, 28, 35, 40, 42
		10		3	10				
8	CA mg/day	−	ces. sect.	3	16	15	20		−7, −3, 0, 7 12, 14, 21, 25, 31, 39, 42, 46
		10		5	22				

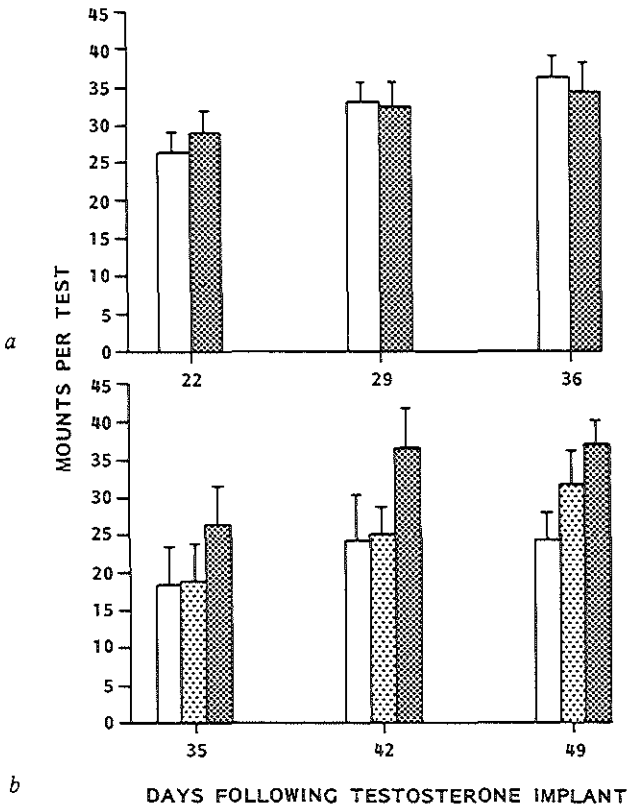

Fig. 5. Frequency (mean ± SE) of T-induced (Silastic implant) mounting behavior of adult female rats exposed to AN *(a)*, FL *(b)* or the solvents (open bars) during days 11–22 of fetal life. AN: 35 mg/kg b.w./day (dark dots); FL: 5 mg/day (middle density dots) or 10 mg/day (dark dots) into pregnant rats. For experimental details, see table 2.

in mount frequencies between the 5- and 10-mg FL females. There were 3 females that never showed mounting behavior: 2 (20%) of the controls, and 1 (5%) of the FL females (in 5-mg group).

Since the FL data were different from results reported by Clemens et al. [3], the experiment was repeated. In experiment 7 only the high dose FL (10 mg/day) was administered during pregnancy and T in adulthood was given in increasing dosages. First the rats received a 0.5-cm Silastic implant for 14 days, then another 0.5-cm implant was added, and 14 days later again an implant (1 cm) was added. During the last 2 weeks, the females had a total

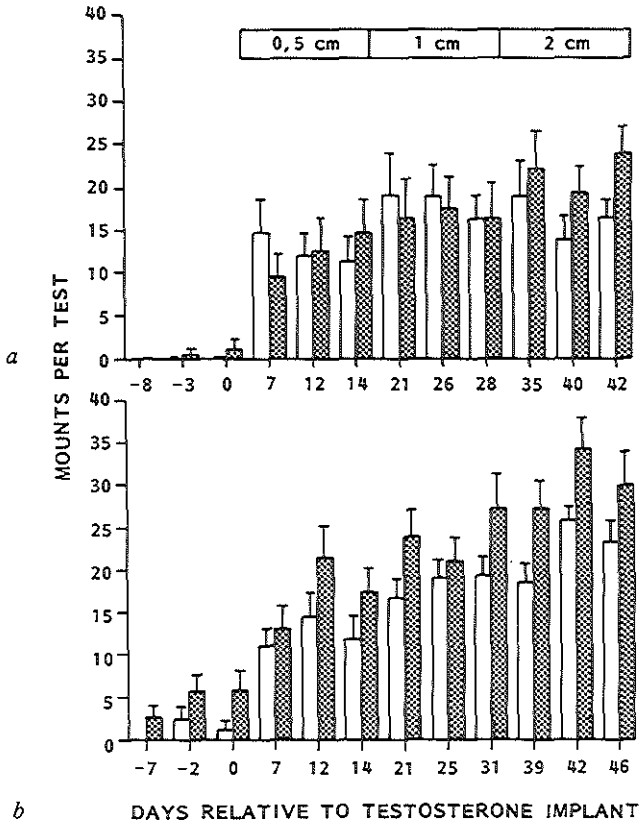

Fig. 6. Frequency (mean ± SE) of T-induced (Silastic implants) mounting behavior of adult female rats exposed prenatally (days 11–22) to FL *(a)* (10 mg/day to mother), the solvent, or CA *(b)* (10 mg/day to mother). Animals were tested 3 times before and 9 times following T treatment. Total length of s.c. Silastic T implants is indicated in horizontal bar, top of figure. See also table 2. Dotted bars refer to experimental rats (FL or CA) and open bars to their controls.

of 2-cm Silastic implant filled with crystalline T (fig. 6, top). This dose is similar to what females received in experiments 1–3, 5, 6 and 8.

Again there was no effect of prenatal FL treatment on adult T-induced mounting behavior. There was only a significant effect of tests ($F(8/128) = 57.7$, $p < 0.0001$). Further analysis essentially showed that with increasing dose of T the mean mount frequencies per test increased ($\alpha = 0.05$, $HSD = 8.0$).

In experiment 8, the results of which are depicted in figure 6, ANOVA revealed a very significant effect of tests ($F(8/288) = 16.6$, $p < 0.0001$) and a trend for groups ($F(1/36) = 3.0$, $p \approx 0.10$), which indicates that females prenatally exposed to CA display more mounting behavior than controls (overall means $\pm$ SEM 24.0 ± 1.2 and 17.9 ± 0.9). The increasing dose of T caused increasing mount frequencies ($\alpha = 0.05$, HSD = 6.2). During the last 3 T-tests (maximum T 'stimulation') there were 2 (10%) of the CA females and none of the controls that did *not* display mounting behavior.

Discussion

Several weeks of T treatment caused the vast majority of females to display relatively high levels of mounting behavior (i.e. mounts with pelvic thrusts and intromission-like mounts). Mean values per 15 min test ranged from roughly 20 to 40 mounts. Of a total of 250 adult ovariectomized females treated with a relatively low dose of T (replacement dose for castrated males) for 2 or 3 weeks, 11 only (4.4%) never showed mounting behavior.

With regard to the fetal position, it was found that in none of the experiments did male contiguity per se affect mounting behavior. Females located between two brothers during fetal life did not display more adult T-induced mounting behavior than females which developed prenatally between two sisters. Females from all-female litters (15 of those females were obtained) displayed high levels of mounting behavior. These results are in line with earlier data from our department [6, 12]. We are inclined to believe that, at least for Wistar rats, the adjacent fetal brother(s) do not seem to be responsible for the 'organization' of adult T-induced mounting behavior of female rats. Other investigators reached similar conclusions (e.g. for Sprague-Dawley rats [5], for mice [13]).

In one experiment of the present studies (exp. 4a and c: no manipulations during pregnancy), it was found that the presence of one or more males located caudally in the same uterine horn significantly increased adult female mounting. In the other experiments no such effect was found. Differences in experimental procedures may partially account for this discrepancy. Surgical manipulations (i.e. stress?) during pregnancy might eliminate the 'organizational' effect of a male located caudally. This could explain why no caudal male effect was found in experiments 2 and 3: quite severe surgical stress on days 9 or 11 of pregnancy. Why there was no significant caudal-male(s) effect in experiment 1 remains unknown. Nevertheless, we do believe that a male

located caudally during fetal life does play some role in the 'organization' of adult T-induced mounting behavior of female rats.

On the basis of our results we have to reject *the male contiguity hypothesis* (i.e. androgen transfer through interamniotic diffusion) originally proposed by Clemens [4]. Recently, working with mice, Gandelman and Kozak [13] were unable to replicate Clemens' results. This was even more surprising since the mouse is a species which has been shown to be particularly sensitive to fetal male contiguity on a large repertoire of behaviors (other than T-induced mounting!) [e.g. 15, 16].

The caudal-male hypothesis, originally put forward by Meisel and Ward [5], with androgen transfer from fetal brothers to sisters through uterine vasculature, might be valid. We found support for this idea. The prenatal 'masculinization' of females could, in this way, be 'additional'. We believe that the major prenatal 'androgenic organization' of female rats (at least Wistar rats) occurs through endogenous androgens produced in the placenta [6, 7, 12]. The caudal-male hypothesis has recently been challenged by vom Saal et al. [17]: they found support for the Clemens' hypothesis, diffusion across fetal membranes (rat strain not specified).

With respect to the various prenatal antiandrogen treatments, it was found that none of the treatments affected adult T-induced female mounting. One could assume that the antiandrogens had not reached the fetuses. This seems very unlikely since the genital appearance of all newborn experimental males was such that they looked like females: the somatic development was impaired. In adulthood the vast majority of these males had many anomalies in genital or gonadal development and functioning [14]. The present antiandrogen data, therefore, do not support the hypothesis of 'prenatal androgenization' of female rats, at least for female Wistar rats. Such effects have been reported for females of other strains (Long-Evans, Sprague-Dawley) [e.g. 1, 18].

It is intriguing that many of our present results differ from various other experimental published data. Besides several differences in experimental procedures, there is one consistent difference: the strain of rats. We have always worked with Wistar rats, whereas other studies dealt with Sprague-Dawley [5, 11, 19], Long-Evans [18, 20] or Holtzman rats [3]. Strain differences in hormonal sensitivity have been reported for lordotic behavior in male rats when Long-Evans and Sprague-Dawley rats were compared [21], or even when males of two Wistar substrains were compared [22]. It seems possible that Wistar females, compared with females from other strains, are prenatally only marginally affected by adrogens from whatever source. Further research into this interesting possibility is necessary.

Conclusion

The presence or absence of males in utero is not the major factor determining adult T-induced mounting behavior of female Wistar rats. When no stress is experienced during pregnancy, female rats can be affected by male brothers located caudally in the same uterine horn so that they show more T-induced mounting behavior in adulthood. Stress during pregnancy seems to eliminate this phenomenon. Prenatal treatment with various potent antiandrogens does not interfere with adult mounting behavior of female Wistar rats. Future research into the possibility of a strain difference is needed to solve the apparent discrepancy between the present results and earlier reported effects of adjacent prenatal brothers or antiandrogen treatment on adult T-induced mounting behaviors. The idea of a prenatal 'masculinization' of female Wistar rats for adult mounting behavior does not find much support in the present data.

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5.2 Perinatal flutamide and mounting and lordosis behavior in adult female Wistar and Sprague Dawley rats.

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Perinatal flutamide and mounting and lordosis behavior in adult female Wistar and Sprague–Dawley rats

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The present study was designed to investigate the possible role of perinatal androgens acting via the androgen receptor, as opposed to the estrogen receptor, for the differentiation of adult mounting and lordosis behavior in female rats. Female Wistar (W) and Sprague–Dawley (SD) rats were exposed to the anti-androgen flutamide prenatally (days 11–22 of pregnancy) and/or neonatally (days 1–10). The females were ovariectomized in adulthood and repeatedly tested for mounting and lordosis behavior. Flutamide, given both pre- and neonatally to SD rats, reduced adult T-induced female mounting. Flutamide administered only prenatally or only neonatally did not lower adult mounting behavior of SD rats. Mounting behavior of female W rats, following pre- and/or neonatal flutamide treatment was not affected. Lordosis behavior was also not altered by perinatal flutamide treatment of either strain. The results of the present study, no effect of *pre*natal flutamide, do not support the hypothesis that female rats require prenatal androgen receptor-mediated actions of testosterone in the organization of neural tissues for the occurrence of adult mounting and lordosis behavior. Only in SD rats androgenic organization of adult mounting behavior via the androgen receptor seems to occur both pre- and neonatally. Since clear behavioral effects of prenatal flutamide treatment have been published earlier in Long–Evans females, we suggest strain differences in sensitivity for perinatal androgenization in female rats. Future research into that possibility is needed.

INTRODUCTION

Ovariectomized female rats treated with testosterone in adulthood readily show mounting behavior when pair-tested with an estrous female (e.g. refs. 4,12,29,30,33–35,38). Lordosis behavior in ovariectomized female rats can be elicited by estradiol with or without additional progesterone (e.g. refs. 16,17). Also testosterone, with or without progesterone, can induce lordosis behavior in ovariectomized female rats[13–15,33,35]. This is probably due to conversion of testosterone (T) to estradiol (E_2)[18].

Fairly high levels of testosterone are circulating in the female rat around the time of birth[2,3,10,27,28,39]. The origin of the prenatal androgens in female rats is still a matter of debate (e.g. ref. 30). Ward and Renz[37] postulated that the adrenals might be the source of the androgen(s). Another speculation came from the work of Clemens and collaborators[9]: they suggested that fetal male androgens may reach the female fetus by way of direct diffusion across the amniotic membranes late in gestation. A different mechanism has been proposed by Meisel and Ward[21]. They suggested that vascular bloodflow, supposedly flowing from the cervix to the ovary, transports androgens to prenatal females such that females would be masculinized by androgens, secreted by males, located 'upstream' from the female. A third hypothesis assumed the placenta as a major source of androgens in female rat fetuses (e.g. ref. 36).

In analogy to what is known about the perinatal organizing action of endogenous testosterone for adult sex behavior in *male* rats (e.g. refs. 1,19), it is assumed[38] that perinatal testosterone in *female* rats also has an organizing effect on adult mounting and lordosis behavior. There is a good deal of evidence to support the idea that both masculinizing and defeminizing actions of perinatally secreted androgens in rats are caused, ultimately, by the action(s) of

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estrogen[1]. However, there is also some evidence that perinatally secreted testosterone, acting via its androgen receptor, has organizing effects on adult sexual behavior in the rat. Support for this latter supposition was derived from studies in which the action of T or its metabolites was suppressed prenatally by anti-androgens[9,16,17,31,37]. As adults, such females displayed less mounting and more lordosis behavior when paired with an estrous female or with an active male partner, respectively.

To shed further light on the possible role of androgen receptor-mediated actions of testosterone in the organization of adult female mounting and lordosis behavior, 3 consecutive experiments were carried. In these studies female rats were pre- and/or neonatally exposed to the anti-androgen flutamide or the solvent and tested in adulthood for sexual behaviors. Flutamide was chosen because of its known androgen receptor blocking capabilities[22,24], its anti-androgenic effects on normal male genital development[7,23], and because it does not interfere with pregnancy, delivery and lactation (e.g. ref. 9). Some of the results have been published as a chapter in a book[5].

GENERAL METHODS

Animals

In Expts. 1 and 2, albino Wistar (HSD-CPB : WU, Harlan, Zeist, The Netherlands) and in Expt. 3 Sprague–Dawley rats (Harlan Sprague–Dawley Inc., Indianapolis, IN, U.S.A.) were used. They were housed 2–4 to a cage, with water and food available ad libitum. Lights in the animal room were on for 14 h/day between 17.30 and 07.30 h, and temperature ranged from 22 to 24 °C.

Treatments

Female rats were time-mated, day of mating = day 0 of pregnancy. Pregnant rats were daily treated with 5 or 10 mg of the non-steroidal anti-androgen flutamide (FL, Sch 13521, 4′-nitro-3′-trifluoromethyl-isobutyranilide, Schering, Bloomfield, NJ, U.S.A.) or 0.1 ml propylene glycol (solvent), from days 11 through the end, day 22, of pregnancy. Neonatal treatment consisted of s.c. injections in the back, for details see relevant experiment. Pups were weaned at 21 days of age and ovariectomized via bilateral lumbar incisions under light ether anaesthesia in early adulthood (see Table I for age of ovariectomy and other details).

Adult testosterone administration occurred by way of silicone rubber implants (inner diameter 1.5 mm, outer diameter 2.1 mm) filled with crystalline testosterone placed s.c. in the back under light ether anaesthesia. In Expts. 1 and 3, 2.0 cm long implants were used; in Expt. 2, 0.5 and 1.0 cm implants were used. Stimulus animals were sexually active male and ovariectomized female Wistar rats brought into estrus with 30 μg estradiol benzoate (EB) 24–48 h prior to

TABLE I

Overview of the experimental designs to study the effects of prenatal exposure to the anti-androgen flutamide on testosterone induced mounting behavior in two strains of rats (Wistar (W) and Sprague–Dawley (SD))

Number expt. (strain)	*Treatment*		*Number of litters*	*Number of female young used*	*Age (weeks) at ovex*	*Age (weeks) at onset of T*	*Treatment manner*	*Mounting behavior studied relative to start of T-treatment (days)*
	Prenatal (mg/day; days 11–22)	*Neonatal (mg/inj.; days 1,3,5,7,9)*						
1 (W)	0	0	4	10	4.5	17	Silastic implant (2 cm SR-3)	35, 42, 49
	5	0	3	10				
	10	0	3	10				
2 (W)	0	0	2	8	14	18.5	Silastic implants (0.5, 1, 2 cm SR-3)	−8, −3, 0, 7, 12, 14, 21, 26, 28, 35, 40, 42
	10	0	3	10				
	0	0.25	2	10				
	10	0.25	3	7				
3 (SD)	0	0	3	17	7–8	13	Silastic implant (2 cm SR-3)	−6, −1, 6, 13, 20, 27, 34, 41
	10	0	4	19				
	0	0.25	6	17				
	10	0.25	5	13				

testing followed by 2.5 mg progesterone (P) 3–4 h before testing.

Behavior tests

Behavior tests were carried out during the dark period of the day in a dimly lit room in semicircular cages measuring 62 × 40 × 36 cm, and lasted 15 min each.

Pair test with estrous female. The experimental female was first put in the cage for a 5-min adaption period, then a stimulus female was also brought in the cage and mounts with pelvic thrusts and intromission-like behaviors were scored.

Pair test with sexually active male. After a 5-min adaptation period of the stimulus male, the experimental female was put in the cage. The lordosis responses of the females to the mounts of the male were recorded. The test lasted until the female had received 10 mounts (Expt. 2), or at the longest 10 min (Expt. 3).

Hormone assay and collection of blood

After the behavior tests had been carried out, blood was collected from the orbital plexus under light ether anaesthesia for the testosterone radioimmunoassay[2]. The interassay and intraassay coefficients of variation were 13.4% and 5.6%, respectively.

Statistics

Mounting data were analysed using one- or two-way ANOVA[25]. Further analysis with the LSD-procedure[20]. For analysis of the number of females mounted, χ^2 analyses were carried out[25].

EXPERIMENT 1

Prenatal flutamide and adult mounting behavior in female Wistar rats

Methods

Twenty-one hemi-hysterectomized rats received during the second half of pregnancy, daily s.c. injections (0.1 ml) with flutamide 5 mg ($n = 9$) or 10 mg ($n = 6$) or propylene glycol (solvent; $n = 6$). At weaning 10 female pups of each treatment group were randomly chosen for later behavioral testing. In addition to partner preference behavior tests (results presented earlier[6]), these females were pair-tested with an estrous female 35, 42 and 49 days following T-implantation (see Table I). Blood was collected 82 days after implantation of T.

Results

Mean (± S.E.M.) mount frequencies are depicted in Fig. 1.

Two-way ANOVA showed no group difference ($F_{2,27} = 1.83$, n.s.), a significant effect of tests ($F_{2,54} = 12.81$, $P < 0.0005$), and no significant groups × tests interaction ($F_{4,54} = 0.86$, n.s.). Further analysis (LSD (5%) = 7.05) indicated lower mount frequencies during test 1 than during tests 2 and 3; the latter two did not differ. There was no significant difference in mount frequencies between the 5- and 10-mg flutamide females. There were 3 females that never showed mounting behavior: 2 (20%) of the controls, and 1 (5%) of the flutamide females (in 5 mg group). The blood testosterone data are presented in Fig. 6; there were no significant differences between the 3 groups.

We conclude from this experiment that prenatal exposure to flutamide does not interfere with adult testosterone-induced mounting behavior in female Wistar rats.

EXPERIMENT 2

Pre- and neonatal flutamide and adult sexual behavior in female Wistar rats

Since no effects were found of exposure to flutamide prenatally, in the present experiment female pups were exposed to flutamide pre- and/or neonatally.

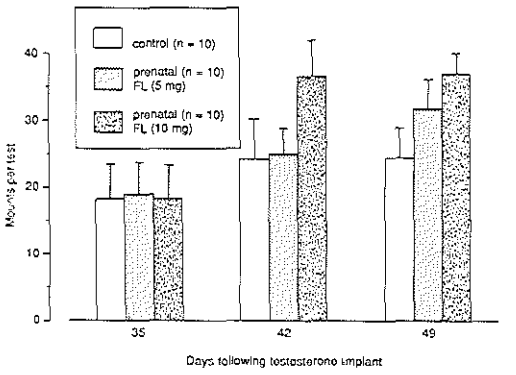

Fig. 1. Frequency (mean ± S.E.M.) of mounts of ovariectomized and testosterone-treated adult female Wistar rats which had been exposed to flutamide or propylene glycol (open bars) during days 11–22 of fetal life. FL: 5 mg/day (lightly dotted) or 10 mg/day (heavily dotted) into pregnant rats. For experimental details see Table I.

Methods

Prenatally, 10 mg/day flutamide was administered to the mother (see Table I). Neonatally, on days 1, 3, 5, 7 and 9 the pups were injected s.c. in the back with 0.25 mg flutamide or 0.05 ml olive oil (solvent). The first injection was given between 9 and 12 h after birth. Eventually, 4 groups of females were formed: (1) control (prenatal propylene glycol and neonatal olive oil; $n = 8$); (2) prenatal flutamide and neonatal solvent (pre-FL; $n = 10$); (3) prenatal solvent and neonatal flutamide (neo-FL; $n = 10$); (4) pre- and neonatal flutamide (preneo-FL; $n = 7$). In adulthood, 12 consecutive pair tests with an estrous female were carried out (see Table I), 3 without T treatment and 9 with increasing dosages of T (3 tests under each treatment condition). Firstly, the rats received a 0.5 cm T-implant for 14 days, then another 0.5 cm T-implant was added, and 14 days later a third implant (1 cm) was added. During the last two weeks the females had a total of 2 cm silastic implant filled with crystalline T, comparable to Expt. 1. Blood was collected after tests 6, 9 and 12.

Two pair-tests with a sexually active male were carried out on days 53 and 54 after T-implantation. The second test was preceded by an s.c. injection with 0.5 mg P 4–6 h before testing.

Results

Mounting behavior

Mean (± S.E.M.) mount frequencies are depicted in Fig. 2. Mean values of 3 consecutive tests with the same treatment are used in statistical analysis.

The mounting data before and after the onset of T-treatment were analysed separately. The numbers of females that showed at least one mount (in one of the 3 tests) before T-treatment were: controls 2 of 8, pre-FL 1 of 10, neo-FL 4 of 10 and preneo-FL 3 of 7. χ^2 analyses on these ratios did not show significant differences.

Two-day ANOVA of T-induced mounting behavior (blocks 2–4; tests 4–12) showed no group difference ($F_{3,31} = 0.52$, n.s.), a significant effect of tests ($F_{2,62} = 4.67$, $P = 0.013$) and a borderline significant interaction ($F_{6,62} = 2.03$, $P = 0.075$). Further analysis of this effect of tests (LSD (5%) = 2.65) showed lower mount frequencies during test block 2 than during blocks 3 and 4; the latter two did not differ. From looking at the figure it seems clear that the effect of tests is mainly due to the control and pre-FL females, which showed higher mount frequencies during blocks 3 and 4 than during block 2. The neo-FL and preneo-FL females did not increase over test blocks 2–4.

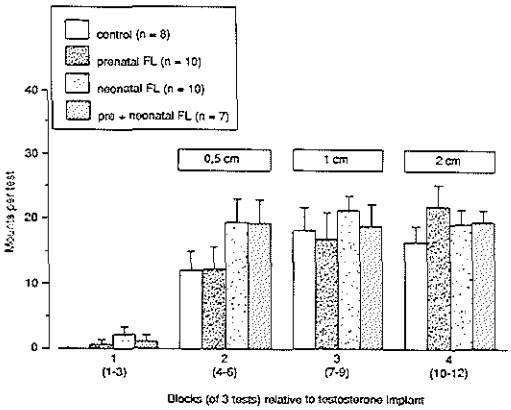

Fig. 2. Frequency (mean ± S.E.M.) of T-induced mounting behavior of adult female Wistar rats exposed prenatally (days 11–22) to flutamide (10 mg/day to mother) or propylene glycol (solvent) and neonatally (days 1,3,5,7,9) to flutamide (0.25 mg/0.05 ml) or olive oil. Total length of s.c. implants is indicated in horizontal bars, top of figure. Mean value for each group was calculated by using the mean mount frequency in 3 tests with the same length of implant. See Table I for the relationship between time of tests and start of testosterone treatment.

Lordosis behavior

Fig. 3 shows the mean (± S.E.M.) lordosis quotients (LQ's) of the females after 53 days of T-treatment

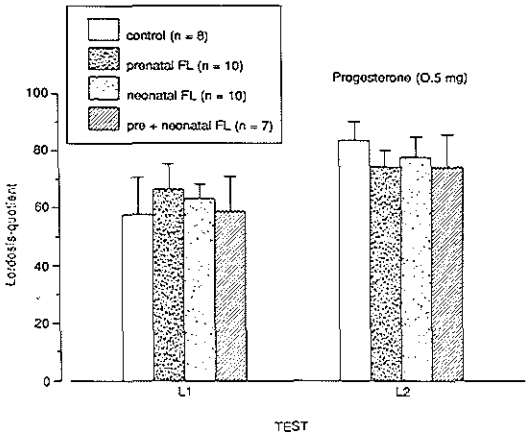

Fig. 3. Mean (± S.E.M.) lordosis quotient of female Wistar rats perinatally exposed to flutamide or solvent (treatment see Fig. 2). Test L1 was carried out after 53 days of T-implantation; test L2 1 day later 4–6 h after an s.c. injection with 0.5 mg P.

without (test L1) and with one progesterone (P) injection (test L2).

Two-way ANOVA of the LQ's revealed no significant group difference ($F_{3,30} = 0.08$, n.s.), a significant effect of tests ($F_{1,30} = 7.21$, $P = 0.012$), and no significant interaction ($F_{3,30} = 0.41$, n.s.). Thus, perinatal flutamide treatment did not affect T-induced lordosis behavior in female Wistar rats. Progesterone facilitated lordosis behavior in these females.

From the present experiment it can be concluded that perinatal exposure to flutamide is (again) unable to interfere with the (possible) organizing action of perinatal testosterone on adult mounting and lordosis behavior in female Wistar rats.

EXPERIMENT 3

Pre- and neonatal flutamide and adult sexual behavior in female Sprague–Dawley rats

The results in the previous experiments differ from the results reported in the literature. Besides several differences in experimental procedures, there is one consistent difference: the strain of rats used. Therefore, we decided to use Sprague–Dawley rats which could be imported directly from the U.S.A.; Long–Evans rats could not be purchased at that time in Holland. The experimental Sprague–Dawley animals were exposed pre- and/or neonatally to flutamide as in Expt. 2.

Methods

Prenatally, 10 mg/day flutamide was administered to the mother (see Table I). Neonatally, on days 1, 3, 5, 7 and 9 the pups were injected s.c. in the back with 0.25 mg flutamide or 0.05 ml olive oil (solvent). The first injection was given between 4 and 6 h after birth. Four groups of females were formed: (1) control ($n = 17$), (2) prenatal flutamide and neonatal olive oil ($n = 19$); (3) prenatal propylene glycol and neonatal flutamide ($n = 17$); pre- and neonatal flutamide ($n = 13$). In adulthood the ovariectomized females were pair-tested 8 times with an estrous female, 2 tests before T and 6 weekly tests after implantation with T (see also Table I). Blood was collected 3 days after the last pair-test (44 days following T-implantation). Four weeks following the last pair-test the implant was removed and the females were left undisturbed for 6 weeks. Lordosis behavior was studied, in an identical way as reported earlier by Gladue and Clemens[16], from the age of 30 weeks on. The subjects were paired twice a week with a sexually active male (6 tests). In one week the animals received 3 daily s.c. injections with EB and were tested on the fourth day. After the test an additional EB-injection was given. The next day (day 5) they were tested 4–6 h following an injection with 0.5 mg P. Daily EB-injections were 0.4 μg in the first week, 0.8 μg in the second and 1.6 μg in the third week.

Results

Mean ($\pm$ S.E.M.) mount frequencies are shown in Fig. 4.

The data on mounting behavior with (tests 3–8) and without testosterone treatment (tests 1 and 2) were analysed separately. The number of females that showed at least one mount prior to the onset of T-treatment (i.e. tests 1 and 2) were: controls 8 of 17, pre-FL 4 out of 19, neo-FL 2 out of 17 and preneo-FL 4 out of 13. χ^2 analysis on these ratios showed that significantly fewer neo-FL females mounted than control females ($\chi^2(df1) = 4.29$, $P < 0.04$).

The data of tests with T-treatment (3–8) were subjected to two-way ANOVA. This revealed no significant group difference ($F_{3,62} = 1.58$, n.s.), a significant effect of tests ($F_{5,310} = 37.76$, $P < 0.0005$) and a significant groups $\times$ tests interaction ($F_{15,310} = 1.84$, $P = 0.029$). Further analysis of this interaction (LSD(5%) = 6.89) showed gradually increasing mounting frequencies. The increase was significant for all groups between tests 3 and 4. The increase between tests 4 and 6 was significant for all groups except the preneo-FL females. From looking at the

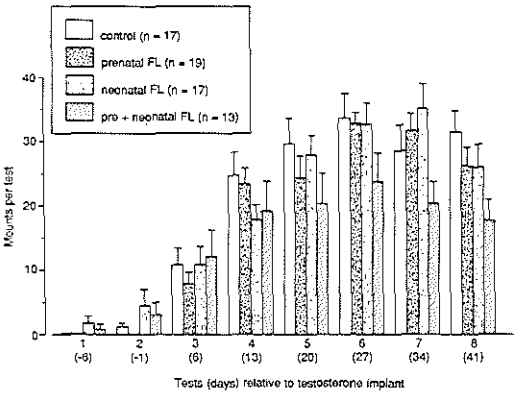

Fig. 4. Frequency (mean $\pm$ S.E.M.) of T-induced (silastic implant) mounting behavior of adult female Sprague–Dawley rats exposed prenatally (days 11–22) to flutamide (10 mg/day to the mother) or propylene glycol (solvent) and neonatally (days 1,3,5,7,9) to flutamide (0.25 mg/0.05 ml) or olive oil. See also Table I.

figure it appears that over the last 3 tests the frequencies are stabilized. In these tests the preneo-FL females had lower mount frequencies than controls, pre-FL and neo-FL females; the latter 3 groups did not differ. In test 5 the preneo-FL females had lower frequencies than the control and neo-FL females, but the difference between preneo-FL and pre-FL was not statistically significant. In test 3 there were no differences and in test 4 the neo-FL females mounted less than the control females.

No differences were observed in androgen-induced clitoral growth and development in adulthood between controls and females given flutamide pre- and neonatally.

Lordosis behavior

Mean (± S.E.M.) LQ's are depicted in Fig. 5.

Only female rats that received 3 or more mounts by the stimulus male are included in the analysis, since a number of females were not mounted by the male, especially when a low dose of EB was used. χ^2 analyses on the number of animals mounted by the male (Fig. 5, left hand panel), showed that only with the low EB (0.4 μg) dose significantly fewer neo-FL females (3/17) were mounted than pre-FL females (12/19; $\chi^2(df1) = 5.88$, $P < 0.02$), although none of them showed a lordosis response. Two-way ANOVA of the LQ-data in the 0.8 and 1.6 μg EB test showed no group difference ($F_{3,42} = 1.87$, n.s.), a significant effect of tests ($F_{1,42} = 53.30$, $P < 0.0005$), and no significant interaction ($F_{3,42} = 0.32$, n.s.). Thus, there is a dose-dependent increase in lordosis behavior. Two-way ANOVA of the LQ data in tests with progesterone (right-hand panel Fig. 5) revealed no group difference ($F_{3,60} = 1.71$, n.s.), a significant effect of tests ($F_{2,120} = 10.04$, $P < 0.0005$), and no significant interaction ($F_{6,120} = 1.25$, n.s.). Further analysis of the effect of tests (LSD(5%) = 6.0) showed that the LQ's in the 0.4 μg EB dose were significantly lower than in the 0.8 μg and 1.6 μg EB dose.

The data of this experiment show that adult mounting behavior of female rats was markedly affected (i.e. lowered) when they were exposed to flutamide both pre- and neonatally. Lordosis behavior was unaffected.

Hormone levels (Expts. 1–3)

Mean (± S.E.M.) testosterone concentrations in serum are shown in Fig. 6.

As can be seen from the figure adult serum T-concentrations in ovariectomized females, bearing one or more silastic implants, were not affected by perinatal

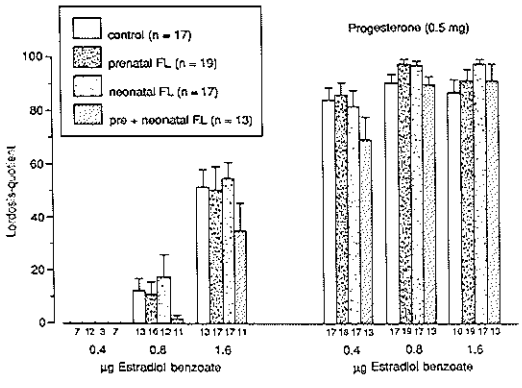

Fig. 5. Mean (± S.E.M.) lordosis quotient of female Sprague–Dawley rats perinatally exposed to flutamide or solvent (treatment see Fig. 4) and adult response to varying doses of estrogen with (right panel) and without (left panel) addition of 0.5 mg progesterone given 4–6 h before testing. The digits below the bars indicate the number of females that were mounted by the stimulus male 3 or more times.

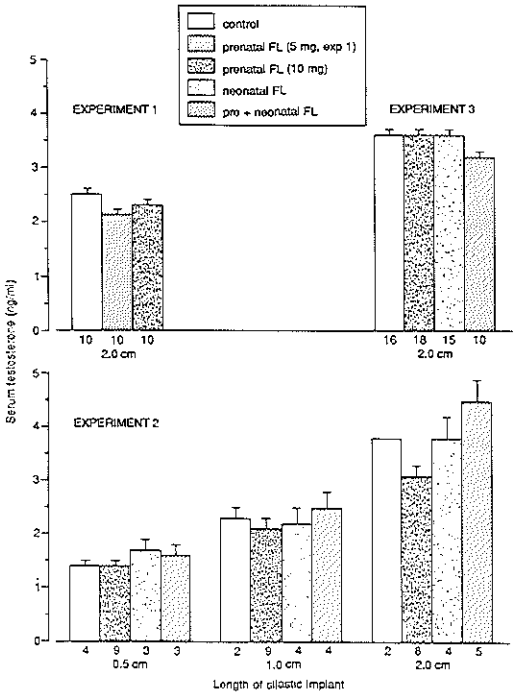

Fig. 6. Mean (± S.E.M.) serum testosterone levels of ovariectomized female rats perinatally treated with flutamide or solvent. In adulthood, they received a silastic implant (length indicated below the bars) filled with crystalline testosterone s.c. in the back. The digits below the bars indicate the number of blood samples analysed.

FL-treatment. Higher T-concentrations were found in Expt. 2 with increasing length of the implant (overall means (± S.E.M.): 0.5 cm: 1.5 ± 0.1 ng/ml; 1.0 cm: 2.3 ± 0.2 ng/ml; 2.0 cm: 3.7 ± 0.2 ng/ml). Lower T-levels were found with longer duration of implantation: for the 2.0 cm implant after 14 days of implantation (Expt. 2): 3.7 ± 0.2 ng/ml, after 44 days (Expt. 3): 3.5 ± 0.1 ng/ml and after 82 days (Expt. 1): 2.3 ± 0.1 ng/ml. This indicates that serum T-concentrations gradually decrease, presumably due to encapsulation of the implant, since most of the implants still contained a fair amount of crystalline T at removal. It should be pointed out that the serum T-levels at the time of behavioral testing were presumably well within the range of adequate levels (2–4 ng/ml) for maintaining mounting behavior in castrated male rats[11].

DISCUSSION

From the present experiments it is clear that flutamide reduced adult T-induced female mounting when it was given both pre- and neonatally to Sprague–Dawley rats. In Wistar rats flutamide treatment had no such effect. Lordosis behavior was not affected by perinatal flutamide treatment of either strain.

The present studies were designed to test the hypothesis originally put forward by Ward and Renz, and Clemens and collaborators, that prenatal androgens acting via the androgen receptor are able to organize adult mounting and lordosis behavior in female rats[8,9,16,17,37]. Blocking the action of prenatally circulation androgens with the anti-androgen flutamide was reported to decrease adult mounting behavior in females[9]. In the present experiments *pre*natal flutamide alone was unable to lower mounting behavior. There is no easy explanation for the discrepancies between our results and those of Clemens et al.[9]. One could assume that in the present experiments flutamide did not reach the fetuses. However, the somatic data (anogenital distance at birth; unpublished findings) indicate that the flutamide did reach the fetuses. In all 3 experiments the anogenital distance of the prenatally flutamide exposed males was highly significantly reduced, such that at birth the sex could not be determined by visual inspection. There is also a difference in the methodology used between our experiments and the work of Clemens et al.[9]. We waited to see the animals develop and determined the gender at the time of weaning by visual inspection of the external genitalia. Clemens and coworkers performed laparotomies on the flutamide-exposed pups within 24 h after birth to locate the testis or ovary; controls were sham-operated or were not. It could be speculated that this neonatal operation interfered selectively with females treated with flutamide and not with control females, because a laparotomy in which a gonad has to be located could be more 'stressful' than a sham-operation. However, this seems unlikely, because if the neonatal operation is responsible for lower T-induced mounting behavior it is difficult to understand why no such effect was found in the low dose females, which were identically treated neonatally. Finally, there is a difference in the strain of rats used: Wistar and Sprague–Dawley in the present experiments; Long–Evans in Clemens' experiment. Possible strain differences in sensitivity for circulating androgens may account for this discrepancy. The finding that pre- plus neonatal flutamide was able to lower mount frequencies in Sprague–Dawley females, while it did not in Wistar rats, needs some comment. An explanation may be that the neonatal treatment started too late in the Wistar rats, roughly between 9 and 12 h after birth, whereas in the Sprague–Dawley rats this was between 4 and 6 h from birth. This could be critical, since androgens during the first few hours after birth have been shown to be very important for 'organizing' the neonatal (male) rat brain[26]. Furthermore, neonatal female rats have high endogenous testosterone levels only during the first 6 h after birth[2,30]. Therefore, neonatal anti-androgen treatment should ideally start immediately after birth. In future experiments this has to be taken into account.

The idea of a prenatal 'masculinization', through androgen receptor-mediated actions of testosterone, of adult T-induced mounting behavior in female Wistar and Sprague–Dawley rats does not find much support in the present data. Pre- plus neonatal flutamide treatment lowered mounting behavior only in Sprague–Dawley females. The organizing action of testosterone is presumed to be mediated through its estradiol metabolite[1]. Flutamide blocks the androgen receptor-mediated actions of testosterone, but is assumed not to interfere with aromatization of testosterone, nor with the possible organizing effect of E_2[23,24]. Yet, flutamide has some diminishing effect on female mounting behavior in Sprague–Dawley rats (present study) as well as long-Evans rats (Clemens et al.)[9]. Two possible explanations can be considered: (1) Flutamide has also (some?) aromatase inhibiting properties, as suggested by Gladue et al.[18]; (2) androgens and estrogens are both implicated in the masculinization process in the female rat, analogous to what has been suggested for the masculinization in

the male ferret by Tobet and Baum[32]. Further research is needed to solve this intriguing possibility.

In the present experiments flutamide was unable to interfere with the defeminizing effect of prenatal testosterone, i.e. lowering of adult lordosis behavior in testosterone-treated Wistar and estradiol-treated Sprague–Dawley rats. Gladue and Clemens[16,17], however, found that prenatally flutamide-treated Long–Evans females showed higher LQ's than controls in tests with EB (daily doses 0.175 or 0.25 μg) and P. In tests with EB alone, pre-FL females showed higher LQ's at the 1, 2 or 10 μg EB-level. Thus, it seems that the prenatal anti-androgen flutamide treatment enhances EB-induced lordosis in Long–Evans females, but not in Sprague–Dawley rats. As was discussed earlier for mounting behavior, a strain difference may exist in the organization of this behavior in female rats. A strain difference was suggested earlier for lordosis behavior in Sprague–Dawley and Long–Evans male rats by Whalen et al.[40]. They found that male Long–Evans rats showed higher frequencies of lordosis than Sprague–Dawley males. This prompted them to say that 'SD males are more fully defeminized by their testes than LE males' (p. 81). They concluded that Long–Evans males are thus more sensitive to exogenous estrogen in adulthood.

Female SD rats, *pre*natally exposed to flutamide, were more readily mounted by the stimulus male, i.e. they were more attractive, compared to *neo*natally flutamide-treated females when given low dose of flutamide in adulthood. No lordosis responses were elicited, which indicates that the females of the groups were equally unreceptive. We have no explanation for this finding. More research is needed to substantiate and elucidate this finding.

Conclusion

Prenatal or neonatal treatment with the anti-androgen flutamide does not interfere with adult mounting and lordosis behavior in female Wistar and Sprague–Dawley rats. Pre- plus neonatal flutamide treatment lowered adult mounting behavior in Sprague–Dawley females, but not in Wistar females. Lordosis behavior was not affected in either strain.

The results of the present study do not support the hypothesis that female rats in general require prenatal androgen receptor activation for the organization of neural tissues for the occurrence of adult mounting and lordosis behavior. Such effects have been published in Long–Evans rats. A possible strain difference in sensitivity for perinatal androgenization is suggested. Future research into that possibility is needed.

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Hoofdstuk 6

Perinatale programmering van volwassen partner preferentie van vrouwelijke ratten.

On the organization of partner preference behavior in female Wistar rats.

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On the Organization of Partner Preference Behavior in Female Wistar Rats

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BRAND, T. AND A. K. SLOB. *On the organization of partner preference behavior in female Wistar rats.* PHYSIOL BEHAV 49(3) 549–555, 1991.—The possible prenatal organizing effects of testosterone (T) on adult sexual partner preference, i.e., sexual orientation in female rats, were studied through prenatal exposure (days 11–22) of female fetuses to the antiandrogens flutamide (Sch 13521; 4'-nitro-3'-trifluoromethylisobutyranilide; 5 or 10 mg/day; Experiment 1) or anandron [RU 23908; 5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-2,4-imidazolidinedione; 35 mg/kg/day; Experiment 2]. The neonatal organizing effects of T were further studied by giving T, dihydrotestosterone (DHT) or oil within 9 h after birth to female pups (Experiment 3). In adulthood sexual orientation was ascertained, after ovariectomy followed by hormone treatment, in an automated open field (AOF), with stimulus animals behind wire mesh, and in a 3-compartment box (3-CB), with stimulus animals tethered. When given the choice between an estrous female and a sexually active male in the AOF, flutamide females, as well as controls, preferred the male partner. After long-term T treatment and 3 weekly pair-tests with an estrous female, flutamide females as well as controls switched their preference to the estrous female partner. In anandron females similar results were obtained. Thus the prenatal antiandrogens had no significant effect on sexual orientation in female rats. This suggests that adult sexual orientation in female rats is not organized prenatally through endogenous T. The change in preference after sexual experience corroborates earlier findings from our laboratory. When given the choice between an estrous female and a sexually active male in the 3-CB (sexual interaction with incentives possible), neonatally DHTP-treated females preferred the male; neonatally TP- or oil-treated females showed no preference. Following long-term T treatment most females preferred the estrous female incentive. TP females had higher preference scores than neonatally DHTP or oil females. It thus seems that adult T-activated partner preference behavior of female rats is affected by neonatally administered T, possibly through estradiol, but not by DHT.

Female rats Prenatal antiandrogen Anandron Flutamide Neonatal hormone injections Testosterone Dihydrotestosterone Estradiol Partner preference behavior Sexual orientation

PARTNER preference behavior, i.e., sexual orientation, is one of the precopulatory behaviors of the female rat. Several studies have appeared about the hormonal *activation* of sexual orientation in female rats. Intact estrous female rats prefer a sexually active male over an estrous female (14). Ovariectomized female rats, given a single injection of estradiol benzoate (EB) or testosterone propionate (TP), tested between 1 and 9 days later preferred the vicinity of a sexually active male over an estrous female (7, 12, 14, 15). Ovariectomized females, given a single injection of dihydrotestosterone benzoate (DHTB), tested between 1 and 6 days later did not show a preference for the estrous female or the sexually active male (12). Long-term treatment (10 to 14 days) with EB or TP also caused a heterosexual preference, i.e., a preference for the active male (6, 8, 18). When pair-tested with an active male, the subsequent preference behavior changed towards the estrous female incentive (18).

Sexual behavior in both sexes is activated by gonadal steroids in adulthood. In male rats it is "organized" by gonadal hormones perinatally, i.e., masculinized and defeminized (1). Such an "organization" of adult sexual behavior has been suggested to occur perinatally in *female* rats, but is still a matter of debate (3).

We have recently found that *male* rat partner preference behavior is to some extent "organized" *neo*natally by T, presumably through its estradiol (E_2) metabolite (5). Concerning female rats, it is known that female pups have measurable blood levels of circulating testosterone prenatally and during the first 24 h after birth (2, 19, 20, 23). It is also known that there is a clear sex dimorphism: male pups have much higher blood levels of testosterone than female pups (2, 19, 20, 23). Little is known about the possible "organizing" effects of this testosterone on sexual orientation in the *female* rat. Two studies addressing this question have been performed (9,13) in which sexual interaction with the stimulus animals was not possible. de Jonge et al. (9) injected female pups with 1 mg TP or oil within 24 h after birth. When given TP in adulthood, following ovariectomy, neonatally androgenized females preferred the estrous female over an active male. Neonatally oil-injected females had no preference for either stimulus animal or showed a "weak" preference for the sexually active male. Meyerson et al. (13) injected female pups with 0.5 mg TP or oil at Day 5. Neonatally androgenized females, ovariectomized in adulthood and treated with EB or TP, preferred the estrous female over the active male. Thus, exogenous neonatal testosterone "organized" a sexual preference for an estrous female in adult hormone-treated female rats. The question remained whether neonatal testosterone itself organized or whether its E_2 and/or DHT metabolite programmed the neonatal female brain for adult sexual orientation. It is also possible that endogenous T could be active *pre*natally in organizing adult sexual orientation.

since female fetuses have considerable amounts of circulating T prenatally [e.g., (2, 19–21)].

The present study was designed to address these questions. The contribution of testosterone, acting via androgen receptors, in the possible *pre*natal organization of partner preference behavior was studied through prenatal exposure of female fetuses to antiandrogens (flutamide in Experiment 1; anandron in Experiment 2). The *neo*natal organizing effects of T were studied by giving T, DHT (the nonaromatizable metabolite) or oil very shortly after birth to female pups (Experiment 3). Adult sexual orientation, i.e., sexual partner preference, was determined in specially designed choice apparatuses (18) where sexual incentives were tethered (sex behavior possible) or were behind wire mesh.

GENERAL METHOD

ANIMALS AND TREATMENTS

Albino Wistar rats (HSD-CPB:WU, Harlan, Zeist, The Netherlands; Wistar Zentral Institut für Versuchtierzucht, Hannover, FRG) were housed 2–4 to a cage, of same sex and treatment. Water and food were available ad lib. Lights in the animal rooms were on for 14 h/day, between 5:30 p.m. and 7:30 a.m., and temperature ranged from 22 to 24°C.

Females were time mated (day of mating = day 0 of pregnancy) and parturition occurred 22 days later. In Experiments 1 and 2 pregnant females were treated (SC injections) with flutamide (Sch 13521, 4′-nitro-3′-trifluoromethylisobutyranilide, Schering, Bloomfield, NJ) or anandron [RU 23908, 5,5-dimethyl-3-(4-nitro-3-(trifluoromethyl)phenyl)-2,4-imidazolidinedione, Roussel-UCLAF, Paris], both nonsteroidal antiandrogens, from days 11 through 22 of pregnancy. Newborn pups were sexed when possible on the basis of genital appearance and marked individually by toe clipping. In Experiment 3 female pups were within 9 hours after birth injected SC in the neck region with testosterone propionate (TP), dihydrotestosterone propionate (DHTP) or solvent (oil). Further neonatal treatment consisted of these injections on days 2, 4, 6, 8 and 10.

Pups were weaned at 21–23 days of age. In adulthood, all females were lightly anaesthetized with ether and ovariectomized via bilateral lumbar incisions, prior to hormone administration. Adult hormone substitution consisted of a silicone rubber implant placed SC in the neck under light ether anaesthesia. Characteristics of the implants: T: inner diameter (i.d.) 1.5 mm, outer diameter (o.d.) 2.1 mm, length 2 cm; DHT: i.d. 1.5 mm, o.d. 2.1 mm, length 3 cm; estradiol (E_2): i.d. 0.5 mm, o.d. 1.0 mm, length 2.5 cm. Stimulus animals were sexually active (usually R × U) Wistar males and ovariectomized female rats that received either nothing or were brought into heat with 30 μg estradiol benzoate (EB) 24 to 48 h prior to testing followed by 2.5 mg progesterone (P) 3 to 4 hours before testing.

BEHAVIORAL TESTS

Automated Open-Field Partner Preference Test

An automated open-field apparatus (AOF; measuring 75 × 75 × 30 cm high) was used, made of black perspex, which was made octagonal by "cutting off" the corners (4,18). Four transparent perspex cages with opaque tops (25 × 20 × 13 cm) were attached to but separated from the arena by a wire mesh wall: 17 cm wide and 8 cm high. The open field received indirect dim red light, reflected from the walls and ceiling of the room, originating from 4 fluorescent lights of 40 W each. A white background noise of 64 dB was supplied to the testing room. Three or four days before the first test the animals were adapted to the AOF for at least 15 min per animal. During behavioral tests two of these cages contained a stimulus animal: a sexually active male and an estrous female, or an estrous and a nonestrous female. After each test (15 min) the transparent cages were rotated clockwise and the next experimental female was put in the center of the arena. Through a television camera situated 2 m above the center of the open field, the X and Y coordinates of the position of the white rat were monitored and automatically stored on a floppy disk of a personal computer. Various parameters could be assessed with computer processing. To quantify partner preference a method was used, adopted from Edwards and Pfeifle (10). A preference score was calculated for each test by subtracting the time spent in front of one stimulus animal during the 15 min test from that in front of the other.

Three-Compartment Partner Preference Test

A test box made of gray perspex with transparent front was used which had 3 compartments (60 × 30 × 40 cm each) with a small opening (13 × 12 cm) in both partitions near the front window. These openings could be closed by a guillotine door. The left and right compartment contained stimulus animals. These incentives, a sexually active male and an estrous female or an estrous female and a nonestrous female, were wearing a leather harness which was attached with a stainless steel string to the rear of the compartment. The stimulus animals thus had a limited action radius. They were adapted to the tethering device during one hour in the week before testing.

Behavioral testing lasted 15 minutes. Before testing all three animals were put in the box, one in each compartment (the two incentives tethered), with the sliding doors closed, for 15–20-min adaptation. At the beginning of the test the sliding doors were removed and the experimental animal could freely move around and interact with the stimulus animals. Time spent in each compartment was measured and various sexual behaviors were scored. To quantify partner preference a comparable method was used as in the automated open-field test. A preference score was calculated for each test by subtracting the time spent in one compartment from that in the other.

STATISTICAL ANALYSIS

Data were subjected to one- or two-way analysis of variance (16). Significant overall effects ($p<0.05$) were further analyzed with the LSD procedure (11).

EXPERIMENT 1: PRENATAL FLUTAMIDE

METHOD

Twenty-one hemi-hysterectomized rats received during the second half of pregnancy daily SC injections (0.1 ml) with 5 mg flutamide (n = 9) or 10 mg flutamide (n = 6) or propylene glycol (solvent; n = 6). At weaning 10 female pups out of each treatment group were randomly chosen for later behavioral testing. At 5 weeks of age all females were ovariectomized. The first partner preference test in the automated open field, without hormone treatment, was carried out at the age of approximately 14 weeks. Stimulus animals were a sexually active male and an estrous female. The next day the experimental females received a silastic T implant.

Subsequently 3 partner preference tests in the AOF were car-

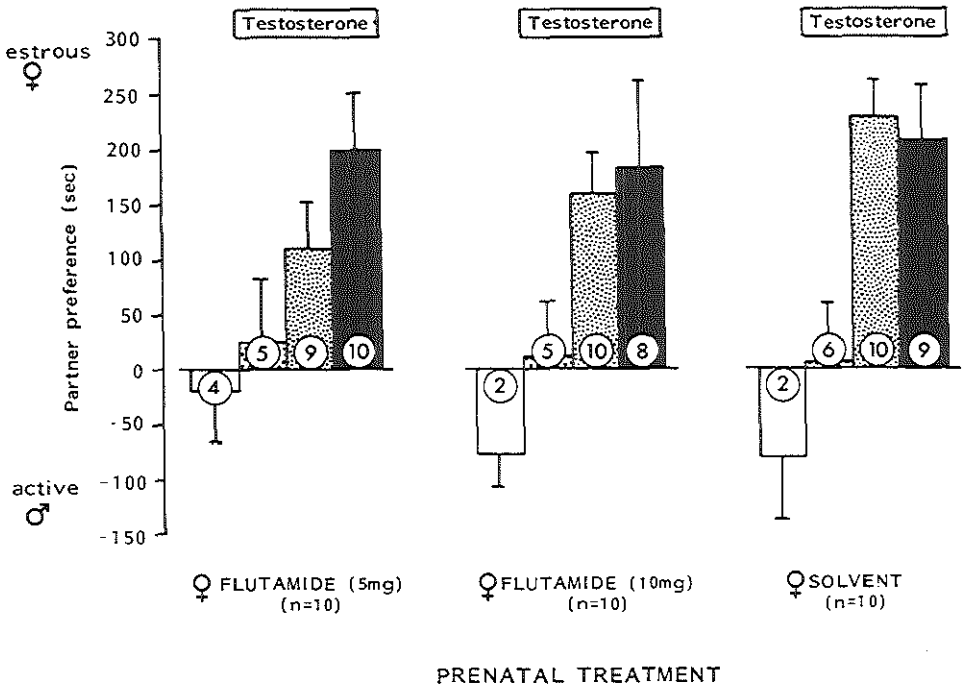

FIG. 1. Mean (±SEM) preference (seconds) for an estrous female over a sexually active male of female rats prenatally treated with flutamide (5 or 10 mg/day, days 11–22 of pregnancy) or solvent (propylene glycol). The bars represent consecutive tests (1 to 4) per group. Tests were conducted in the automated open field, in which sexual interaction was not possible. Between tests 2 and 3 the females were pair-tested with an estrous female; between tests 3 and 4 with a sexually active male. The encircled numbers indicate the number of females per group that preferred the estrous female, i.e., had a positive score. Group size is indicated at the bottom. The horizontal bars at the top indicate when the females were treated with testosterone.

ried out 24, 52 and 76 days after the implantation of T. Between the second and third partner preference test each female was pair-tested three times for mounting behavior with an estrous female. Between the third and fourth partner preference test each female was pair-tested with a sexually active male. Only the partner preference data will be presented here. The data on mounting behavior have been published elsewhere (3).

RESULTS

Mean partner preference scores (choice: estrous female vs. active male) are shown in Fig. 1. From looking at the figure it is clear that in the first test most females preferred the male vicinity. This preference changed in the course of testing towards a clear cut preference for the female partner. Statistical analysis revealed a significant effect of testing, $F(3,81)=35.39$, $p<0.0005$, no significant difference between groups, $F(2,27)=0.21$, n.s., and no significant groups × tests interaction, $F(6,81)=1.20$, n.s. Further analysis of the effect of testing [LSD(5%) = 58 s] showed a significant decrease in preference for the active male from test 1 to 2 and an increase in preference for the estrous female from test 2 to 3 and 4. Tests 3 and 4 did not differ. It is of interest to note that in test 3, after sexual interaction with an estrous female, and in test 4, after sexual interaction with a sexually active male, virtually all females preferred the estrous partner [8 to 10 out of 10, $p<0.02$, binomial test (17)].

EXPERIMENT 2: PRENATAL ANANDRON

METHOD

Female rats were daily SC injected during the second half of pregnancy with olive oil (1 ml/kg b.wt.) containing anandron 35 mg/ml with (AB, n = 3) or without 5% benzyl-alcohol (A, n = 2), with solvent (= olive oil) only (O, n = 3) or 1 ml/kg b.wt. saline (S, n = 2). At weaning the offspring of the four groups of experimental female rats contained the following numbers: AB: n = 7; A: n = 5; O: n = 11; S: n = 10. The females were ovariectomized at the age of 7 weeks.

The first partner preference test in the AOF (stimulus animals: sexually active male and estrous female), without hormone treatment, was carried out at the age of 8 weeks. At 10 weeks of age the experimental females received a silastic implant containing T. Two more partner preference tests in the AOF were carried out, 8 (test 2) and 43 days (test 3) following onset of T treatment. Between the second and third test in the automated open field 3 weekly pair-tests for mounting behavior with an estrous female were carried out. Only the partner preference data will be pre-

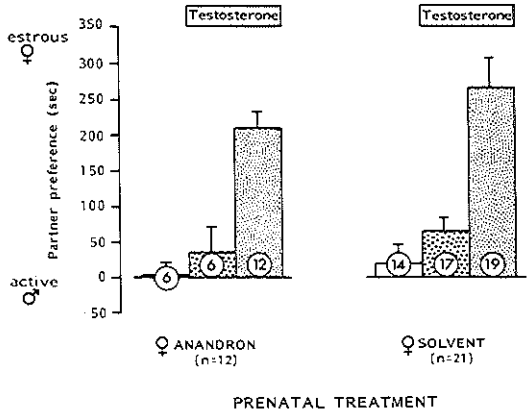

FIG. 2. Mean (±SEM) preference (seconds) for an estrous female over a sexually active male of female rats prenatally treated with anandron (RU 23908, 35 mg/kg b.wt./day, days 11–22 of pregnancy) or solvent (olive oil or saline). The bars represent consecutive tests (1 to 3) per group. Tests were conducted in the automated open field, in which sexual interaction was not possible. Between tests 2 and 3 the females were pair-tested with an estrous female. The encircled numbers indicate the number of females per group that preferred the estrous female. Group size is depicted at the bottom. The horizontal bars at the top indicate when the females were treated with testosterone.

sented here. The data on mounting behavior have been published elsewhere (3).

RESULTS

Since there were no differences between the groups AB and A, and between O and F, two groups were formed for statistical analysis. Mean partner preference scores of the two groups are shown in Fig. 2. From the figure it seems as if during the first tests females do not have a clear preference but in the last test they clearly prefer the estrous female incentive. Statistical analysis revealed a significant effect of testing, $F(2,62) = 31.75$, $p<0.0005$, no significant difference between groups, $F(1,31) = 1.97$, n.s., and no significant groups × tests interaction, $F(2,62) = 0.18$, n.s. Further analysis of this effect of testing [LSD(5%) = 62.0 s] showed that in test 3, i.e., after sexual interaction with an estrous female, the mean preference score differed significantly from the scores in tests 1 and 2. From looking at the number of females with a positive score, i.e., with preference for the estrous female, it appeared that in all tests a significant proportion of the control females preferred the estrous female [14 to 19 out of 21, $p<0.001$, binomial test (17)]. Of the anandron females in tests 1 and 2 only 50% (6 out of 12) preferred the estrous female; in test 3 all anandron females preferred the estrous partner.

EXPERIMENT 3: NEONATAL TP OR DHTP

In Experiments 1 and 2 no effects were found of prenatal antiandrogen treatment on partner preference behavior of female rats treated with gonadal hormones in adulthood. In an earlier study we found that neonatal testosterone plays an organizing role in the masculinization of partner preference behavior in *male* rats (5). Therefore, in the next experiment *female* rats were treated with TP, DHTP or oil neonatally to investigate whether or not this affects adult hormone-activated partner preference behavior.

Since T is metabolized to DHT and E_2 these steroids were administered in adulthood: firstly, DHT alone, secondly combined with E_2 in order to mimic testosterone.

METHOD

Three groups of neonatally injected female rats were formed: 1) TP treatment (0.5 mg, n = 9), 2) DHTP treatment (0.5 mg, n = 10), 3) oil treatment (0.05 ml, n = 10). The females were ovariectomized at the age of 8 weeks. The first partner preference test in the AOF was carried out when the females were 16 weeks old, i.e., 3 weeks after onset of DHT treatment. Stimulus animals: an estrous and a nonestrous female. After this test they received a second SC implant filled with E_2. One and 3 weeks later (tests 2 and 4) the females were tested again in the AOF. One week after AOF tests 2 and 4 the females were subjected to a 15-min partner preference test in the 3-compartment box (tests 3 and 5). A pair-test for sexual behavior with an estrous female was carried out between tests 3 and 4. After test 5 both implants were removed and the females were left undisturbed for 6 weeks.

At the age of about 30 weeks the females were tested again for partner preference in the 3-compartment cage (incentives: sexually active male versus estrous female) without hormone treatment (test 6). The next day all animals received a T implant and 3 weeks later a final partner preference test (incentives: active male and estrous female) was carried out (test 7).

RESULTS

Partner Preferences

Mean partner preference scores (choice: estrous female vs. nonestrous female) are shown in Fig. 3.

The data of tests 1, 2 and 4 (open bars) in the AOF were analyzed separately from those of tests 3 and 5 (dotted bars) in the 3 compartment box.

Statistical analysis of the AOF data revealed no effect of testing, $F(2,52) = 0.92$, n.s., no difference between groups, $F(2,26) = 0.46$, n.s., and no groups × tests interaction, $F(4,52) = 1.75$, n.s. From looking at the number of females that preferred the estrous female it appeared that in test 1 only 1 out of 10 DHTP females preferred the estrous partner ($p<0.02$, binomial test), whereas 5 out of 9 TP females and 6 out of 10 oil females did so.

The preference scores in tests 3 and 5 did not differ significantly from each other, $F(1,26) = 0.50$, n.s. There was no difference between groups, $F(2,26) = 1.02$, n.s., and no groups × tests interaction, $F(2,26) = 1.18$, n.s.

At the age of about 30 weeks the females were tested again in the 3-CB for their preference for an estrous female or a sexually active male, first without hormone treatment (test 6) and then 3 weeks after implantation of testosterone (test 7). Partner preference scores are shown in Fig. 4.

Statistical analysis revealed a significant group difference, $F(2,25) = 3.82$, $p<0.04$, a significant effect of testing, $F(1,25) = 34.86$, $p<0.0005$, and a significant interaction, $F(2,25) = 4.68$, $p<0.02$. Further analysis of this interaction [LSD(5%) = 130.9 s] showed that in test 6 (open columns) the groups did not differ; in test 7 (dotted columns) TP females differed from DHTP and oil females: the last two groups did not differ. T treatment had a significant effect on the partner preference of TP and DHTP fe-

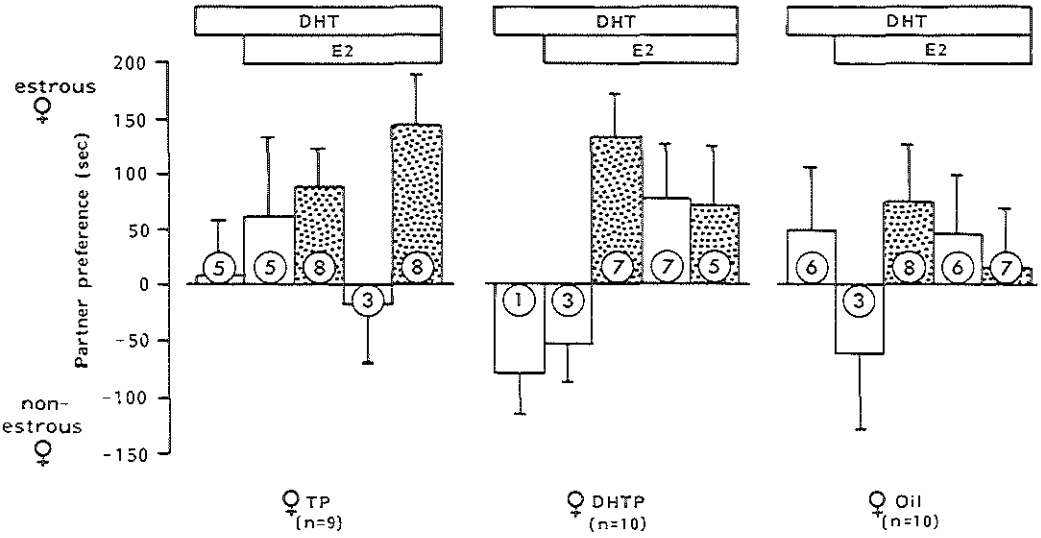

FIG. 3. Mean (±SEM) preference (seconds) for an estrous female over a nonestrous female of female rats neonatally treated with TP (♀TP), DHTP (♀DHTP) or oil (♀Oil). Open bars represent tests in the automated open field; sexual interaction was not possible. Dotted bars represent tests in 3-compartment box; sexual interaction was possible. The encircled numbers indicate the number of females per group that preferred the estrous female. Group size is depicted at the bottom. The horizontal bars at the top indicate the hormones with which the females were treated.

males; it changed from no preference (TP females) or a male preference (DHTP females) towards a preference for the female partner in heat. It is of interest to note that in test 6 (no hormone

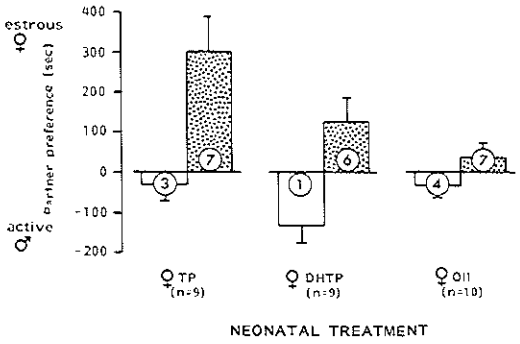

FIG. 4. Mean (±SEM) preference (seconds) for an estrous female over a sexually active male of female rats neonatally treated with TP (♀TP), DHTP (♀DHTP) or oil (♀Oil) in tests in the 3-compartment box; sexual interaction was possible. Open bars represent test without hormone treatment. Dotted bars represent test after 3 weeks T treatment. The encircled numbers indicate the numbers of females per group that preferred the estrous female. Group size is depicted at bottom.

treatment) only 1 out of 9 DHTP females preferred the estrous female ($p=0.02$, binomial test).

Sexual Behavior

In the 15-min pair-test with an estrous female, after long-term DHT plus E_2-treatment, all females showed mounting behavior. One-way ANOVA (mounts plus "intros" combined) revealed a borderline significant group difference, $F(2,26)=2.74$, $p=0.083$. Further analysis [LSD(5%)=9.5] showed that neonatally TP-treated females (mean±SEM=26.2±4.2) showed more mounts than neonatally DHTP-treated females (15.8±2.7). The neonatally oil-treated females (24.2±3.1) did not differ from either group. Statistical analysis of the intromission-like mounts revealed a significant difference between groups, $F(2,26)=3.58$, $p<0.05$. Further analysis [LSD(5%)=3.9] revealed that neonatal TP females had more "intromissions" (6.8±1.9) than neonatal oil-treated females (1.6±0.5). The neonatally DHTP-treated females (4.3±1.4) had intermediate scores and did not differ from the 2 groups.

Also, sexual interactions with the active male during partner preference test 7, after 3 weeks T treatment, were analysed. One-way ANOVA showed an overall group difference for total number of mounts plus "intromissions," $F(2,25)=10.03$, $p=0.001$. Further analysis [LSD(5%)=10.6] revealed that neonatal TP females displayed more mounts (mean±SEM=25.8±5.4) than neonatal DHTP females (5.1±3.1) and oil females (6.6±1.8). One-way ANOVA of the "intromissions" showed similar results [groups: $F(2,25)=5.84$, $p=0.008$, LSD(5%)=2.8 "intros"]: TP females higher "intromission" frequencies (4.4±1.7) than DHTP

females (0.7 ± 0.4) and oil females (0.2 ± 0.1). Thus, neonatally TP-treated females displayed more intromission-like behaviors than controls following DHT + E_2 or T treatment. This is in line with earlier studies [e.g., (22)].

DISCUSSION

The following interesting findings emerge from the first two experiments. Ovariectomized female rats, prenatally exposed to flutamide or solvent, preferred a sexually active male over an estrous female. Ovariectomized females, prenatally exposed to anandron or solvent, showed no clear cut preference for either incentive. Testosterone treatment (1 to 3 weeks) without sexual experience rendered all mean preference scores positive, although only the control females in the anandron experiment showed a significant preference for the estrous female partner. Continuous T treatment and three weekly pair-tests for mounting behavior with an estrous female made virtually all females to prefer the estrous female incentive.

Thus prenatal exposure to flutamide or anandron had no effect on sexual orientation of adult ovariectomized female rats. One could argue that the antiandrogens did not reach the fetuses. This is very unlikely. The antiandrogens had clearly affected the male brothers: at birth they were indistinguishable from the females. Apparently, the masculinization of the external genitalia was blocked by the prenatal antiandrogens. We have no explanation for the finding that in the first test the animals in the flutamide experiment (experimentals as well as controls) preferred the male partner, whereas the anandron animals showed no partner preference in their first test. The two groups differed in age of ovariectomy and of first partner preference test: flutamide experiment: ovariectomy at 5 weeks and test at 14 weeks of age; anandron: 7 and 8 weeks, respectively.

The distinct preference for the estrous female after sexual experience (either with a female or with a male) is in line with earlier reports (6,18). Slob et al. (18) studied ovariectomized females which were treated long-term with TP. They found that it was indeed the heterosexual experience itself that made the T-treated females to switch their slight preference for a male to a clear cut preference for an estrous female. de Jonge et al. (6) reported that sexually naive ovariectomized females, during long-term adult TP treatment, preferred the male. Such females, after many tests with an estrous female, preferred the estrous female in a choice situation. Thus sexual experience with a male or with a female both affected adult partner preference of long-term TP-treated females.

In the present experiments the *pre*natal exposure to antiandrogens had no significant effect on adult partner preference behavior in female Wistar rats. This suggests that adult sexual orientation in female Wistar rats is not organized *pre*natally by androgens acting via androgen receptors. Additional studies are required to show whether the estradiol metabolite of T (formed in the CNS) may serve a programming role prenatally, or whether no steroidal contribution to the "programming" of partner preference behavior in female rats occurs prenatally.

The findings of Experiment 3 can be summarized as follows: neonatally DHTP-treated females, with DHT in adulthood, showed a preference for the nonestrous partner over the estrous partner. The neonatally TP- and oil-treated females, with adult DHT treatment, had no preference for either partner. Treatment with E_2 in addition to DHT caused none of the neonatally treated females to show a clear cut preference. After removal of the implants, given the choice between an estrous female and a sexually active male, then the neonatally DHTP-treated females preferred the male. Females neonatally treated with TP or oil showed no preference. Following long-term treatment with T (3 weeks) most females preferred the estrous female incentive. Neonatally TP-treated females had higher preference scores than neonatally DHTP- or oil-treated females. It thus seems that adult T-activated partner preference behavior of female rats is affected by neonatally administered T, but not by DHT. The results are partly in line with earlier reports by Meyerson et al. (13) and de Jonge et al. (9). These studies reported that neonatally TP-treated females, when given TP as adults, preferred an estrous partner over a sexually active male. However, neonatally oil-treated females, with TP in adulthood, either preferred the active male (13), or showed no preference (9). In the present study females slightly preferred the estrous female. We have no explanation for the differences between the control females in the 3 studies. From the last experiment we conclude that adult partner preference behavior of female rats can to some extent be organized by neonatal T treatment. We assume that estrogenic metabolites of T may be responsible for this "organizing" effect, since neonatally administered DHT, which can not be aromatized to E_2, had no effect.

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Hoofdstuk 7

Perinatale programmering van volwassen partner preferentie en seksueel gedrag van mannelijke ratten.

7.1 Neonatal organization of adult partner preference behavior in male rats.

T. Brand & A.K. Slob.
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Neonatal Organization of Adult Partner Preference Behavior in Male Rats

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BRAND, T. AND A. K. SLOB. *Neonatal organization of adult partner preference behavior in male rats*. PHYSIOL BEHAV 49(1) 107–111, 1991.—Male rats were castrated or sham castrated shortly after birth. Castrated males were then injected every other day on days 0–10 with testosterone propionate (TP, 0.5 mg), dihydrotestosterone propionate (DHTP, 0.5 mg) or the oil vehicle (0.05 ml); sham-castrated males received oil injections. In adulthood, when substituted with DHT, DHT + E_2, or T (silastic implants), sexual partner preference was measured in an automated open field (AOF), in which wire mesh prevented sexual interaction with incentives, and in a 3-compartment box (3-CB), in which sexual interaction with tethered incentives was possible. Choices were an estrous female and a nonestrous female or an estrous female and a sexually active male. In adulthood, following long-term treatment with DHT or DHT + E_2, the males did *not* show any partner preference when sexual interaction with incentives was prevented. Following sexual experience with an estrous female these males preferred the estrous over the nonestrous female, although this change could also be due to long-term hormone treatment. In the 3-CB, a clearcut preference emerged for the estrous female over the nonestrous conspecific, although the neonatally DHTP- or oil-treated males scored lower than the neonatally TP-treated or control males. Six weeks after removal of the hormone implants, when tested in the 3-CB (estrous female vs. active male), the males showed no partner preference. Unexpectedly the control males showed a low preference for the active male. Three weeks T-treatment made all males show a preference for the estrous female (in 3-CB). The neonatally DHTP or oil males displayed significantly lower preference scores than the neonatally TP or control males. In conclusion, neonatal testosterone plays a role in the "masculinization" of partner preference behavior in male rats, probably through its estrogenic metabolite.

Male rats Neonatal castration Neonatal hormone injections Partner preference behavior Sexual orientation

MOST studies concerning male rat sexual behavior focus on performance aspects, i.e., mounts, intromissions and ejaculations. In 1976 Beach (3) called attention to the study of precopulatory behaviors. An important aspect of precopulatory behaviors is the interest in a potential sexual partner, i.e., sexual partner preference or "sexual orientation."

Several apparatuses have been designed to measure sexual orientation behavior in animals [e.g., circular open field (11,21); residential plus maze (14); bi-level chamber (13); tether-box apparatus (27)]. Typically, an experimental animal is given a choice between two incentive animals, an estrous female versus a sexually active male [e.g., (11, 21, 25)] or versus a nonestrous female [e.g., (14–16)]. The experimental animal can behaviorally interact with the tethered stimulus animals or interaction may be prevented by wire mesh. In studies from our laboratory several devices have been used: a residential plus maze (14–20), an automated open field (4,25) and a 3-compartment box (5,6,25).

From the available literature [e.g., review Adkins-Regan (1)] the *activating* effects of hormones on sexual partner preference of male rats seem quite clear. Castration of adult male rats leads to disappearance of preference for an estrous female both versus a sexually active male and versus a nonestrous female. Substitution with testosterone or estradiol restores the preference for an estrous female (4,15).

Relatively little is known about the *organizing* effects of gonadal hormones on sexual orientation in the male rat. Four studies have been published that address this question [(9, 16, 22, 26); see Table 2]. In these experiments male rats were castrated between 0 and 36 h following birth and tested in adulthood for partner preference behavior with or without substitution with testosterone or estradiol. Although these males usually showed a preference for the estrous female, which led to the conclusion that neonatal testicular hormones did not play a very significant role in adult sexual orientation, the studies were not conclusive. Only in one study were males castrated neonatally and subsequently treated with testosterone in an attempt to control for the absence of "organizing" hormones (22).

The present study was designed to investigate the possible role of neonatal androgens, testosterone and dihydrotestosterone, in the organization of adult gonadal hormone activated partner preference behavior of male rats. Partner preference behavior was tested with and without the possibility of sexual interaction with the stimulus subjects.

METHOD

Animals and Treatments

Female Wistar rats were time mated. Between 6 and 14 hours after birth the male pups were castrated, sham castrated or left undisturbed. Hypothermia was used as anesthesia. Further neona-

TABLE 1

EXPERIMENTAL DESIGN OF THE ADULT PARTNER PREFERENCE TESTING IN MALE RATS

Test No.	Test Apparatus	Choice	Age (weeks)	Treatment
1	AOF		16	3 weeks with DHT implant
2	AOF	estrous ♀-nonestrous ♀	18	5 weeks DHT plus 2 weeks E_2 implant
3	3-CB		19	6 weeks DHT, 3 weeks E_2
	Pair test with estrous ♀		20	
4	AOF		21	8 weeks DHT, 5 weeks E_2
		estrous ♀-nonestrous ♀		
5	3-CB		23	10 weeks DHT, 7 weeks E_2 implants removed following this test
6	3-CB		30	Following this test: T-implantation
		estrous ♀-active ♂		
7	3-CB		33	3 weeks with T-implant

AOF = automated open field; 3-CB = 3-compartment box; DHT = dihydrotestosterone; E_2 = estradiol; T = testosterone.

tal treatment per litter consisted of SC injection in the neck region of testosterone propionate (TP, 0.5 mg/0.05 ml oil), dihydrotestosterone propionate (DHTP, 0.5 mg/0.05 ml oil) or solvent (olive oil, 0.05 ml) on neonatal days 0, 2, 4, 6, 8 and 10. Eventually 4 groups of male rats were formed: 1) neonatal castration and TP injection (NC-TP, n = 10), 2) neonatal castration and DHTP injection (NC-DHTP, n = 10), 3) neonatal castration and oil injection (NC-Oil, n = 8), 4) neonatal sham castration and oil injection or neonatal oil injection only [N(S)-Oil, n = 19].

The pups were weaned at 3 weeks of age, housed 2–4 of same treatment to a cage. Water and food were available ad lib. Lighting in the animal room was artificial only for 14.5 h per day (lights on: 5:30 p.m. to 8:00 a.m.) and temperature ranged from 22 to 24°C. At the age of 8 weeks the intact males were castrated via a midline abdominal incision under ether anaesthesia. When 3 months old all subjects were lightly anaesthetized with ether and given a silastic implant (Talas, Ommen, The Netherlands; length 3 cm, inner diameter 1.5 mm, outer diameter 2.1 mm, SR3), containing DHT, under the skin of the back.

Stimulus males were sexually active RxU Wistar males. Stimulus females were ovariectomized Wistar rats that received either nothing or were brought into behavioral estrus by injecting 30 μg estradiol benzoate (EB) 24 to 48 h prior to testing followed by 2.5 mg progesterone (P) 3 to 4 hours before testing.

Behavioral Tests

Automated open-field (AOF) partner preference test. For three tests an automated open field (measuring 75 × 75 × 30 cm high) was used (3,23). This square open-field apparatus, made of black perspex, was made octagonal by "cutting off" the corners with black perspex walls. Four transparent perspex cages with opaque top (25 × 20 × 13 cm) were attached to but separated from the arena by a wire mesh wall: 17 cm wide and 8 cm high; they were left empty in tests without incentives. In tests with stimulus animals two opposite cages contained an incentive; the other two cages were left empty.

The open field received indirect dim light, reflected from the walls and ceiling of the room, originating from 4 red fluorescent lights of 40 W each. A white background noise of 64 dB was supplied to the testing room. Through a television camera situated 2 m above the center of the open field, the X and Y coordinates of the position of the subject were monitored and automatically stored on a floppy disk of a personal computer. Various parameters could be assessed with computer processing. In this experiment time spent in an area measuring 25 × 25 cm in front of each incentive during the 15-min test was used to calculate a preference score.

3-Compartment box (3-CB) partner preference test. A test box made of gray perspex, first described by Slob et al. (23), with transparent front was used. The box had 3 compartments (60 × 30 × 40 cm each) with an opening of 13 cm wide and 12 cm high in both partitions near the front window. These openings could be closed by guillotine doors.

In the left and right compartment, stimulus animals could be placed. These incentives, a sexually active male and an estrous female or an estrous female and a nonestrous female, were wearing a leather harness which was attached with a stainless steel string to the rear of the compartment. The stimulus animals thus had a limited action radius. They were adapted to the tethering device during one hour in the week before testing.

Behavioral tests lasted 15 minutes. Before a test all three animals were put in the box, one in each compartment, the two incentives tethered, with the guillotine doors closed, for 15–20 minutes adaptation. At the beginning of the test the guillotine doors were removed and the experimental male could freely move around and interact with the stimulus animals. Time spent in each compartment was measured and various sexual behaviors were scored.

To quantify partner preference a preference score was calculated for each test by subtracting the time spent with one partner from time spent with the other partner [adapted from Edwards and Pfeifle (8)].

Test Procedure

Behavioral tests were begun when the males were 3.5 months old, 2 weeks after DHT-implantation (see also Table 1). First they were adapted to the automated open field (AOF) for 15 min without stimulus animals. The next week the first preference test

in the AOF was carried out (test 1) with an estrous and a non-estrous female as stimulus animals. After the test they received a second SC implant filled with estradiol (E_2; length 2.5 cm, inner diameter 0.5 mm, outer diameter 1.0 mm). One and 3 weeks later the males were tested again in the AOF (tests 2 and 4). One week after each of these tests the males were subjected to a 15-min partner preference test in a 3-compartment box (tests 3 and 5). A 15-min pair-test for sexual behavior with an estrous female was carried out between tests 3 and 4. The animals were observed, but the behaviors were not systematically scored. All males displayed mounting behavior readily, although not all males intromitted and ejaculated. After test 5 both implants were removed and the males were left undisturbed for 6 weeks.

At the age of about 30 weeks the males, which were now without hormone substitution, were tested again for partner preference in the 3-compartment boxes with a sexually active male and an estrous female (test 6). The next day a silastic implant (length 2 cm, inner diameter 1.5 mm, outer diameter 2.1 mm) filled with testosterone (T) was placed SC in the neck and 3 weeks later a final partner preference test (test 7) was carried out with an active male and an estrous female.

Statistical Analysis

The data were subjected to one- or 2-way ANOVA (23). Significant overall effects were further analysed with the LSD procedure (12).

RESULTS

Mean partner preference scores (choice: estrous female vs. nonestrous female) are shown in Fig. 1. The data of tests 1, 2 and 4 in the automated open field (AOF) were analysed separately from those of tests 3 and 5 in the 3-compartment box (3-CB).

Statistical analysis of the AOF data revealed a significant effect of testing, $F(2,86) = 18.73$, $p<0.0005$, but no significant group difference, $F(3,43) = 0.61$, n.s., or significant interaction, $F(6,86) = 0.55$, n.s. Further analysis [LSD(5%) = 53.0 s] showed that in test 4 the preference score differed significantly from the scores in tests 1 and 2. In other words, in tests 1 and 2 (depicted combined in Fig. 1) there was no preference for either partner; in test 4 the males showed a clear preference for the estrous female.

The preference scores in tests 3 and 5 (depicted combined in Fig. 1) did not differ significantly from each other, $F(1,43) = 0.04$, n.s. There was a significant group difference, $F(3,43) = 5.98$, $p<0.002$, but no significant test × group interaction, $F(3,43) = 1.19$, n.s. Further analysis [LSD(5%) = 93.3 s] indicated that males which were neonatally castrated and treated with either DHTP or with oil showed much lower preference scores than males neonatally castrated and treated with TP or control males.

At the age of about 30 weeks the males were tested again in the 3-compartment box for their partner preference for a sexually active male or an estrous female, first without hormonal substitution (test 6) and again 3 weeks after the implantation of testosterone (test 7). Partner preference scores are shown in Fig. 2. Statistical analysis showed a significant group difference, $F(3,43) = 3.63$, $p<0.02$, a significant effect of testing, $F(1,43) = 226.09$, $p<0.005$, and a significant interaction, $F(3,43) = 4.18$, $p<0.01$. Further analysis [LSD(5%) = 110.0 s] revealed that without hormonal stimulation the groups did not differ, although the difference between NC-TP males and NS-Oil males (104 s) approached significance. It is of interest to note that almost all control males preferred the male partner [17 out of 19: $p<0.001$, Binomial test, (24)]. In the neonatally oil-treated males a similar trend occurred

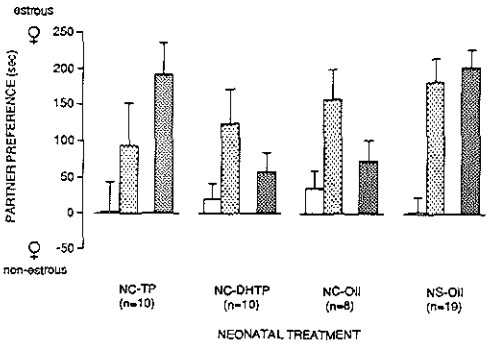

FIG. 1. Mean (± SEM) preference scores (seconds) of male rats castrated at birth and neonatally treated with TP (NC-TP), DHTP (NC-DHTP), oil (NC-Oil), or of sham-castrated males (NS-Oil), for an estrous female over a nonestrous female. Adult treatment consisted of DHT (test 1) or DHT + E_2 (tests 2–5). Testbox was an automated open field (AOF) or a 3-compartment box (3-CB). Group size is indicated in parentheses. Open column: tests 1 + 2 combined, AOF, incentives behind wire mesh; lightly shaded column: test 4, AOF, incentives behind wire mesh; heavily shaded column: tests 3 + 5 combined, 3-CB, incentives tethered.

(6 out of 8 preferred the male). Three weeks exposure to testosterone made virtually all males prefer the estrous female over the male. The NC-DHTP and NC-Oil males showed significantly lower preference scores than the NC-TP or NS-Oil males.

DISCUSSION

From this study it is clear that castrated adult male rats treated long-term with DHT or DHT + E_2 did *not* show any partner preference when behavioral physical contact with the incentives was prevented (AOF, tests 1 and 2). When behavioral interaction was possible (3-CB, tests 3 and 5) a preference emerged for the

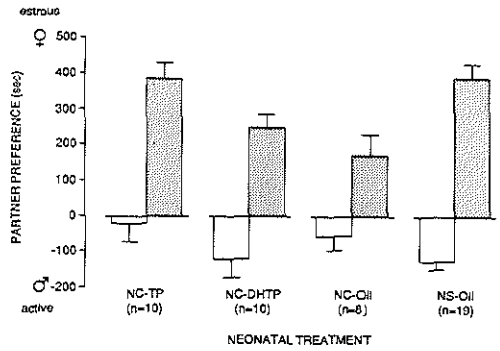

FIG. 2. Mean (± SEM) preference scores (seconds) of male rats castrated at birth and neonatally treated with TP (NC-TP), DHTP (NC-DHTP), oil (NC-Oil), or of sham-castrated males (NS-Oil), for an estrous female over a sexually active male. Tests were carried out in a 3-compartment box. Open column: test 6, without any hormone treatment; shaded column: test 7, after 3 weeks of testosterone treatment.

TABLE 2

STUDIES ON ORGANIZATION OF SEXUAL ORIENTATION IN THE MALE RAT

		Neonatal		Adulthood		
Study	Strain of Rats	Time of Castration After Birth	Time and Nature of Treatment	Treatment	Choice	Partner Preference
Meyerson et al. (22)	Sprague-Dawley	24–36 h	day 2 and 4: TP or oil	TP: 5 weeks later EB	estrous ♀ active ♂	TP: estrous ♀ EB: neonatally TP: estrous ♀ neonatally oil: active ♂
Eliasson and Meyerson (9)	Sprague-Dawley	<24 h or intact	none	none	estrous ♀ active ♂	estrous ♀ (neonatally castr lower preference than intact males)
Merkx (16)	Wistar	<24 h	none	SC implant T, E_2 or DHT	estrous ♀ nonestrous ♀	T: estrous ♀ E_2: estrous ♀ DHT: no preference
Vega Matuszczyk et al. (26)	Wistar	Caesarean section: 0 or 4 h or sham	none	EB + P: 3 weeks later SC implant T	estrous ♀ active ♂	EB + P: sham: estrous ♀ 0 h: active ♂ 4 h: no preference T: 0 and 4 h: estrous ♀
Present study	Wistar	6–14 h or sham	days 0–10: TP, DHTP or oil	SC implant DHT, DHT + E_2;	estrous ♀ nonestrous ♀	DHT: no preference DHT + E_2: estrous ♀ neonat. sham or TP stronger preference than neonat. DHTP or oil
				7 weeks later T	estrous ♀ active ♂	T: estrous ♀ neonat. sham or TP stronger preference than neonat. DHTP or oil

T(P) = testosterone (propionate), E_2(B) = estradiol (benzoate), DHT(P) = 5α-dihydrotestosterone (propionate), P = progesterone.

estrous female (over the nonestrous conspecific). It seems as if behavioral sexual experience with an estrous female made these males prefer an estrous over a nonestrous female. However, the possiblity cannot be ruled out that this change in preference could also be due to longer exposure to adult hormone treatment.

It was further found that partner preference behavior was partially organized in the neonatal period. Castration on the day of birth plus DHTP or oil treatment caused males, treated long-term with DHT + E_2 in adulthood, to show a lower preference for an estrous female. Neonatal treatment with TP prevented this effect of neonatal castration; such males showed a preference for an estrous female identical to control males.

When the males in the present study, without adult hormone treatment, were offered the choice between an estrous female and an active male, they showed no preference or, unexpectedly, a low preference for the active male. When they were treated long-term with testosterone, all groups showed a preference for the estrous female, although the neonatally DHTP- and oil-treated males scored lower than the neonatally TP-treated or control males.

In an earlier study from our laboratory, using a residential plus-maze (no interaction with incentives possible), neonatally castrated rats tested in adulthood with T or E_2 substitution preferred an estrous female over a nonestrous female. Substitution with DHT in adulthood resulted in the males showing no partner preference; moreover, they eschewed the company of both stimulus females [(16); see also Table 2 for overview of relevant literature]. Merkx (16) concluded that neonatal hormones had little effect on adult sociosexual preference behavior. Eliasson and Meyerson (9) reported that neonatally castrated male rats in adulthood *without* testosterone substitution had lower preference scores for an estrous female over an intact male in comparison with intact control males. In an earlier study, Meyerson et al. (22) castrated male rats 24–36 h after birth and gave TP or oil on days 2 and 4. In adulthood with TP substitution, all males preferred an estrous female over an active male. Vega Matuszczyk et al. (26) castrated male rats either immediately after caesarean section or 4 h later. When treated with T in adulthood by SC implant, both groups preferred the estrous female. In general one could say that the available literature suggests that in male rats neonatal testicular hormones have little effect on adult T-activated sociosexual preference behavior. Such a conclusion is mainly derived from experiments in which there was no control for the lack of neonatally circulating hormones. In the present study we tried to control for the absence of endogenous steroids neonatally. It was shown that neonatal castration without subsequent TP substitution significantly lowered adult DHT + E_2- or T-activated partner preference. Neonatal DHTP administration, in contrast to TP, failed to substitute for the "androgen" deficiency. This suggests that the estrogenic metabolite of the endogenous neonatal testosterone (2) partially organizes adult partner preference behavior in male rats.

From the present data it is impossible to determine the degree to which sexual behavioral experience contributed to the observed organizational effects of hormones on sexual preference behavior. In female rats, however, it has been shown that sexual behavioral experience affected partner preference behavior (7,25) and not the duration of exogenous steroid treatment (25). Repeated partner preference testing, with incentives behind wire mesh and without intervening sexual pair tests, could resolve this problem in the male rat. Current research in our laboratory addresses this question.

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7.2 Adult partner preference and sexual behavior of male rats affected by perinatal endocrine manipulations.

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Adult Partner Preference and Sexual Behavior of Male Rats Affected by Perinatal Endocrine Manipulations

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Intact adult male rats, in which aromatization of testosterone to estradiol was prevented pre- and/or neonatally by ATD (1,4,6-androstatriene-3,17-dione), were repeatedly tested for partner preference behavior (choice: estrous female vs active male). In consecutive tests increasing preference scores for the female were found. Neonatal ATD males showed significantly lower preference scores for an estrous female than controls or prenatal ATD males. Prenatal ATD caused preference scores only slightly lower than those of controls. Ejaculation frequencies were markedly reduced or even absent in neonatal ATD males. Prenatal ATD treatment only had no or a moderately lowering effect on ejaculation frequency. Lordosis behavior of adult intact males was more facilitated following *neo*natal ATD treatment than following *pre*natal ATD treatment. In a number of tests the serotonergic drug 8-OH-DPAT was injected prior to testing for sexual partner preference and copulatory behavior. DPAT significantly increased preference for an estrous female in all groups of males when interaction was possible, but had no effect when sexual interaction was prevented by wire mesh. DPAT was able to increase the number of ejaculators in nonejaculating groups (i.e., perinatally ATD-treated males). "Premature ejaculations," i.e., ejaculations with the first intromission, were frequently observed with DPAT treatment in all groups of males. In conclusion, the availability of neonatal estrogen (derived from testosterone) organizes, at least partially, the preference for an estrous female normally shown by adult male rats. The lack of neonatal estrogen causes males to be less masculinized, both in partner preference behavior and ejaculatory behavior, and less defeminized in lordosis behavior. Treatment with a serotonin agonist (8-OH-DPAT) compensates, at least partially, the perinatal ATD effects on adult partner preference behavior and on ejaculatory behavior. © 1991 Academic Press, Inc.

It has been shown that the estradiol (E_2) metabolite of testosterone (T) is essential during a critical perinatal period for masculinization and defeminization of sexual behavior in the male rat (e.g., Baum, 1979). *Neonatal* administration to male rats of ATD (1,4,6-androstatriene-3,17-dione), which blocks the conversion of T to E_2, increased in adulthood the animals' ability to display *lordosis behavior* either with or without

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exogenous estradiol (E_2) and progesterone (P) (McEwen, Lieberburg, Chaptal, and Krey, 1977; Davis, Chaptal, and McEwen, 1979; Vreeburg, van der Vaart, and van der Schoot, 1977). *Prenatal* exposure to ATD also enhanced lordosis behavior in castrated male rats, treated with estradiol and progesterone in adulthood (Clemens and Gladue, 1978; Whalen and Olsen, 1981; Whalen, Gladue, and Olsen, 1986).

Adult mounting behavior was unaffected in *pre*natally ATD treated males, castrated on Day 35 and subsequently treated with TP (Whalen and Olsen, 1981), as well as in *neo*natally ATD-treated gonadally intact male rats (Vreeburg *et al.*, 1977; Davis *et al.*, 1979). Ejaculatory capacity was blocked by neonatal administration of ADT (androst-4-ene-3,6,17-trione), a compound related to ATD, also blocking the conversion of T to E_2, in male rats that were castrated at birth and injected with TP for 5 days (Booth, 1978). In these male rats penile development or the occurrence of intromissions was not affected.

Adult partner preference behavior organization has received less attention (e.g., Adkins-Regan, 1988). We have recently reported that this behavior is affected by neonatal castration (Brand and Slob, 1991). It was found that neonatally castrated and oil- or DHTP-treated male rats showed a significantly lower preference for an estrous female (vs a sexually active male) than control males when tested in adulthood for partner preference behavior under testosterone substitution. This suggests that T, through its estradiol metabolite, may play a role in the programming of this behavior.

The present experiments were carried out to study sexual partner preference behavior of adult intact male rats which were exposed to ATD before and shortly after birth. This approach was chosen because it is known that ATD interferes selectively with the aromatization of endogenous testosterone (Kaplan and McGinnis, 1989), but not with the normal somatic development of the internal and external genitalia (Vreeburg *et al.*, 1977). The latter development is mediated through DHT, the 5α-reduced metabolite of T. Thus, somatically normal male rats with a possibly differentially programmed CNS were studied.

The effects of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), a serotonergic drug with sexual behavior stimulating properties in male rats (e.g., Ahlenius, Larsson, Svensson, Hjorth, Carlsson, Lindberg, Wickström, Sanchez, Arvidsson, Hacksell, and Nilsson, 1981), were also studied. The effects of perinatal ATD treatment on "defeminization" were investigated in adult males, with and without progesterone, when paired with a sexually active male.

GENERAL METHODS

Animals

Experimental animals were Wistar albino rats; stimulus animals were F1 hybrids of two inbred Wistar strains (R × U). They were housed two

to four to a cage with food and water available *ad lib* and kept in a 14 hr light–10 hr dark cycle (lights on: 5:30 PM to 7:30 AM). Temperature in the animal room ranged from 22 to 24°C.

Treatments

Female rats were always time mated. Prenatal treatment of the offspring consisted of ATD, propylene glycol (solvent), or nothing. In experiment 1, ATD was given to pregnant females ($n = 4$) in a 19-cm-long Silastic implant (inner diameter 1.5 mm; outer diameter 2.1 mm), placed sc in the back on Day 11 of pregnancy through parturition. For implantation and removal of the Silastic tube the mothers were lightly anesthetized with ether. Other females ($n = 8$) did not receive an implant. In experiment 2, eight pregnant rats were injected with ATD (5 mg/0.1 ml; $n = 8$) and four with solvent (propylene glycol, 0.1 ml/day; $n = 4$) from Days 10 to 22 of pregnancy.

Within 9 hr after birth small Silastic implants (inner diameter 1.5 mm; outer diameter 2.1 mm; length 5 mm) were placed sc in the back of the newborn male and female pups under ice anesthesia. The implants contained ATD or were left empty (one treatment per litter). The implants were removed when the pups were 10 days (Experiment 1) or 21 days of age (Experiment 2).

Pups were weaned at 21 days of age and housed two to four to a cage of same sex and treatment. They were left undisturbed until the time of behavioral testing, which started at the age of 11 weeks. Most behavioral tests were carried out without any adult treatment, but some tests (see under Method for each experiment) were conducted after an injection with the serotonergic drug 8-OH-DPAT, a $5HT_{1a}$ receptor agonist, 30 min prior to testing. This drug was dissolved in saline at a concentration of 0.1 or 0.2 mg/ml. The animals were sc injected with a volume of 2 ml/kg; i.e., they received dosages of 0.2 or 0.4 mg/kg.

Ovariectomized stimulus females were brought into behavioral estrus by injecting 30 μg estradiol benzoate (EB) 24–48 hr followed by 2.5 mg progesterone (P) 3 to 4 hr prior to testing. These hormones were dissolved in olive oil.

Behavioral Testing

Three-compartment partner preference test. A test box made of gray perspex with a transparent front was used (Slob, de Klerk, and Brand, 1987) which had three compartments (60 × 30 × 40 cm each) with a small opening (13 × 12 cm) in both partitions near the front window. These openings could be closed by a sliding door. Stimulus animals could be put in the left and right compartments. These incentives, an estrous female and a sexually active male, wore either a leather harness which was attached with a stainless-steel string to the rear of the compartment or no harness and were placed behind a wire mesh separation halfway

down the lateral compartment. Tethered animals had a limited action radius. They were adapted to the tethering device during 1 hr in the week before testing. The experimental animal could only see, smell, and hear the incentives when physical interaction was prevented by wire mesh.

Behavioral tests lasted 15 min. Before testing all three animals (one experimental, two incentives) were put in the box, one in each compartment, with the sliding doors closed, for 15–20 min of adaptation. At the beginning of the test the sliding doors were removed and the experimental animal could freely move around and interact with the stimulus animals or sit before the wire mesh separation. Time spent in each compartment was recorded. To quantify partner preference, a preference score was calculated for each test by subtracting the time spent in the compartment containing the sexually active male from time spent near the estrous female (method adopted from Edwards and Pfeifle, 1983). Thus, a positive score indicates preference for the estrous female; a negative score indicates preference for the sexually active male. In the tests in which interaction was possible, the sexual behaviors with the incentives also were scored. Data on ejaculation frequencies and number of intromissions prior to the first ejaculation are presented.

Pair test with estrous female. In these tests, lasting 15 min, semicircular cages measuring 62 × 40 × 36 cm were used. Before the test the experimental male was put in the cage for a 5-min adaptation period. At the beginning of the test an estrous female was put in the cage. Various behaviors were scored. For this report number of ejaculations and number of intromissions prior to the first ejaculation were analyzed.

Pair test with sexually active male. For these tests, carried out also in semicircular cages, sexually active males were used. After a 5-min adaptation period of the stud males, the experimental males were put in the boxes. The lordosis responses of the experimental male to the mounting of the stud male were recorded. The test lasted until the experimental male had received 10 mounts or, at the longest, 10 min. Since many males were not very attractive and did not receive 10 mounts, we have included in the analyses the lordosis quotients of those experimental males that received three or more mounts by the stud male.

Hormone Assay

Testosterone concentrations were estimated in serum by radioimmunoassay, without chromatography, using the prevailing method in our laboratory (e.g., Baum, Brand, Ooms, Vreeburg, and Slob, 1988). The interassay and intraassay coefficients of variation were 13.4 and 5.6%, respectively.

Statistics

Most partner preference data were analyzed using one- or two-way ANOVA (Perlman, 1986). For further analysis the least significant dif-

ference (LSD) procedure (Kirk, 1968) was used. For analyzing the number of animals displaying a certain type of behavior χ^2 analyses were employed.

EXPERIMENT 1

Method

In this experiment four groups of males were formed: (1) control (n = 20; out of five litters); (2) prenatal ATD (pre-ATD; n = 6; two litters); pre- and neonatal ATD (preneo-ATD; n = 12; two litters); (4) neonatal ATD (neo-ATD; n = 10; three litters).

In adulthood, these gonadally intact male rats were tested for partner preference behavior at the ages of 11, 13, 24, and 25 weeks. Incentives were tethered in tests 1, 2, and 4 and freely moving behind wire mesh in test 3.

Four pair tests for sexual behavior with an estrous female (S1–S4) were carried out at 12, 20, 21, and 30 weeks of age. In tests S2 and S3 the effects of 8-OH-DPAT (0.2 mg/kg sc 30 min prior to testing) were assessed in a cross-over design. For the sake of clarity, all saline data are presented as test S2 and the DPAT data as test S3.

Lordosis behavior of the intact males was studied at the age of 18 weeks in two tests using a sexually active male (L1, without progesterone (P); 1 day later (L2), after 0.5 mg P 4–7 hr prior to testing).

Blood was collected under light ether anesthesia from the orbital plexus at 14.5 weeks of age.

Results

Partner preferences. Mean partner preference scores are shown in Fig. 1. The data of tests 1, 2, and 4, with tethered incentives, were analyzed separately from those of test 3, with incentives behind wire mesh.

In tests 1, 2, and 4 there was a significant group difference ($F(3/44) = 41.50$; $P < 0.0005$), a significant effect of tests ($F(2/88) = 99.41$; $P < 0.0005$) and a significant Groups × Tests interaction ($F(6/880) = 2.92$; $P = 0.012$). Further analysis of this interaction (LSD(5%) = 145.1 sec) revealed gradually increasing preference scores for the estrous female. In tests 1 and 2 preneo- and neo-ATD males had preference scores significantly lower than those of pre-ATD and control males; the last two groups did not differ. Most of the control (16–19 of 20) and pre-ATD males (5–6 of 6) preferred the estrous female, whereas the majority of the preneo-ATD males (10–11 of 12) and the neo-ATD males (7–8 of 10) preferred the sexually active male. In test 4 virtually all males preferred the estrous female, although the ATD-treated groups had scores significantly lower than those of the controls. Moreover, the preneo-ATD males scored significantly lower than the pre-ATD males, but did not differ from the neo-ATD males.

One-way ANOVA of test 3 revealed an overall group difference

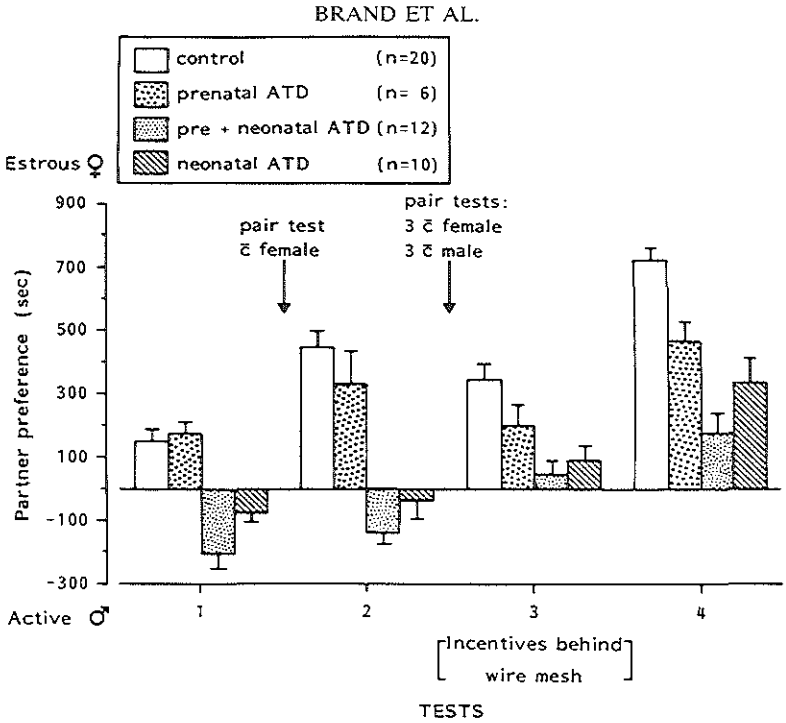


FIG. 1. Mean (± SEM) preference (seconds) for an estrous female over a sexually active male of intact adult male rats after perinatal ATD or control treatment. They were tested four times (15 min/test) for partner preference behavior in a three-compartment box. To calculate a preference score, time spent with the sexually active male was subtracted from time spent with the estrous female. A positive score indicates preference for the female; a negative score: preference for the male. In tests 1, 2 and 4 sexual interaction with tethered incentives was possible; in test 3 sexual interaction was prevented by wire mesh. Between tests 1 and 2 the males were pair tested with an estrous female (left arrow). Between tests 2 and 3 six pair tests were carried out, three with an estrous female and three with a sexually active male (right arrow).

($F(3/44) = 5.38$; $P = 0.003$). Further analysis (LSD(5%) = 117.0 sec) showed that all ATD-treated males had preference scores lower than those of controls. The preneo-ATD males showed lower preference for the estrous female than the pre-ATD males. As in test 4, the neo- and preneo-ATD males did not differ.

Sexual behavior with female during partner preference testing. Sexual behavior during partner preference tests 2 and 4 is depicted in Fig. 2. It seems clear from the figure that neonatal ATD treatment as such (alone or in combination with prenatal ATD) reduced the number of males ejaculating. Hence all males receiving ATD neonatally, i.e., neo- and preneo-ATD combined, were taken together forming group 1 and all males not receiving ATD neonatally, i.e., control and pre-ATD combined, were taken together to form group 2. In preference tests 2 ($\chi^2 = 25.93$,

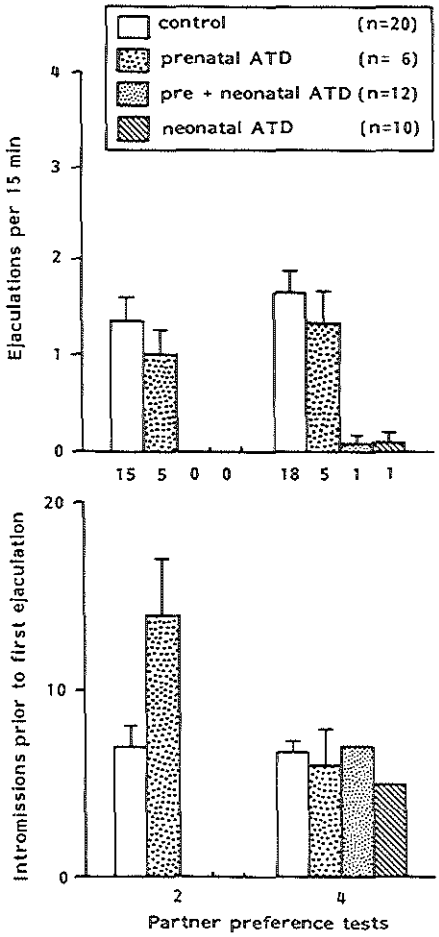


FIG. 2. (Top). Mean (± SEM) ejaculation frequency of intact adult male rats during partner preference tests 2 and 4 as depicted in Fig. 1. The data of test 1 were not reliable and are therefore not presented; in test 3 sexual interaction was prevented by wire mesh. The digits below the bars represent the number of males per group that ejaculated during partner preference testing. (Bottom). Mean (± SEM) number of intromissions prior to the first ejaculation of the males that ejaculated during partner preference testing (responders only).

$P < 0.001$) and 4 ($\chi^2 = 26.98$, $P < 0.001$) fewer group 1 males ejaculated than group 2 males, indicating that indeed neonatal ATD treatment lowered the number of males that ejaculated. Control males and pre-ATD males did not differ in ejaculation frequency in tests 2 and 4 (two-way ANOVA: groups ($F(1/24) = 0.86$, ns), tests ($F(1/24) = 1.66$; ns), interaction ($F(1/24) = 0.003$, ns)).

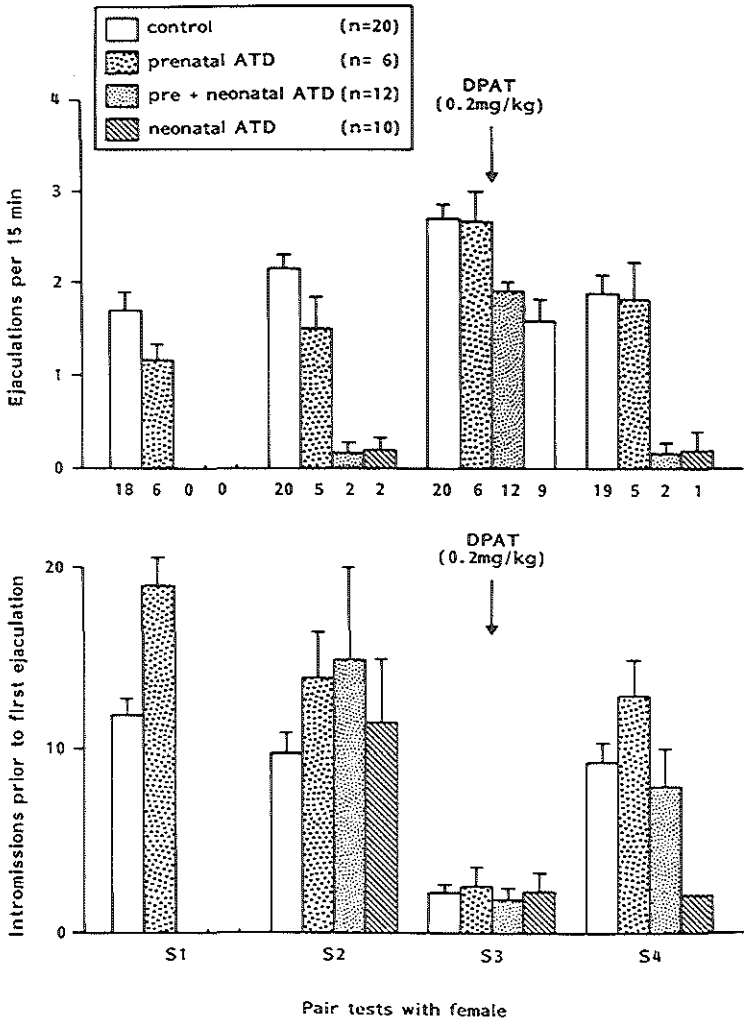


FIG. 3. (Top). Mean (± SEM) ejaculation frequency of intact adult male rats during 15-min pair tests with an estrous female after perinatal ATD or control treatment. Tests were carried out in a semicircular arena. The digits below the bars indicate the number of males per group that ejaculated during pair testing. In test S3 the males were injected sc with 0.2 mg/kg 8-OH-DPAT 30 min prior to testing. (See text for details). (Bottom). Mean (± SEM) number of intromissions prior to the first ejaculation of the males that ejaculated during pair testing (responders only).

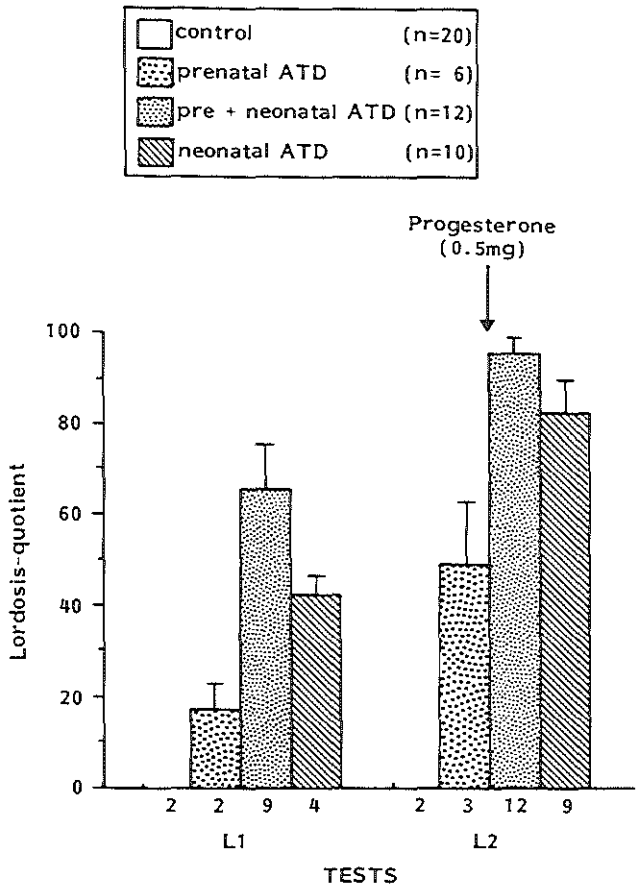


FIG. 4. Mean (± SEM) lordosis quotients of intact adult male rats during pair tests with a sexually active male after perinatal ATD or control treatment. The digits below the bars indicate the number of males per group being mounted by the sexually active male three or more times. In test L2 the males were tested 4–7 hr after a sc injection with 0.5 mg progesterone.

Sexual pair tests with estrous female. The ejaculation data of the four pair tests with an estrous female are shown in Fig. 3. The non-DPAT test data (S1,S2,S4) were analyzed separately from the DPAT test data (S3). Two groups were formed for statistical analysis: group 1, with neonatal ATD, group 2, without neonatal ATD. Without DPAT more group 2 males (i.e., without neonatal ATD) than group 1 (i.e., with neonatal ATD) males ejaculated (S1, $\chi^2 = 37.00$; S2, $\chi^2 = 27.12$; S4, $\chi^2 = 26.86$; all three $P < 0.001$). Two-way ANOVA on ejaculation behavior showed that pre-ATD males ejaculated somewhat less than controls ($F(1/24) = 3.51$; $P < 0.07$). There was no significant effect of tests ($F(2/48) = 2.04$;

TABLE 1
Endogenous Serum Testosterone (T) Levels (Mean ±SEM) in Gonadally Intact Adult Male Rats after Perinatal ATD Treatment

Treatment	T level (ng/ml)
Control	2.4 ± 0.4
Prenatal ATD	2.2 ± 0.3
Pre- and neonatal ATD	2.2 ± 0.4
Neonatal ATD	2.4 ± 0.4

ns) and no interaction ($F(2/48) = 0.72$, ns). DPAT stimulated ejaculation frequency in all males (test S3). One-way ANOVA of the ejaculation frequency showed an overall significant difference between groups ($F(3/44) = 7.89$; $P < 0.0005$). Further analysis (LSD(5%) = 0.56 ejac) revealed that the neo- and preneo-ATD males still had ejaculation frequencies lower than those of controls and pre-ATD males.

As can be seen in Fig. 3 (bottom) DPAT treatment decreased significantly the number of intromissions prior to the first ejaculation in all groups.

Lordosis behavior with active male. Lordosis data are shown in Fig. 4. Significantly more preneo-ATD control males were mounted by the sexually active male in test L1 ($\chi^2 = 11.31$, $P < 0.001$) and L2 ($\chi^2 = 21.16$, $P < 0.001$). In test L2 (after progesterone 4–7 hr before testing) also more neo-ATD males ($\chi^2 = 15.09$, $P = 0.0001$) were mounted than controls. Lordosis quotients (one-way ANOVA) in test L1 showed a significant group difference ($F(3/13) = 3.83$; $P < 0.04$). Further LSD (5%) = 42.0 analysis showed that preneo-ATD males had higher lordosis quotients than control and pre-ATD males. In test L2 there was also a significant group difference ($F(3/22) = 10.08$; $P < 0.001$). Further LSD(5%) = 40.3 analysis revealed that ATD-treated males showed significantly higher LQs than controls. The difference between preneo-ATD and pre-ATD males approached significance.

Hormone data. The mean levels of testosterone at 14.5 weeks of age can be seen in Table 1. One-way ANOVA showed no significant group differences ($F(3/41) = 0.31$, ns). From these data it appears that perinatal ATD treatment had no effect on endogenous testosterone levels in these gonadally intact adult male rats.

EXPERIMENT 2

In the previous experiment the experimental males could interact behaviorally with the stimulus animals. Through this interaction and subsequent behavioral experience the preference behavior could be affected.

Therefore a new experiment was carried out in which adult partner preference behavior was studied when interaction with the stimulus animals was initially prevented by wire mesh.

Method

Three groups of males were formed: (1) control (n = 14; out of four litters); (2) prenatal ATD (pre-ATD; n = 12; four litters); pre- and neonatal ATD (preneo-ATD; n = 18; six litters).

As adults, from 11 weeks onward, the males were subjected to 13 weekly partner preference tests in the three-compartment box. In tests 1–7 wire mesh prevented sexual interaction. In tests 8–13 sexual interaction with the tethered incentives was possible. In tests 6, 10, and 12, 8-OH-DPAT was sc injected 30 min before testing (0.2 mg/kg in tests 6 and 10; 0.4 mg/kg in test 12). In tests 4, 5, 9, 11, and 13 saline (2 ml/kg) was injected 30 min before testing. In fact, tests 5 and 6 were carried out in a cross-over design. The saline data are presented as test 5 and the DPAT data as test 6.

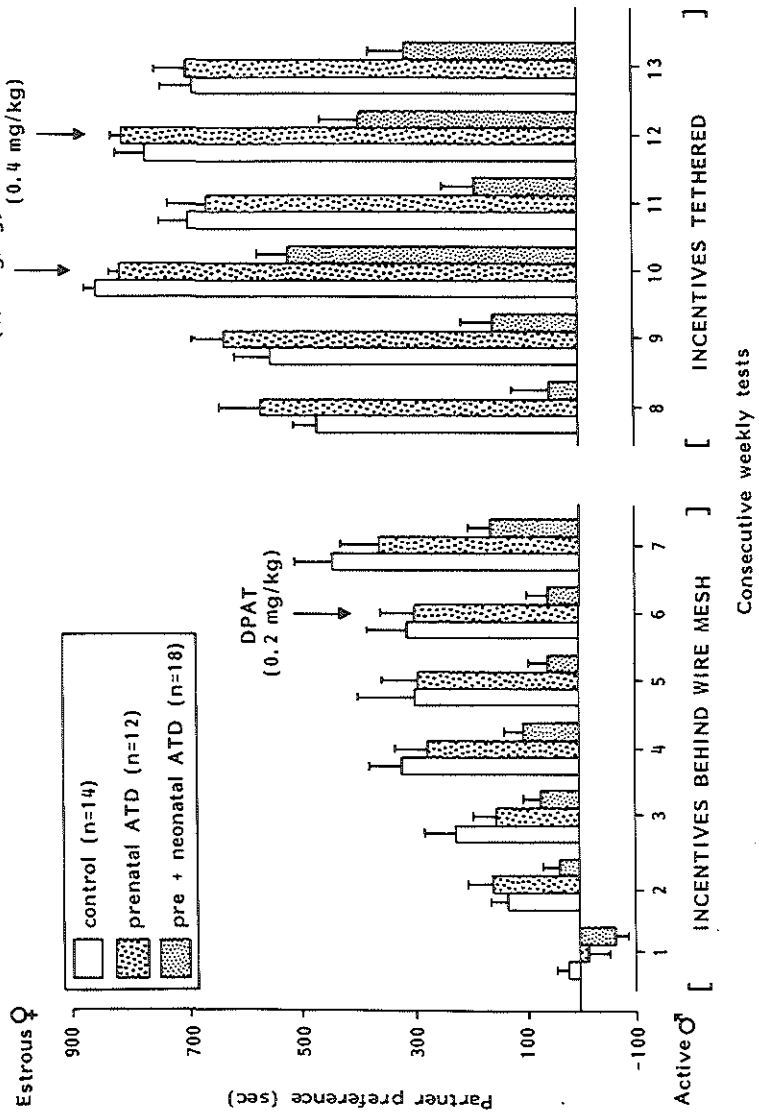
One and 2 weeks following the last partner preference test the males were pair tested with a sexually active male and with an estrous female.

Results

Partner preferences. Mean partner preference scores are shown in Fig. 5. Two-way ANOVA of tests 1–7 revealed a significant group difference ($F(2/41)$ = 18.67, $P < 0.0005$), a significant effect of tests ($F(6/246)$ = 15.76, $P < 0.0005$), and no significant interaction (12/246) = 1.12, ns). Further analysis of the group difference (LSD(5%) = 70.7 sec) revealed that preneo-ATD males had significantly lower preference scores than pre-ATD and control males; the latter two groups did not differ. Further analysis of the effect of tests (LSD(5%) = 72.6 sec) showed overall gradually increasing preference scores for the estrous female (test 1, overall mean: −24 sec, test 7: 304 sec). The differences were significant between tests 1 and 2, 2 and 4, as well as between 6 and 7. 8-OH-DPAT given in test 6 did not significantly affect partner preference.

It is of interest that in test 1 most control males (10 out of 14), half of the pre-ATD males (6 out of 12), and only a few preneo-ATD males (5 out of 18) preferred the estrous female. In test 7 nearly all males preferred the estrous female.

Two-way ANOVA on data of tests 8–13 revealed a significant group difference ($F(2/41)$ = 119.51, $P < 0.0005$), a significant effect of tests ($F(2/205)$ = 17.70, $P < 0.0005$), and no significant interaction ($F(10/205)$ = 0.64, ns). Further analysis of the group difference (LSD(5%) = 66.1 sec) showed that preneo-ATD males had lower preference scores than pre-ATD and control males; the latter two groups did not differ. Further analysis of the effect of tests (LSD(5%) = 92.7 sec) showed overall


control (n=14)
prenatal ATD (n=12)
pre + neonatal ATD (n=18)
DPAT (0.2 mg/kg)
DPAT (0.2 mg/kg)
DPAT (0.4 mg/kg)
Partner preference (sec)
900
700
500
300
100
-100
Estrous ♀
Active ♂
[INCENTIVES BEHIND WIRE MESH]
[INCENTIVES TETHERED]
1 2 3 4 5 6 7 8 9 10 11 12 13
Consecutive weekly tests

increasing preference scores for the estrous female (test 8, overall mean: 325 sec; test 13: 536 sec) with two peaks, namely test 10 (overall: 706 sec) and 12 (624 sec), coinciding with the 8-OH-DPAT treatment.

Sexual behavior with tethered estrous female. The sexual interaction data obtained during partner preference tests 8–13 are shown in Fig. 6. Tests without DPAT treatment (8, 9, 11, 13) were analyzed separately from tests with DPAT (10, 12). Since only a few preneo-ATD males ejaculated in tests without DPAT, a χ^2 analysis was carried out on the number of males ejaculating. This showed that more controls ($\chi^2 = 22.7$, $P < 0.001$) and pre-ATD males ($\chi^2 = 16.4$, $P < 0.001$) ejaculated than preneo-ATD males.

Comparing control and pre-ATD males during the non-DPAT tests (2-way ANOVA), revealed a significant overall difference between groups ($F(1/24) = 15.20$, $P = 0.001$), a significant effect of tests ($F(3/72) = 7.22$, $P < 0.0005$), and no significant interaction ($F(3/72) = 0.77$, ns). Further analysis of the effect of tests (LSD(5%) = 0.54 ejacs) showed an increasing number of ejaculations per test, reaching significance between tests 8 and 9, as well as between 9 and 11. Controls ejaculated more often than pre-ATD males.

Analysis of DPAT tests 10 and 12 revealed a significant group difference (2-way ANOVA; $F(2/41) = 7.82$, $P = 0.001$), a significant effect of tests ($F(1/41) = 29.97$, $P < 0.0005$), and no significant interaction ($F(2/41) = 1.60$, ns). Further analysis of the group difference (LSD(5%) = 0.58 ejacs) showed that with DPAT treatment controls ejaculated significantly more than pre- and preneo-ATD males; the last two groups did not differ. The ejaculation frequency was significantly higher in test 12 than in test 10. As can be seen in Fig. 6, DPAT treatment significantly increased the number of males ejaculating, especially in the preneo-ATD group.

The number of intromissions prior to the first ejaculation was decreased by 8-OH-DPAT treatment. This treatment resulted sometimes in "premature ejaculation," i.e., ejaculation during the first intromission: with low dose (0.2 mg/kg) DPAT in 21% of males (8/38 ejaculators), with high dose (0.4 mg/kg) in 81% of males (35/43 ejaculators).

Sexual pair tests with estrous female and with active male. The results are depicted in Fig. 7. Fewer preneo-ATD males ejaculated than pre-

FIG. 5. Mean (± SEM) preference (seconds) for an estrous female over a sexually active male of intact adult male rats after perinatal ATD or control treatment. They received 13 weekly tests for partner preference behavior using the three-compartment box. In tests 1–7 sexual interaction with the incentives, an estrous female and a sexually active male, was prevented by wire mesh; in tests 8–13 sexual interaction with the tethered incentives was possible. The preference scores were calculated as described in the legend to Fig. 1. In tests 6, 7, and 12 the experimental males were injected sc with 8-OH-DPAT (30 min prior to testing) in dosages indicated in the figure.

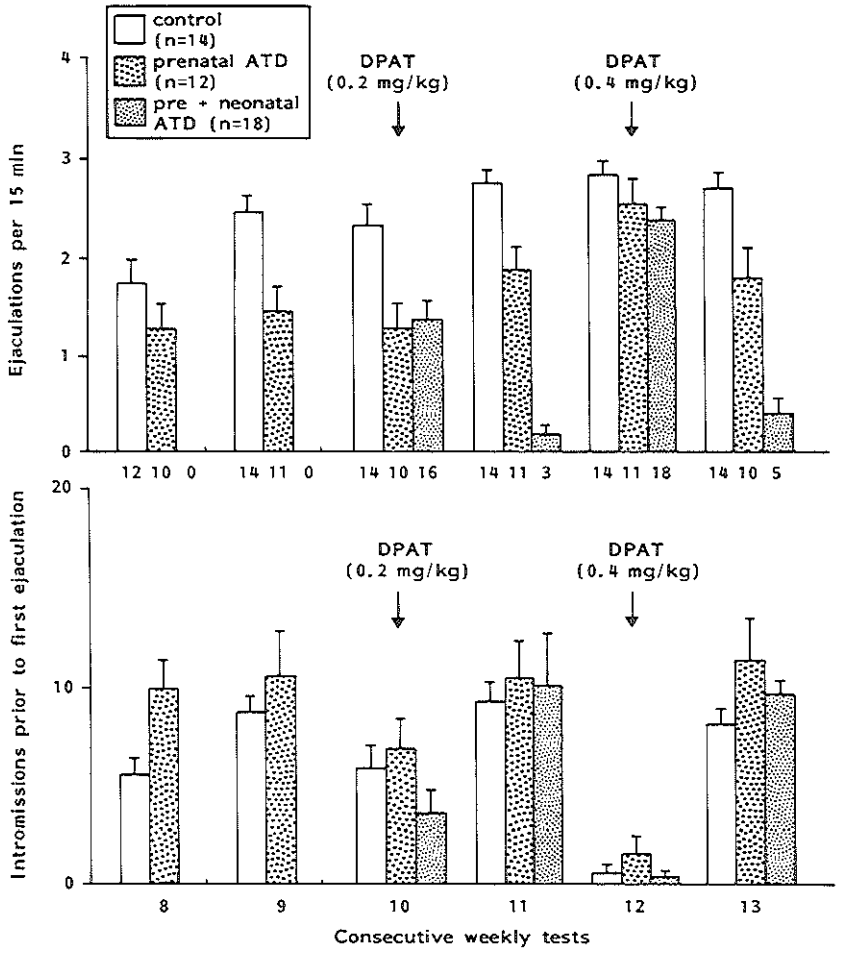


FIG. 6. (Top). Mean (± SEM) ejaculation frequency during partner preference tests 8–13 (see Fig. 5) of intact adult male rats after perinatal ATD or control treatment. The digits below the bars indicate the number of males per group that ejaculated during partner preference testing. In tests 10 and 12 the males were injected sc with 8-OH-DPAT (30 min prior to testing) in dosages of 0.2 and 0.4 mg/kg, respectively. (Bottom). Mean (± SEM) number of intromissions prior to the first ejaculation of the males that ejaculated during partner preference testing (responders only).

ATD males ($\chi^2 = 10.46$, $P = 0.001$) and controls ($\chi^2 = 15.37$, $P < 0.0001$). The number of males ejaculating in the pre-ATD and control group did not differ ($\chi^2 = 0.02$, ns). One-way ANOVA of the mean ejaculation frequencies of the control and pre-ATD males showed a significant group difference ($F(1/24) = 6.54$, $P < 0.02$). In fact, controls

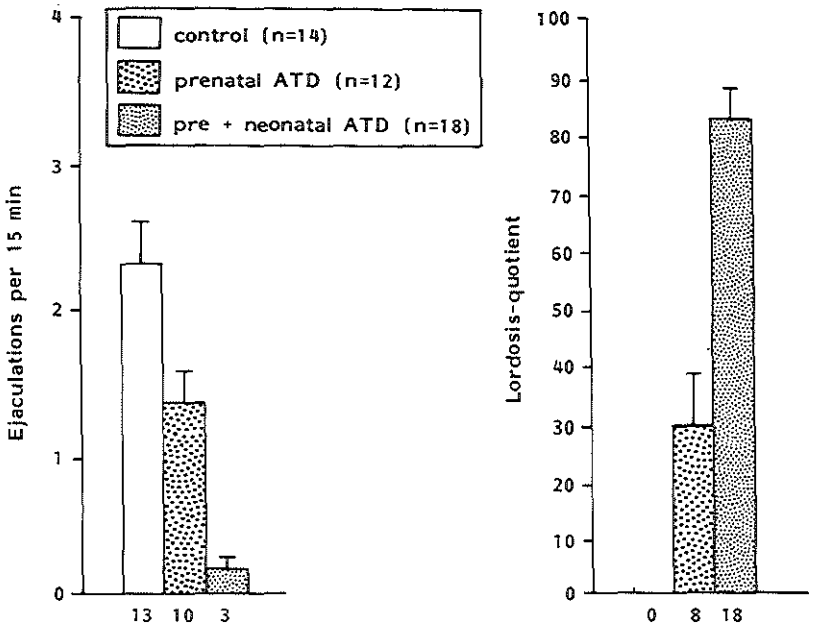


FIG. 7. (Left). Mean (± SEM) ejaculation frequency during a 15-min pair test with an estrous female in a semicircular arena of the same males as in Fig. 6. The digits below the bars indicate the number of males per group that ejaculated during the pair test. (Right). Mean (± SEM) lordosis quotient during a pair test with a sexually active male of the same males as the left hand panel. The males were still intact and did not receive any exogenous steroids. The numbers below the bars indicate the number of males per group being mounted three or more times by the sexually active male.

ejaculated more often than pre-ATD males. Looking at lordosis behavior (right hand panel) it appeared that more preneo-ATD males were mounted by the stud male than pre-ATD males ($\chi^2 = 4.34$, $P < 0.04$) and than controls ($\chi^2 = 28.07$, $P < 0.001$). The pre-ATD males were also more readily mounted than the control males ($\chi^2 = 10.53$, $P < 0.002$). One-way ANOVA of the lordosis quotients ($F(1/24) = 29.22$, $P < 0.005$) showed that preneo-ATD males had higher LQs than pre-ATD males.

DISCUSSION

This study, carried out with intact male rats, clearly shows that adult partner preference behavior can be added to the list of behaviors in the male rat that are (at least partially) "organized" during a critical period. It seems that testosterone through its estradiol metabolite is responsible for this programming. The critical period seems to be mainly (early) neonatal, since prenatal ATD treatment only slightly affected partner

preference. The main results of the present experiments corroborate earlier findings from our lab (Brand and Slob, 1991), but are not in line with data reported by Davis *et al.* (1979). The latter authors could not find changes in adult partner preference following ATD treatment during postnatal Days 2–10. It is suggested that Davis *et al.* started their ATD treatment (Postnatal Day 2) too late. In the present experiments ATD treatment was started on Day 1, when high levels of testosterone are circulating (e.g., Baum *et al.*, 1988).

Neonatal ATD administration impairs the ability of most males to ejaculate. Prenatal ATD was able to lower the ejaculation frequency in one experiment (experiment 2), but not in the other (experiment 1). The route of administration may be relevant in this respect, presumably higher blood levels of ATD were achieved after sc injection to the mother (experiment 2) than following treatment with a silastic implant placed sc in the back (experiment 1).

The ejaculation data of the *neo*-ATD males are not in line with those of Vreeburg *et al.* (1977) and Davis *et al.* (1979). Vreeburg *et al.* reported that males injected with 1 mg ATD on the day of birth (Day 1) and on Days 3, 5, 10, and 15 postnatally, showed normal male copulatory behavior in adulthood. The discrepancy between our results, i.e., a loss of the ejaculation capacity, and the normal ejaculation response reported by Vreeburg *et al.* (1977) may be due to the treatment regimen. The latter investigators injected ATD with intervals of at least 2 days between injections. We used a silastic implant that continuously released ATD. Davis *et al.* (1979) used male rats treated with a 5-mm long Silastic implant containing ATD (controls received an empty implant) on Days 2–10. Tested as adults (about 90 days of age) there were no differences in the ejaculation behavior of 14 ATD and 12 control males. As discussed earlier for partner preference behavior, the difference in the time of onset of treatment may account for the difference with our findings; i.e., Davis *et al.* (1979) started their ATD treatment too late. The results of Booth (1978) are in line with the present experiments. She castrated male rats at birth and treated them with an aromatase inhibitor ADT in combination with T. Masculinization was suppressed, such that none of the ADT + T treated rats ejaculated in adulthood. This suggests that estradiol plays an important role in the organization of this behavior.

The ejaculation data of the *pre*-ATD males in the present study are ambiguous: in experiment 1 no effect was found, in experiment 2 the ejaculation frequency was lowered. We have suggested above that the route of ATD administration (Silastic implant vs sc injection) may be relevant in this regard. However, ambiguous results have also been published by Gladue and Clemens (1980) and Whalen and Olsen (1981) who both used prenatal injections to the mother as the manner of ATD administration. Whalen and Olsen found no significant effects, whereas

Gladue and Olsen found a slightly lower percentage of animals ejaculating in the 5 mg ATD group.

Perinatal ATD treatment and in some tests also prenatal ATD treatment rendered males to be more attractive than controls; i.e., they were more readily mounted by the stud male. This corroborates data from de Jonge and Meyerson (1982) who reported that neonatally castrated males were more approached by male rats than intact males in a choice apparatus. Thus, the lack of endogenous gonadal hormones neonatally rendered such males to be (more) attractive to normal males. In the present study, preneo-ATD males were hardly defeminized since they showed high lordosis quotients. Pre-ATD males were somewhat defeminized, more so than preneo-ATD males, but less than control males. It should be kept in mind that in the present experiments all adult males were gonadally intact and did not receive any exogenous steroids.

It is known from the literature that male rats perinatally treated with ATD, castrated in adulthood and treated with EB with or without P, show high levels of lordosis behavior (Vreeburg *et al.*, 1977; McEwen *et al.*, 1977; Clemens and Gladue, 1978; Fadem and Barfield, 1981; Whalen *et al.* 1986). Davis *et al.* (1979) reported increased lordosis quotients in gonadally intact adult male rats which were neonatally treated with ATD. From our data we conclude that ATD treatment at least partially prevents the defeminization of intact males. Neonatal ATD treatment seems to have more effect than prenatal ATD treatment. This suggests that the defeminization of the male rat occurs mainly neonatally, presumably through the estradiol metabolite of testosterone.

The facilitatory effects of progesterone for lordosis behavior in our ATD treated intact male rats is in line with other studies (e.g., van de Poll and van Dis, 1977). In the latter study intact males showed higher lordosis quotients than adult castrated males following adult estrogen plus progesterone treatment. The fact that our control males did not show lordosis behavior could be due to a lack of sufficient levels of endogenous estradiol, since estradiol treatment stimulates lordosis behavior in intact male rats (van de Poll and van Dis, 1977). It could be hypothesized that ATD-treated males in the present study had higher endogenous E_2 levels and/or their CNS is more sensitive to E_2. However, higher E_2 levels and/or a more E_2-sensitive CNS are at variance with a decrease in female partner preference, since estradiol treatment stimulates preference for an estrous female vs an active male (Meyerson, Eliasson, and Hetta, 1979). Further research is needed to elucidate these possibilities.

8-OH-DPAT, a known stimulator of male rat sexual behavior (e.g., Ahlenius *et al.*, 1981), increased the preference scores for an estrous female in all groups when sexual interaction was possible (experiment 2). It was, however, unable to totally undo the effect of neonatal ATD. DPAT was able to increase the number of males that ejaculated in the

preneo-ATD male group. DPAT lowered the number of intromissions preceding the first ejaculation. This resulted quite often in premature ejaculation, i.e., ejaculation with the first intromission. This phenomenon occurred in about 20% of the males with low dose DPAT and in about 80% with high dose DPAT, regardless of perinatal treatment. DPAT treatment has no lasting effects on partner preference behavior and on male sexual behavior: tests 1 week after DPAT tests showed results similar to those of tests preceding DPAT. This indicates that the neonatal ATD effects cannot be overcome by one learning experience. This suggests that ATD has indeed permanently affected the CNS. Such males behave differently to the activating action of their own testicular hormones. Apparently, 8-OH-DPAT is able to bypass, in an unknown way, the affected CNS structures. Perhaps DPAT activated alternative CNS-networks that activate normal male sexual orientation and behavior.

In conclusion, the present data suggest that partner preference behavior of male rats is programmed neonatally, presumably by estradiol derived from endogenous testosterone. A lack of neonatal estrogen impairs adult ejaculatory behavior (such males are less masculinized) and enhances lordosis behavior in intact male rats (they are less defeminized). Treatment with 8-OH-DPAT (a serotonin agonist) abolishes most effects of perinatal ATD treatment on adult partner preference behavior as well as on ejaculatory behavior.

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7.3 Adult sexual orientation and behavior of male rats neonatally treated with aromatase inhibitors.

T. Brand & A.K. Slob.

ADULT SEXUAL ORIENTATION AND BEHAVIOR OF MALE RATS NEONATALLY TREATED WITH AROMATASE INHIBITORS

T. BRAND AND A.K. SLOB

Abstract. Intact adult male rats, in which the aromatization of testosterone (T) to estradiol (E_2) was prevented neonatally by ATD (1,4,6-androsta-triene-3,17-dione) or Atamestan (1-methyl-1,4-androstadiene-3,17-dione), were repeatedly tested for partner preference behavior (choice: estrous female vs sexually active male) and for sexual behavior (with estrous female or sexually active male) in adulthood. Gonadally intact adult male rats, neonatally treated with ATD, showed less preference for an estrous female, were less masculinized, i.e. showed fewer ejaculations, and less defeminized, i.e. showed more lordoses than controls. Similar effects were found in neonatally Atamestan treated males. The α_2-adrenoreceptor blocker yohimbine hydrochloride was able to partially undo the effects of neonatal ATD treatment by increasing the number of males that ejaculated. Yohimbine had no such effect in neonatally Atamestan treated and control males. Treatment with estradiol benzoate in adulthood had no effect on male sexual behavior. Progesterone, injected 4-7 h prior to testing, had no effect on partner preference and sexual behavior displayed during partner preference testing in a choice box. The present results support the hypothesis that neonatally circulating T, presumably through its metabolite E_2, is essential in organizing adult sexual orientation and sexual behavior in male rats.

Estradiol, derived from aromatization of testosterone, is essential during a critical period around the time of birth for masculinization and defeminization of adult sexual behavior in male rats (e.g. 2). The role of estradiol in organizing adult sexual orientation, as indicated by partner preference behavior, has received less attention (1). In a recent study (5) it was found that neonatally castrated and oil or DHTP treated male rats showed significantly lower preference for an estrous female (vs. an active male) than control males when tested in adulthood under testosterone substitution. We suggested that T, through its metabolite estradiol, plays a role in the organization of sexual

orientation in male rats. In other experiments (4) male rats were perinatally treated with the aromatase inhibitor ATD (1,4,6-androstatriene-3,17-dione) and tested for sexual orientation in adulthood. It was found that neonatal ATD treatment significantly lowered preference for an estrous female. The present study was carried out: 1) to replicate the organizing effect of ATD on adult sexual orientation and 2) to study the effects of another aromatase inhibitor, Atamestan (1-methyl-1,4-androstadiene-3,17-dione; 14).

In the above mentioned study (4) we also found that neonatally ATD-treated males were less masculinized, i.e. that they seldom ejaculated, and were less defeminized, i.e. showed more lordosis behavior, than controls. Whether or not neonatal Atamestan treatment has similar effects is unknown. Therefore, sexual behavior with an estrous female or with a sexually active male were studied, both during partner preference tests and during pair-tests. Finally, the effects of yohimbine, an α_2-receptor blocker with sexual behavior stimulating properties in intact and castrated male rats (6-8, 19), were investigated on adult sexual behavior of neonatally ATD or Atamestan treated and control males.

MATERIALS AND METHODS

Animals

Wistar albino rats were used; stimulus animals were F1 hybrids of two inbred Wistar strains (R x U). They were housed 2-4 to a cage, with water and food available ad lib. and kept in a 14 h light-10 h dark cycle (lights on 5.30 pm to 7.30 am). Temperature in the animal room ranged from 22 to 24 °C.

Treatments

Female rats were time mated. Between 5 and 9 h after birth small silastic implants (inner diameter 1.5 mm; outer diameter 2.1 mm; length 5 mm) were placed s.c. in the back of the newborn male and female pups under ice anaesthesia. The implants contained ATD (approximately 8 mg) or Atamestan (approximately 6 mg) or were left empty. The implants were removed when the pups were 21 days of age, they were weaned and housed 2-4 to a cage of same sex and treatment. At weaning, three experimental groups of males were formed: (1) controls (n=12; out of 5 litters); (2) neonatal ATD (neo-ATD; n=11; 8 litters); (3) neonatal Atamestan (neo-ATA; n=14; 2 litters). Most behavioral tests of these intact males

were carried out without any adult treatment, some tests were conducted after one or more additional injections with estradiol benzoate or progesterone (see test procedure for details). In one test yohimbine hydrochloride was injected intraperitoneally 25 min prior to testing at a concentration of 2 mg/ml in a volume of 0.1 ml distilled water/100 g body weight at a dose of 2 mg/kg.
Ovariectomized stimulus females were brought into behavioral estrus by injecting 30 µg estradiol benzoate (EB) 24–48 h prior to testing followed by 2.5 mg progesterone (P) 3 to 4 h prior to testing. Steroid hormones were dissolved in olive oil.

Behavior tests
Behavior tests were carried out during the dark period of the day in a dimly lit room and lasted 15 min.

Three compartment partner preference test. A test box made of gray perspex with transparent front was used (18) which had 3 compartments (60 x 30 x 40 cm each) with a small opening (13 x 12 cm) in both partitions near the front window. These openings could be closed by a sliding door. In the lateral compartments stimulus animals could be put. These, an estrous female and a sexually active male, were either tethered by a leather harness which was attached with a stainless steel wire to the rear of the compartment, or were without harness and placed behind a wire mesh separation halfway down the lateral compartments. Tethered animals had a limited action radius. They were adapted to the tethering device during one hour in the week before testing. When physical interaction was prevented by wire mesh, the experimental animal could only see, smell and hear the stimulus animal, but not interact behaviorally.
Before testing all three animals (one experimental, two stimulus animals) were put in the box, one in each compartment, with the sliding doors closed, for 15–20 min adaptation. At the beginning of the test the sliding doors were removed and the experimental animal could freely move around and interact with the stimulus animals or sit before the wire mesh separation. Time spent in each compartment was recorded. To quantify partner preference, a preference score was calculated for each test by subtracting the time spent in the compartment containing the sexually active male from time spent near the estrous female (method after Edwards and Pfeifle, 12). Thus, a positive score indicates

preference for the estrous female; a negative score preference for the sexually active male. In the tests in which interaction was possible also various sexual behaviors with the incentives were scored.

Pair test with estrous female. In these tests semicircular cages, measuring 62 x 40 x 36 cm, were used. The experimental male was put in the cage for a 5 min adaptation period, then a stimulus female was also brought in the cage and various sexual behaviors were scored.

Pair test with sexually active male. For these tests, carried out in semicircular cages, sexually active males were used. After a 5 min adaptation period of the stimulus male, the experimental male was put in the cage. The lordosis responses of the experimental males to the mounting of the stimulus male were recorded. The test lasted until the experimental male had received 10 mounts or at the longest 10 minutes. Since many males did not receive 10 mounts, we included in the analyses the lordosis quotients of those experimental males that received 3 or more mounts by the 'stud' male.

Test procedure

The males were subjected to 4 consecutive partner preference tests (at ages 12, 14, 16 and 17 weeks) in the 3-compartment-box. In test 1 sexual interaction was prevented by wire mesh; in tests 2-4 sexual interaction with the tethered stimulus animals was possible. A s.c. injection with progesterone (0.5 mg) was given 4-7 h prior to partner preference test 4. Also, 4 pair tests with an estrous female were carried out (S1-S4 at ages 18, 20, 22 and 29 weeks); tests S1 and S4 without any pretreatment, test S2 after 3 s.c. injections with 20 µg EB/day, test S3 25-30 min after one i.p. injection with 2 mg/kg Yohimbine. Lordosis behavior was studied in a pair test with a sexually active male (test L; age 19 weeks), without any treatment. Blood was collected from the orbital plexus under light ether anaesthesia at 30 weeks of age.

Hormone assay

Testosterone concentrations were estimated in serum by radioimmunoassay, without chromatography, using the method prevailing in our laboratory (e.g. 3). The interassay and intraassay coefficients of variation were 13.4 % and 5.6 %, respectively.

Statistics

One- or two-way ANOVA (16), followed by the least significant difference (LSD)-procedure (15) were used for analysing partner preference data. Differences in the number of animals displaying a certain type of behavior were analysed using Fisher's exact two-tailed probability analysis (16) or the Binomial test (17), when appropriate. All data are presented as mean ± SEM.

RESULTS

Partner preferences

Partner preference scores are depicted in fig. 1.

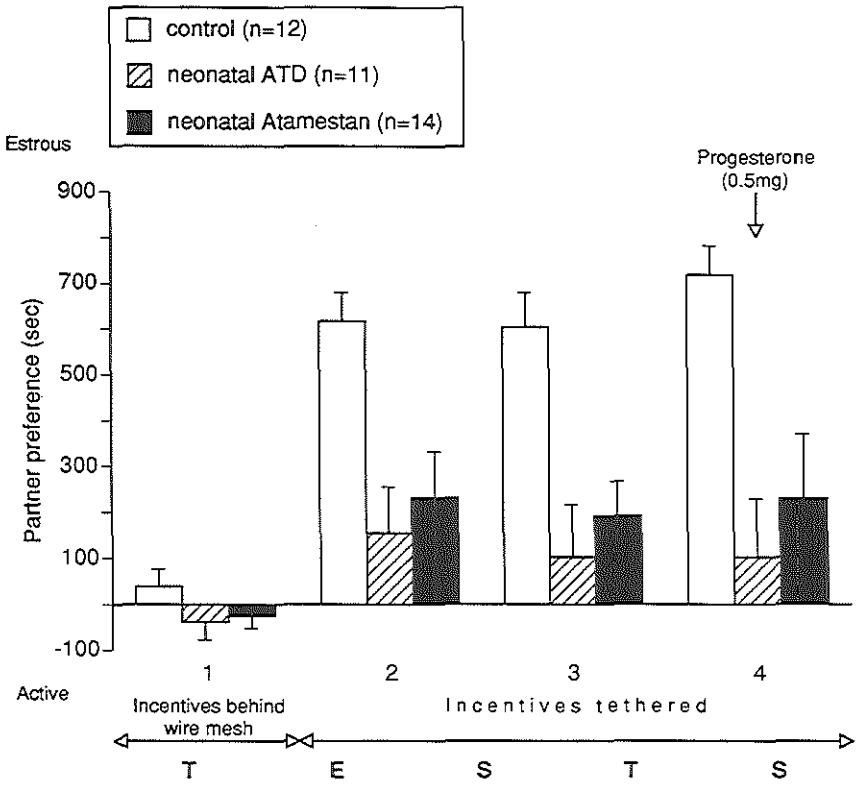

FIG. 1. Mean (± SEM) preference (seconds) for an estrous female over a sexually active male of intact adult male rats after neonatal ATD, Atamestan or control treatment. They were tested 4 times (15 min/ test) for partner preference behavior in a 3-compartment-box. Between 4-7 h before test 4 the experimental males were injected with 0.5 mg progesterone.

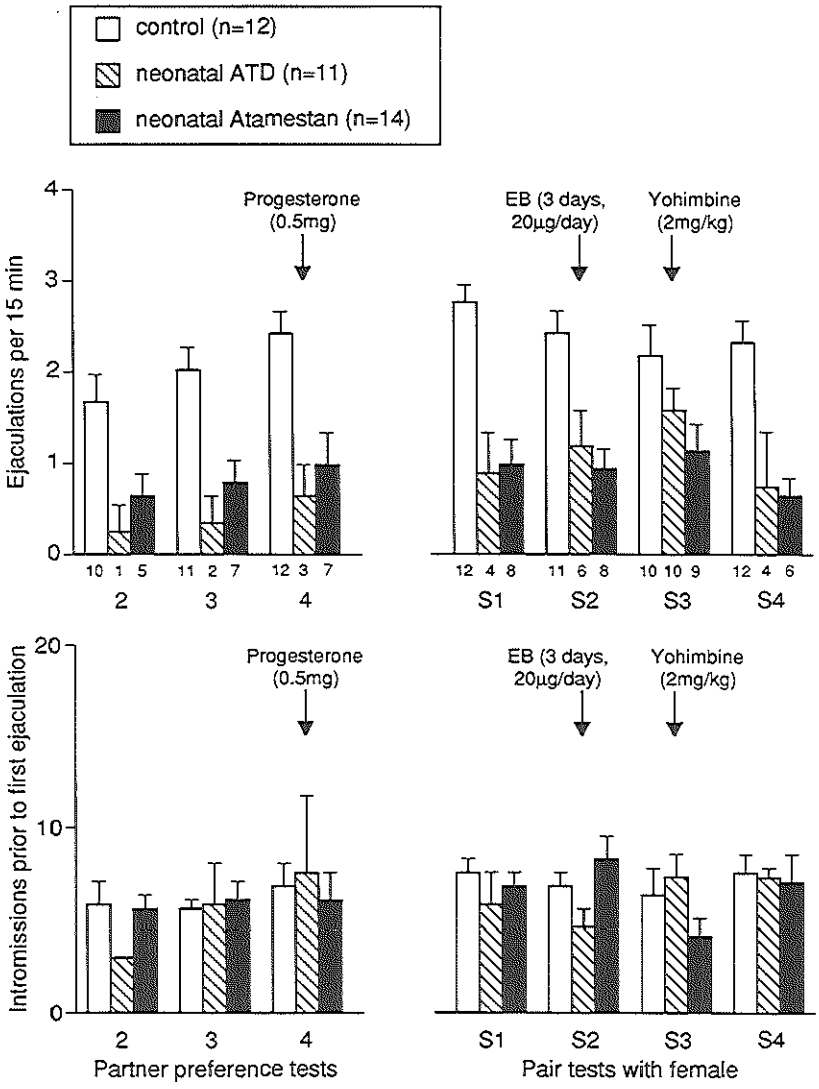

FIG. 2. Top. Mean (± SEM) ejaculation frequency during partner preference tests (2-4) and pair tests with an estrous female (S1-S4) of intact adult male rats after neonatal ATD, Atamestan or control treatment. The partner preference tests were carried out in a 3-compartment-box; the pair tests in a semicircular arena. Adult treatment consisted of progesterone (0.5 mg 4-7 prior to test) before test 5, EB (3 consecutive days before test, 20 µg/day) before test S2 and Yohimbine hydrochloride (i.p. 2 mg/kg 30 min prior to test) in test S3. The digits below the bars indicate the number of males per group ejaculating during the test.Bottom. Mean (± SEM) number of intromissions prior to the first ejaculation of the males that ejaculated during partner preference and pair tests.

One-way ANOVA of test 1 did not show a significant group difference ($F(2/34)=1.44$, n.s.). Two-way ANOVA of tests 2-4 revealed a significant group difference ($F(2/34)=11.32$, $p<0.0005$), no significant effect of tests ($F(2/68)=0.41$, n.s.), and no significant groups x tests interaction ($F(4/68)=0.28$, n.s.). Further analysis of the group difference (LSD(5%)=233.2 sec) showed that control males had significantly higher preference scores than neo-ATD and neo-ATA males; the latter two groups did not differ.

Sexual behavior with estrous female

Ejaculation frequency and number of intromissions prior to the first ejaculation during partner preference and pair testing are shown in fig. 2.

Fisher's exact two-tailed probability analysis, performed on the total number of males ejaculating at least once during partner preference tests 2, 3 and 4 (combined), showed that a lower number of neo-ATD and neo-ATA males ejaculated than controls (control vs neo-ATD: $p<0.001$; control vs neo-ATA: $p<0.02$). Neo-ATD and neo-ATA males did not differ.

Two-way ANOVA on the ejaculation frequencies displayed during pair testing, showed a significant group difference ($F(2/34)=17.66$, $p<0.0005$), no significant effect of tests ($F(3/102)=1.70$, n.s.), and no significant interaction ($F(6/102)=1.27$, n.s.). Further analysis of this group difference (LSD(5%)=0.65 ejac) showed that males neonatally treated with ATD or Atamestan had lower ejaculation frequencies than controls in all 4 pair tests.

Yohimbine only stimulated the number of neonatally ATD treated males that ejaculated: after a single injection (test S3) almost all ATD treated males ejaculated (10 out of 11), whereas in tests before and after Yohimbine (S1 and S4, respectively) only 4 out of 11 males ejaculated (10 of 11 vs 4 of 11, $p<0.001$, Binomial test). Such stimulatory effect of Yohimbine was not found in males neonatally treated with Atamestan or control males. Adult treatment with EB had no effect on any group. The number of intromissions prior to the first ejaculation was not affected by any neonatal or adult treatment.

Lordosis behavior with active male

Lordosis data are shown in fig. 3. In this experiment many males were not very attractive, i.e. they were not readily mounted by the stimulus male.

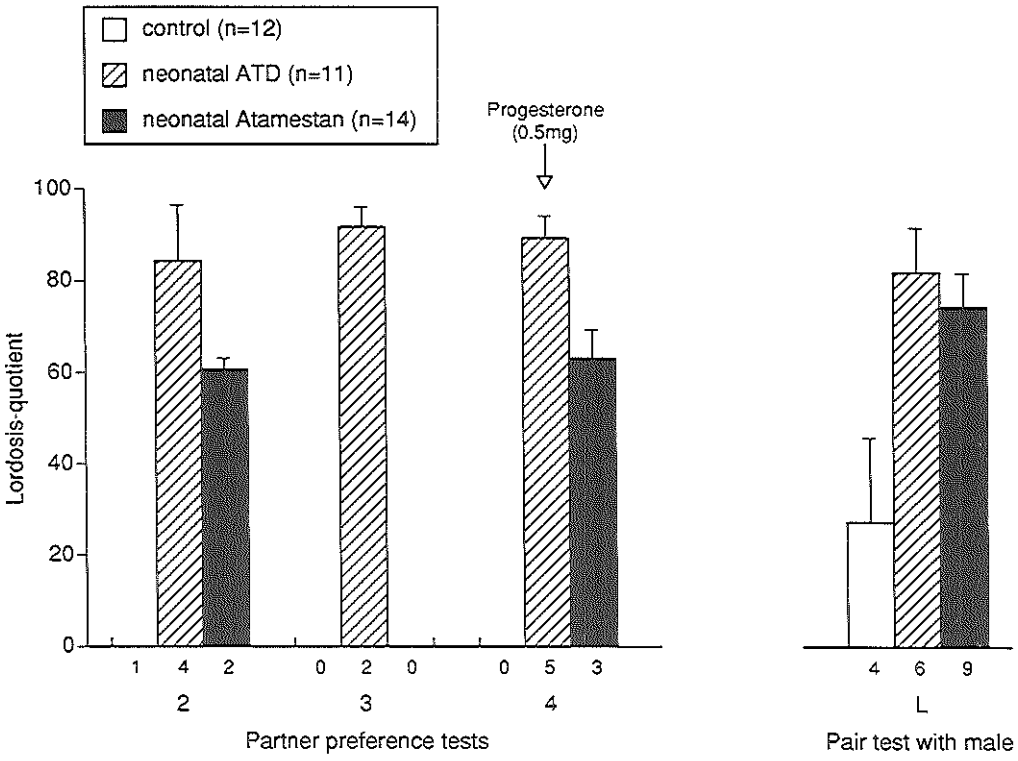

FIG. 3. Mean (± SEM) lordosis quotients of intact adult male rats during partner preference tests 2-4 and pair test with a sexually active male (test L) after neonatal ATD, Atamestan or control treatment. The pair test with the male was carried out in a semicircular arena. The digits below the bars indicate the number of males per group that were mounted 3 times or more by the sexually active male and that contributed to the calculated lordosis quotients. Test 4 was carried out 4-7 h after a s.c. injection with 0.5 mg progesterone.

The number of males that had received 3 or more mounts by the stimulus male in at least one of the partner preference tests 2-4 were: control: 1 of 12, ATD: 6 of 11, and Atamestan: 3 of 14. Fisher's exact two-tailed probability analysis did not show differences between groups (control vs neo-ATD, $p=0.07$; control vs neo-ATA, $p=1.00$; neo-ATD vs neo-ATA, $p=0.11$). It is remarkable that neo-ATD and neo-ATA males that were receptive, showed fairly high lordosis quotients; the only control male allowing mounts

from the stimulus male during partner preference testing did not show lordosis.
Fisher's exact two-tailed probability analysis on the number of males being mounted 3 times or more by the sexually active male (during test L) did not reveal statistically significant differences. One-way ANOVA on the lordosis quotients (of the responders) in test L showed a significant group difference ($F(2/16)=5.95$, $p<0.02$). Further analysis (LSD(5%)=35.8) showed that controls had lower lordosis quotients than neo-ATD and neo-ATA males; the latter two groups did not differ.

Hormone data
Testosterone levels at 30 weeks of age can be seen in table 1.

TABLE 1

Serum testosterone (T) levels in 30 weeks' old gonadally intact male rats which had been neonatally treated with ATD or Atamestan

Treatment	Number of samples	T-level (ng/ml) Mean ± SEM	Median
Control	11	2.1 ± 0.3	1.7
Neonatal ATD	9	1.5 ± 0.1	1.5
Neonatal Atamestan	13	1.5 ± 0.2	1.4

One-way ANOVA of the serum T levels showed no significant group difference ($F(2/30)=1.75$, n.s.). From these data it appears that perinatal ATD or Atamestan treatment had no effect on endogenous testosterone levels in these gonadally intact adult male rats.

DISCUSSION

In this study, carried out in gonadally intact male rats, the effects of neonatal ATD treatment on adult sexual orientation found earlier (4) were replicated: neonatally ATD treated males had significantly lower preference scores for an estrous female than controls. Neonatal treatment with Atamestan had similar effects on male sexual orientation. Thus, this study confirms the suggestion from our laboratory (4,5) that estradiol derived from testosterone plays a significant role in organizing adult

male rat sexual orientation. The present data differ from those reported by Davis et al. (10) who found no effects of neonatal ATD treatment on adult sexual orientation. As discussed earlier (4), the difference in effects between the two studies is probably due to the difference in the time of the onset of neonatal ATD treatment, day 2 in the Davis et al. experiment and between 5 and 9 h after birth in the present study.

As in our previous study (4), neonatal ATD treatment lowered ejaculatory behavior in adult males, both during partner preference and pair tests. Comparable results were obtained with neonatal Atamestan treatment. Adult treatment with EB failed to undo the effects of neonatal ATD or Atamestan on ejaculatory behavior. Treatment with Yohimbine significantly increased the number of males that ejaculated in the neo-ATD group, but not in the neo-ATA and control groups. High levels of ejaculatory behavior in the control group probably precluded observation of any stimulatory effects in this group. The stimulatory effect corroborates results obtained by Smith et al. (19), who found an increase in the percentage of gonadally intact adult male rats copulating to ejaculation after i.p. administration of 1.0, 2.0, or 4.0 mg/kg yohimbine.

Consistent with other studies (4,10,13) neonatal ATD treatment prevented defeminization of male rats by their own testicular secretions, i.e. ATD treated males showed somewhat more lordosis behavior than controls. Similar results were obtained in Atamestan treated males.

In the present study progesterone was unable to enhance lordosis behavior in adulthood. This is not in line with some investigations, in which it was reported that exogenous progesterone was able to enhance adult lordosis behavior in gonadally intact ATD treated (4) and control males (20). Procedural differences may account for this discrepancy: Van de Poll and van Dis pretreated their males with estradiol-benzoate, whereas in the present study we did not. Brand et al. (4) studied lordosis behavior in a semicircular cage, whereas in the present experiment this was done with tethered incentives. Adult progesterone treatment did not affect ejaculatory behavior and sexual orientation. The partner preference data of our male rats after progesterone differ from those obtained with female rats by de Jonge and van de Poll (11). These investigators found drastically increased choices for males (vs ovariectomized females) following progesterone injection in TP- or EB-treated

ovariectomized females in a Y-maze (sexual interaction with the incentives possible). Both sex and procedural differences may be involved.

Adult serum testosterone levels were not affected by neonatal ATD or Atamestan treatment. The mean serum T-levels in the present experiment are slightly below those reported for normal males [2.2-2.9 ng/ml; (9)] and those found in the previous study [2.2-2.4 ng/ml; (4)], but well above the level for maintaining normal male sexual behavior [(0.7 ng/ml; (9)].

In conclusion, the present data show that neonatal organization of adult partner preference (sexual orientation) and sexual behavior of male rats by estradiol, derived from endogenous testosterone, is disrupted by neonatal treatment with ATD or Atamestan (aromatase inhibitors, which prevent the conversion of T to E_2). Endogenous serum T-levels in adulthood were not affected by the neonatal treatment. Adult treatment with yohimbine was able to partially undo the effects of neonatal ATD treatment by increasing the number of males ejaculating; yohimbine had no such effect in neonatally Atamestan treated or control males. Adult estradiol treatment failed to affect male sexual behavior of any group. Progesterone treatment had no effect on behaviors displayed in a 3-compartment box: neither sexual orientation nor sexual interactions with the incentives were affected.

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Hoofdstuk 8.

8.1 Samenvatting

Hoofdstuk 1.

Inleiding

In dit hoofdstuk wordt de theoretische achtergrond van de in dit proefschrift beschreven experimenten besproken.

Wanneer een bronstig vrouwtje en een seksueel aktief mannetje worden samengebracht, vertoont het mannetje seksueel gedrag, bestaande uit beklimmingen, met of zonder bekkenstoten, intromissies en ejaculaties. Het vrouwtje vertoont bronstgedrag dat bestaat uit receptief gedrag, lordose genoemd, en diverse proceptieve (uitnodigende) gedragingen, waaronder oortrillen en presenteren. In één ovariële cyclus van 4 of 5 dagen vertonen vrouwelijke ratten dit bronstgedrag slechts gedurende ongeveer 12 uur in de nacht van ovulatie. Na ovariëctomie treedt dit bronstgedrag niet meer op. Toediening van oestradiol (E_2), al dan niet gecombineerd met progesteron (P), laat het seksuele gedrag weer terugkomen. Dit wordt de **activerende** werking van geslachtshormonen genoemd. Bij mannelijke ratten neemt het seksueel gedrag na castratie geleidelijk af. Eerst verdwijnen ejaculaties, vervolgens intromissies en tenslotte beklimmingen. Na toediening van testosteron (T) keren alle seksuele gedragingen vrij snel (1 à 2 weken) weer terug. Ook hier dus een **activatie** van het seksuele gedrag door geslachtshormoon.

Androgenen hebben ook een **differentiërende** werking (gedurende een zogenaamde kritische fase) op de ontwikkeling van in- en uitwendige geslachtsorganen. In de embryonale fase bezitten zowel het mannetje als het vrouwtje twee interne buissystemen, de gangen van Wolff en de buizen van Müller, en uitwendig een sinus urogenitalis en tuberculum genitale. Bij vrouwelijke individuen (met XX-geslachtschromosomen) groeit uit de buizen van Müller een baarmoeder, de eileiders en het bovenste eenderde deel van de vagina. Bij mannelijke individuen (met XY-geslachtschromosomen) verdwijnen de buizen van Müller onder invloed van MIF (Müllerian Inhibitory Factor) en groeien de buizen van Wolff onder invloed van testosteron uit tot de epididymis, ductus deferens en zaadblazen. De indifferente sinus urogenitalis blijft bij de vrouw nagenoeg ongewijzigd. Het tuberculum genitale ontwikkelt zich tot clitoris. Bij de man differentieert de sinus urogenitalis onder invloed van

dihydrotestosteron (DHT; door reductie ontstaan uit T) tot de prostaat en scrotum. Het tuberculum genitale ontwikkelt zich tot penis.

Testosteron, aanwezig gedurende een kritische periode, blijkt ook een **differentiërende** werking te hebben ten aanzien van het volwassen seksueel gedrag van proefdieren. Dit wordt ook wel de **programmerende** of **organiserende** werking van T genoemd. Vrouwelijke cavia's, prenataal blootgesteld aan T, worden geboren met vermannelijkte genitalia, vertonen in volwassenheid meer beklimgedrag na behandeling met T en minder lordosegedrag na behandeling met oestradiol (E_2) en progesteron (P) dan controle vrouwtjes. Latere onderzoeken bij de rat toonden aan dat het seksueel gedrag van mannetjes perinataal wordt geprogrammeerd door T; dit proces wordt vermannelijking of masculinisatie genoemd. Daarnaast blijkt een mannelijke rat grotendeels het vermogen te verliezen om "vrouwelijk" gedrag, waaronder lordosegedrag, te vertonen. Men spreekt van defeminisatie.

Volwassen vrouwelijke ratten vertonen vrij gemakkelijk beklimgedrag, al dan niet na behandeling met T, wanneer ze worden samengebracht met een bronstig vrouwtje. De vraag is of bij vrouwtjesratten, net als mannetjes, perinataal androgenen circuleren en of deze een programmerende werking hebben. Bij vrouwelijke ratten blijken inderdaad perinataal androgenen te circuleren. Uit onderzoek waarin vrouwelijke ratten perinataal werden blootgesteld aan anti-androgenen kwam de suggestie naar voren dat vrouwelijke ratten door hun eigen androgenen in zekere mate worden gemasculiniseerd (meer beklimgedrag) en in zekere mate worden gedefeminiseerd (minder lordosegedrag). De oorsprong van deze bij het wijfje circulerende androgenen was onduidelijk. Als mogelijke bronnen werden door verschillende onderzoekers genoemd: de broertjes in dezelfde uterushoorn, de bijnieren of de placenta. Sommige onderzoekers hebben een masculiniserend effect gevonden van naastgelegen broertjes in utero, andere rapporteerden alleen een effect van caudale (meer naar de cervix uteri gelegen) broertjes in utero. Weer andere onderzoekers vonden geen effect van prenatale nestsamenstelling op volwassen beklimgedrag van vrouwtjes.

Bij de masculinisatie en defeminisatie blijkt E_2, door aromatisatie gevormd uit T, een belangrijke rol te spelen. Dit kan geconcludeerd worden uit studies met vrouwelijke ratten welke E_2 kregen toegediend of mannelijke ratten waarin de vorming van E_2 uit T werd geblokkeerd met behulp van aromatase-remmers.

Het meeste onderzoek bij ratten is verricht naar de activerende en programmerende werking van T en metabolieten (DHT en E_2) op het seksueel gedrag. Tot voor kort werd weinig aandacht besteed aan mogelijke activatie en programmering van pre-copulatoire gedragingen, waaronder partner preferentiegedrag. Voor het meten van partner keuzegedrag zijn diverse testapparaten

ontwikkeld met stimulusdieren in kooitjes. Hierbij is seksuele interactie tussen het experimentele en stimulusdier soms mogelijk of wordt deze voorkómen door metaalgaas. In zulk type onderzoek wordt een preferentie-score berekend, waarbij de tijd doorgebracht in de nabijheid van het ene stimulusdier verminderd wordt met de tijd doorgebracht bij het andere stimulusdier. Dit levert dan een maat voor de preferentie op. Enkele onderzoekers vonden dat partner preferentie of partner keuzegedrag van volwassen mannelijke en vrouwelijke ratten wordt **geactiveerd** door geslachtshormonen. Hierbij bleek E_2, al dan niet afkomstig van T, een belangrijke rol te spelen.

Slechts enkele onderzoeken zijn verricht naar de perinatale **programmerende** werking van T of E_2 op volwassen partner preferentie van mannelijke en vrouwelijke ratten. Bij vrouwtjesratten bleek neonataal toegediend T in staat volwassen partner preferentie te organiseren: vrouwtjes neonataal behandeld met T hadden een meer uitgesproken voorkeur voor een bronstig vrouwtje (versus een seksueel aktief mannetje) dan controles. Bij mannelijke ratten werden weinig aanwijzingen gevonden, dat neonataal T, al dan niet via de E_2-metaboliet, volwassen partner preferentie programmeerde.

Sinds enkele jaren is bekend dat de hersenen van mannelijke en vrouwelijke ratten in histologisch opzicht verschillen. Electronenmicroscopisch onderzoek toonde aan dat de dendrieten van zenuwcellen in de area preoptica van vrouwelijke ratten meer synapsen hadden dan die van mannetjes. Neonatale castratie van mannelijke ratten creëerde een vrouwelijk aantal synapsen; neonatale toediening van T aan vrouwtjes veroorzaakte een mannelijk aantal synapsen. Eind 70-er jaren werd voor het eerst een seksueel dimorfe kern in de area preoptica (SDN-POA) van de rat beschreven, die met de lichtmicroscoop waarneembaar was. Het volume van deze kern bleek bij mannetjes circa 5 tot 8 maal zo groot te zijn als bij vrouwtjes. Neonatale castratie verminderde het volwassen volume; neonatale toediening van T aan vrouwtjes deed een "mannelijk" volume ontstaan. De SDN-POA lijkt betrokken te zijn bij het beklimgedrag van mannelijke en vrouwelijke ratten, aangezien lesies het gedrag doen afnemen. Voor de expressie van bronstgedrag van vrouwelijke ratten lijkt de SDN-POA van gering belang.

Hoofdstuk 2.

Vraagstellingen in het huidige onderzoek

1 Is het volwassen door testosteron geïnduceerd beklimgedrag van vrouwelijke ratten geprogrammeerd door perinataal circulerend testosteron (T)?

2 Speelt de prenatale ligging ten opzichte van broertjes hierbij een rol?
3 Wordt de volwassen partner preferentie van vrouwelijke ratten geprogrammeerd door perinataal aanwezig T?
4 Wordt de volwassen partner preferentie van mannelijke ratten geprogrammeerd door perinataal T en/of dihydrotestosteron (DHT) en oestradiol (E_2)?
5 Welke effecten hebben 8-OH-DPAT en yohimbine (bekende ratten-"aphrodisiaca") op partner preferentie en seksueel gedrag van mannelijke ratten?

Hoofdstuk 3.

Gebruikte stoffen en hun werking

Het werkingsmechanisme van testosteron wordt besproken. De werking van T kan beïnvloed worden door de androgeenreceptor te blokkeren met anti-androgenen (cyproteron-acetaat, dit heeft een steroïdale structuur, anandron of flutamide, beide met een niet-steroïdale structuur) of door de omzetting van T naar E_2 te blokkeren met aromatase-remmers (ATD of atamestan). In een aantal onderzoeken zijn stoffen gebruikt die het seksueel gedrag van ratten kunnen stimuleren: yohimbine, een α_2-adrenerge receptor antagonist en 8-OH-DPAT, een serotonerge $5HT_{1A}$ receptor agonist. De anti-androgenen, de aromatase remmers, alsmede de "aphrodisiaca" worden uitgebreid besproken.

Hoofdstuk 4.

Proefdieren, materialen en methoden

In dit hoofdstuk komen de experimentele details aan de orde. Thans beperken we ons tot bespreking van het automatisch open veld en de 3-compartimenten-kooi. In het automatisch open veld bevinden 2 stimulusdieren zich in 2 perspex kooitjes aan de wand van het open veld. Het experimentele en stimulusdier kunnen elkaar wel horen, zien en ruiken, maar seksuele interactie wordt voorkomen door metaalgaas.
In de 3-compartimenten-kooi bevinden de stimulusdieren zich in de zijcompartimenten. Deze dieren dragen een harnasje dat bevestigd is aan de achterwand van het compartiment. Deze constructie zorgt ervoor dat de stimulusdieren tot ongeveer halverwege kunnen komen. Ook kan halverwege de zijcompartimenten een schot geplaatst worden om seksuele interactie te voorkomen.

Om partner preferentie te kwantificeren wordt voor elk dier per test een preferentiescore berekend: de tijd doorgebracht voor het ene kooitje of in het ene compartiment, verminderd met de tijd doorgebracht voor het andere kooitje of in het andere compartiment. Deze verschilscore is een maat voor partner preferentie.

Hoofdstuk 5.

Perinatale androgenen en volwassen seksueel gedrag van vrouwelijke ratten

De relatie tussen volwassen door T geïnduceerd beklimgedrag van vrouwelijke ratten en perinataal circulerend T is onderzocht door vrouwtjes prenataal gedurende de tweede helft van de zwangerschap (dag 11-22) en/of neonataal (dag 1-10) bloot te stellen aan een anti-androgeen. Er werden drie verschillende anti-androgenen gebruikt: cyproteron-acetaat, anandron en flutamide. Cyproteron-acetaat en anandron werden alleen prenataal toegediend; flutamide pre- en/of neonataal. Op volwassen leeftijd werden de vrouwtjes geovariëctomeerd en getest voor beklimgedrag met een bronstig vrouwtje voor en tijdens behandeling met T. Geen van de drie anti-androgenen had effect op het T-geïnduceerd beklimgedrag van Wistar vrouwtjes; nagenoeg alle dieren beklommen met vrij hoge frequenties (ca. 20-30 beklimmingen per 15 min). Perinatale blootstelling aan flutamide had ook geen effect op het (T-geïnduceerde) lordosegedrag. De anti-androgenen hadden wel duidelijke somatische effecten bij mannelijke jongen: bij hen was de differentiatie van de uitwendige genitalia achterwege gebleven. Voor de prenataal aan anti-androgeen blootgestelde dieren was het bij geboorte niet mogelijk het geslacht te bepalen, alle dieren leken van het vrouwelijk geslacht.

Aangezien door andere onderzoekers na prenatale blootstelling aan flutamide een vermindering van het volwassen beklimgedrag was gerapporteerd van andere dan Wistar vrouwtjes, werd door ons een experiment uitgevoerd waarbij Sprague Dawley ratten werden gebruikt. Er werd geen effect gevonden van alleen prenatale of alleen neonatale behandeling. Alleen vrouwtjes die zowel pre- als neonataal behandeld waren met flutamide vertoonden minder beklimgedrag dan controles. Het lordosegedrag van deze vrouwtjes was niet veranderd.

De resultaten van deze onderzoeken suggereren dat over het algemeen perinatale androgeen werking geen belangrijke rol speelt bij de perinatale organisatie van volwassen beklim- en lordosegedrag van vrouwtjesratten. Er lijkt een stamverschil in gevoeligheid voor perinataal T te bestaan.

Prenatale broertjes

De rol van broertjes in utero werd op verschillende manieren onderzocht. In een eerste experiment werd voorafgaande aan de zwangerschap één uterushoorn verwijderd. In een tweede experiment werd voorafgaande aan de zwangerschap één uterushoorn verwijderd en werd op dag 9 van de zwangerschap het aantal foeten gereduceerd tot 3. In het derde experiment werd het aantal foeten op dag 11 van de zwangerschap gereduceerd tot 1 per uterushoorn. De bevallingen in de eerste twee experimenten werden geobserveerd om zo de exacte prenatale ligging te kunnen bepalen. De vrouwelijke nakomelingen werden op volwassen leeftijd geovariëctomeerd en getest voor T-geïnduceerd beklimgedrag. Er werd geen stimulerend effect gevonden van naastgelegen broertjes op het beklimgedrag; de beklimfrequenties van deze vrouwtjes verschilden niet van die van vrouwtjes zonder naastgelegen broertjes. Wanneer de vrouwtjes anders werden geclassificeerd, namelijk met of zonder broertje aan de caudale (cervix uteri) kant werd een gering doch significant 'masculiniserend' effect gevonden van caudaal gelegen broertjes: vrouwtjes met één of meer caudale broertjes hadden een iets hogere beklimfrequentie dan vrouwtjes met één of meer broertjes aan de kant van het ovarium. In een vierde experiment, eveneens beschreven in dit hoofdstuk, werd ook een 'masculiniserend' effect gevonden van caudale broertjes.
Uit de resultaten van deze onderzoeken blijkt dus een masculiniserend effect van caudaal in de uterus gelegen broertjes. Dit effect is slechts additief, getuige het feit dat ook vrouwtjes zonder caudale broertje(s) hoge beklimfrequenties vertonen.

Hoofdstuk 6.

Perinatale programmering van volwassen partner preferentie van vrouwelijke ratten

In twee experimenten werden vrouwelijke ratten gedurende de dagen 11-22 van de zwangerschap behandeld met een anti-androgeen: flutamide in experiment 1 en anandron in experiment 2. Op volwassen leeftijd werden de vrouwtjes geovariëctomeerd en voor en tijdens behandeling met T getest in een automatisch open veld voor hun partner preferentie voor een bronstig vrouwtje of voor een seksueel aktief mannetje. De prenatale anti-androgene behandeling had geen invloed op de partner preferentie van deze ratten. Onverwacht werd een effect op partner voorkeur gevonden van seksuele ervaring met een bronstig vrouwtje. Vóór de seksuele ervaring was de partner preferentie indifferent of gering voor het vrouwtje, daarna zeer uitgesproken voor het bronstige vrouwtje. In een derde experiment werden vrouwelijke ratten tussen 6 en 14 uur na geboorte en verder gedurende 10 dagen behandeld met TP, DHTP of olie

en op volwassen leeftijd getest voor partner keuzegedrag. Vrouwtjes, neonataal behandeld met TP, hadden een significant hogere preferentie voor het bronstig vrouwtje dan neonataal met DHTP of olie behandelde vrouwtjes; de laatste 2 groepen verschilden onderling niet.
Partner preferentie van vrouwelijke ratten kan dus "geprogrammeerd" worden door neonatale behandeling met TP. Waarschijnlijk wordt dit effect veroorzaakt via omzetting in E_2, aangezien DHTP dit effect niet had.

Hoofdstuk 7.

Perinatale programmering van partner preferentie en seksueel gedrag van mannelijke ratten

In een eerste experiment werden mannelijke ratten tussen 6 en 14 uur na de geboorte gecastreerd en vervolgens vanaf de geboorte gedurende 10 dagen behandeld met TP, DHTP of olie. Een controle groep werd pas op volwassen leeftijd gecastreerd. Partner preferentie werd op volwassen leeftijd getest in een automatisch open veld of in een 3-compartimenten-kooi. De keuzemogelijkheden waren tussen een bronstig en een niet-bronstig vrouwtje, en tussen een bronstig vrouwtje en een seksueel aktief mannetje. Na langdurige behandeling op volwassen leeftijd met onder de huid geïmplanteerd DHT of DHT+E_2 vertoonden de mannetjes geen duidelijke voorkeur wanneer seksuele interactie niet mogelijk was (in het automatisch open veld). In de 3-compartimenten-kooien, wanneer seksuele interactie met de stimulusdieren mogelijk was, ontstond een duidelijke voorkeur voor het bronstige versus het niet-bronstige vrouwtje. De neonataal gecastreerde en met DHTP of olie behandelde mannetjes hadden een significant lagere preferentie voor het bronstige vrouwtje dan neonataal gecastreerde en met TP behandelde mannetjes of de mannetjes die pas op volwassen leeftijd gecastreerd waren. Zes weken na verwijdering van de implantaten, vertoonden de mannetjes geen duidelijke preferentie meer voor een bronstig vrouwtje versus een seksueel aktief mannetje. Na daaropvolgende behandeling met T gedurende 3 weken vertoonden alle mannetjes een voorkeur voor het bronstige vrouwtje. De neonataal met DHTP of olie behandelde mannetje hadden lagere preferentie-scores dan de neonataal met TP behandelde of pas later gecastreerde mannetjes.
Uit deze resultaten blijkt dat neonataal testosteron, mogelijk via oestradiol, volwassen partner preferentie van mannetjes programmeert.
In andere experimenten werden mannelijke ratten pre- en/of neonataal blootgesteld aan een aromatase-remmer. Bij prenatale behandeling met ATD werd de moeder gedurende de dagen

11-22 van de zwangerschap geïnjecteerd of werd op dag 11 van de zwangerschap een siliconen rubber buisje gevuld met ATD geïmplanteerd, dat direct na de bevalling weer werd verwijderd. Voor neonatale behandeling werd tussen 5 en 9 uur na geboorte een klein siliconen rubber buisje gevuld met ATD of atamestan s.c. geïmplanteerd (onder verdoving met ijs). Dit buisje werd er op dag 10 of 21 weer uitgehaald.
Op volwassen leeftijd werden deze mannetjes in een 3-compartimenten kooi getest voor hun partner preferentie voor een bronstig vrouwtje dan wel een seksueel aktief mannetje (seksuele interactie wel of niet mogelijk).
In de eerste tests was geen voorkeur of een geringe preferentie voor het seksueel aktieve mannetje; dit laatste vooral bij de neonataal met ATD behandelde mannetjes. Gedurende opeenvolgende tests (ook wanneer seksuele interactie werd voorkomen) werd de voorkeur voor het bronstige vrouwtje steeds duidelijker, zowel in de behandelde als in de controle dieren. Echter, deze voorkeur voor het bronstige vrouwtje is significant minder van de neonataal behandelde mannetjesratten ten opzichte van controles. Prenatale ATD behandeling had dit effect niet. Neonataal met ATD behandelde mannetjes waren zowel minder gemasculiniseerd (d.w.z. ze vertoonden minder ejaculaties), als minder gedefeminiseerd (ze vertoonden meer lordosegedrag) dan controles.
Er kan geconcludeerd worden dat volwassen partner preferentie en seksueel gedrag van mannelijke ratten neonataal wordt geprogrammeerd, vermoedelijk door endogeen E_2 afkomstig van T door aromatisering.

8-OH-DPAT en yohimbine

Toediening van 8-OH-DPAT aan ATD behandelde of controle mannetjes leidde tot een verhoging van de preferentiescores voor het bronstige vrouwtje in alle groepen. Het was niet in staat het effect van neonataal ATD op te heffen: deze mannen hadden nog steeds lagere preferentiescores dan prenataal met ATD behandelde en controle mannetjes. Na een hoge dosis 8-OH-DPAT vertoonden alle (ook ATD behandelde) mannen één of meer ejaculaties. Bij gebruik van de hoge dosis DPAT ejaculeerde circa 80% van de dieren bij de eerste intromissie. Behandeling met yohimbine deed wel het percentage dieren stijgen dat ejaculeerde in de neonataal met ATD behandelde mannen, maar stimuleerde niet de ejacluatiefrequentie.
Samenvattend kan gezegd worden dat ratte-"aphrodisiaca" in staat lijken de perinataal, geprogrammeerde effecten van aromatase-remmers op volwassen partner preferentie en seksueel gedrag van mannelijke ratten tijdelijk in belangrijke mate teniet te doen.

8.2 Summary

Chapter 1.

Introduction

In this chapter, a theoretical background to the experiments described in this thesis is presented.

When an estrous female is paired with a sexually active male, the male shows mounting behavior, with or without pelvic thrusts, intromissions and ejaculations. The female displays receptive behavior (lordosis) and proceptive behavior (ear wiggling and presenting). Proceptive behavior is assumed to have sexually stimulating properties for the male. A female is said to be estrous (= in heat) when she displays proceptive and receptive behaviors. In the course of the ovarian cycle female rats are only in heat for approximately 12 h in the night of ovulation. Ovariectomy eliminates estrous behaviors completely; treatment with estradiol (E_2), with or without additional progesterone (P), restores these behaviors. This is called the **activating** role of gonadal steroids. Sexual behavior in male rats gradually decreases after castration, first ejaculations disappear, then intrommissions and finally mounts. Treatment with testosterone (T) quite rapidly restores sexual behavior to precastration levels. Thus, in male rats sexual behavior is also **activated** by gonadal hormones.

Androgens have a **differentiating** action on the development of the internal and external genitalia. During fetal development both male and female individuals have two internal genital duct systems, the Wolffian ducts and the Müllerian ducts, and externally an undifferentiated sinus urogenitalis and tuberculum genitale. In female individuals (with XX sex chromosomes) the Müllerian ducts develop into a uterus, oviducts and the upper third of the vagina. In male individuals (with XY sex chromosomes) the gonads differentiate into testes, which produce MIF (Müllerian Inhibitory Factor), causing the Müllerian ducts to regress, and T, causing the Wolffian ducts to develop into epididymides, deferent ducts and seminal vesicles. The undifferentiated urogenital sinus does not change very much in the female. The genital tubercle develops into a clitoris. In the male the urogenital sinus develops into prostate and scrotum under the influence of dihydrotestosterone (DHT), the 5α-reduced metabolite of T. The genital tubercle develops into a penis.

Testosterone has a **differentiating** action on adult sexual behavior of rats. This is called the **programming** or **organizing** action of T. Sexual behavior of the male rat is organized perinatally. This process is called masculinization. The capacity to easily show "female"

behaviors, like lordosis, is for the most part lost during this process and this is called defeminization.

Female rats, treated with T in adulthood, readily display mounting behavior when paired with an estrous female. The question is whether female rats have perinatally circulating androgens, like males do, and whether these androgens "organize" adult sexual behavior (like they do in males). It has been found that female rats have indeed perinatally circulating androgens. From studies in which female rats were perinatally exposed to antiandrogens it was learnt that female rats were somewhat masculinized (showed more mounting behavior) and defeminized (showed less lordosis behavior) by their own androgens. The source of theseandrogens in the female rat is not quite clear. Brothers in the same uterine horn, their own adrenals and the placenta have been suggested by various authors as possible sources. Some researchers found a masculinizing effect of contiguous males in utero, others only found an effect of caudal males (situated nearer to the cervix uteri). Others, again, have not found any effect of prenatal litter composition on adult sexual behaviors of female rats.

Estradiol, derived from T through aromatization, has been shown to play a very significant role in masculinization and defeminization of sexual behaviors in rats. This conclusion is derived from studies in which E_2 was administered to female rats or in which the conversion of T to E_2 was blocked by aromatase inhibitors in male rats.

Most research about the activation and organization of sexually dimorphic behaviors has concentrated on consummatory reproductive behaviors. Less attention has been paid to the possible activation and organization of precopulatory behaviors, like partner preference behavior. To investigate partner preference behavior several apparatuses with stimulus animals in wire mesh cages have been designed. In some experiments sexual interaction was possible whereas in others physical contact was prevented by wire mesh. Usually a preference score is calculated in which the amount of time spent in the vicinity of one stimulus animal is subtracted from the time spent with the other. Partner preference behavior in adult male and female rats is **activated** by gonadal hormones. Estradiol, presumably derived from T, is important in this respect.

Relatively little is known about the **organizing** effects of T or E_2 on adult partner preference behavior. In female rats, neonatally administered T was able to organize adult partner preference; they had higher preference scores for an estrous female (versus a sexually active male) than controls. No clearcut organizing effects of neonatal T or E_2 has been published on adult partner preference behavior in male rats.

Chapter 2.

Aims of the present study

1. Is adult testosterone-induced mounting behavior of female rats organized by perinatally circulating testosterone (T)?
2. What role does the prenatal position in utero relative to a brother play in this respect?
3. Is adult partner preference behavior of female rats organized by perinatal T?
4. Is adult partner preference behavior of male rats organized by perinatal T, DHT or E_2?
5. What effects do 8-OH-DPAT and yohimbine (well known rat "aphrodisiacs") have on partner preference and sexual behavior in the male rat?

Chapter 3.

Substances used and their mode of action

The biomolecular mode of action of T is described. The action of T can be affected by blocking the androgen receptor with antiandrogens (cyproterone-acetate which has a steroidal structure, anandron or flutamide, both non-steroidal antiandrogens) or by blocking the conversion of T to E_2 through aromatase inhibitors (ATD or atamestan). In a number of experiments sexual behavior stimulating drugs have been used, yohimbine, an α_2-adrenergic receptor antagonist and 8-OH-DPAT, a serotonergic $5HT_{1A}$ receptor agonist. The antiandrogens, the aromatase inhibitors and the "aphrodisiacs" are described.

Chapter 4.

Animals, materials and methods

In this chapter the various experimental designs are described. Along opposite sides of an automated open field, 2 stimulus animal are placed in 2 perspex cages. An experimental animal is placed in the open field proper. The experimental and stimulus animals are able to hear, see and smell each other, but sexual interaction between the expeirmental animal and either of the stimulus animals is prevented by wire mesh.

In a 3-compartment-box stimulus animals are placed in the lateral compartments. These animals wear a leather harness connected with a stainless steel string and a paperclip to the rear of the compartment. This limits the radius of action of the animals to halfway the compartment. An experimental animal is free to move from one compartment to the other. A wall made of

gray perspex and wire mesh could be placed halfway down the lateral compartments to prevent sexual interaction.
To quantify partner preference a preference score is calculated in which time spent in the vicinity of (or in the compartment containing) one stimulus animal is subtracted from time spent in the vicinity of or in the compartment containing the other stimulus animal.

Chapter 5.

Perinatal androgens and adult sexual behavior in female rats

The relationship between adult T-induced mounting behavior and perinatally circulating T was investigated by treating female rats prenatally (days 11-22 of pregnancy) and/or neonatally (days 1-10) with an antiandrogen. Three antiandrogens were used, cyproterone-acetate, anandron and flutamide. Cyproterone-acetate and anandron were given prenatally; flutamide pre- and/or neonatally. In adulthood, the females were ovariectomized and tested for mounting behavior with an estrous female before and during treatment with T. None of the three antiandrogens affected adult T-induced mounting behavior in Wistar females, nearly all animals mounted with fairly high frequencies (about 20-30 mounts per 15 min). Perinatal exposure to flutamide had no effects on (T-induced) lordosis behavior. The antiandrogens had clear somatic effects in male pups: the normal differentiation of the external genitalia had not occurred. Animals, prenatally exposed to antiandrogen, could not be separated by sex at birth by visual inspection since they all looked like females.
Other researchers have found that prenatal exposure to flutamide resulted in lower adult mount frequencies in other than Wistar females. Therefore, an experiment was carried out in which Sprague Dawley rats were exposed to flutamide. Pre- or neonatal treatment had no effect. Pre- plus neonatal flutamide treatment significantly lowered adult T-mounting behavior in Sprague Dawley females. Lordosis behavior of these females was not affected.
The results of these investigations suggest that in general perinatal androgens are not very important for the organization of adult mounting and lordosis behavior in female rats. A strain difference in sensitivity for perinatal T is apparent.

Prenatal brothers

The influence of the prenatal position relative to brothers in utero on adult sexual behavior in female rats was investigated. In the first experiment one uterine horn was removed before pregnancy. In the second experiment one uterine horn was removed before pregnancy and the

number of fetuses was reduced to 3 in the other uterine horn on day 9 of pregnancy. In the third experiment the number of fetuses was reduced to 1 in each uterine horn on day 11 of pregnancy. The deliveries were observed in experiment 1 and 2 in order to obtain the prenatal position in utero. Females were ovariectomized in adulthood and tested for T-induced mounting behavior. No masculinizing effects of contiguous brothers in utero were found on adult mounting behavior: females with an adjacent brother did not display higher mount frequencies than females with no adjacent brother(s). When females were classified according to the position of the male brother(s), on the caudal or on the ovarian side, it was found that females with one or more caudal brothers were somewhat 'masculinized' by their brother(s): they had significantly higher mount frequencies than females with one or more brothers on the ovarian side. In experiment 4 it was found that females with males located caudally in the same uterine horn were more masculinized than females without such males. Adjacent males had no influence on the behavioral sexual differentiation of females. The results of this experiment confirm the "caudal male hypothesis" rather than the "contiguity hypothesis". Hemihysterectomy during pregnancy prevented the "caudal male effect". Birth through caesarean section did not interfere with the caudal male effect.

The results of these experiments show a masculinizing effect of brothers located caudally in utero. However, this effect is only additive, since females without caudal brother(s) already displayed fairly high mount frequencies.

Chapter 6.

Perinatal organization of adult partner preference in female rats

In two experiments female rats were treated with an antiandrogen during days 11-22 of pregnancy, flutamide in experiment 1 and anandron in experiment 2. In adulthood, the females were ovariectomized and tested for partner preference behavior for an estrous female or a sexually active male in an automated open field. Prenatal antiandrogen treatment had no effect on adult partner preference in these females. Unexpectedly, an effect of sexual experience with an estrous female on partner preference was found. Before sexual experience the females showed no preference or a slight preference for the estrous female, afterwards they clearly preferred the estrous female. In a third experiment female rats were treated with TP, DHTP or oil between 6 and 14 h after birth and subsequently during 10 days and tested for partner preference in adulthood. The neonatally TP treated females had significantly higher preference scores than the neonatally DHTP or oil treated females. The latter two groups did not differ.

The results of this experiment show that partner preference behavior of females can be organized by neonatally administered T. This effect is presumably caused by E_2, since DHTP had no effect.

Chapter 7.

Perinatal organization of adult partner preference and sexual behavior in male rats

In a first experiment male rats were castrated between 6 and 14 h after birth and subsequently treated with TP, DHTP or oil from the day of birth during 10 days. One group of males was castrated in adulthood. In adulthood, partner preference was tested in an automated open field or in a 3-compartment-box. The choices were between an estrous female and a non-estrous female or between an estrous female and a sexually active male. After long-term treatment with DHT or DHT and E_2 in adulthood male rats did not show a clear preference for either incentive when sexual interaction was prevented (in an automated open field). In 3-compartment-boxes, which allowed sexual interaction between experimental and stimulus animals, a clear preference developed for the estrous female over the non-estrous female. Male rats neonatally castrated and DHTP or oil treated had significantly lower preference scores for the estrous female than neonatally castrated and TP treated or in adulthood castrated males. Six weeks after removal of the DHT and E_2 implants, the castrated male rats did not prefer either incentive. After three weeks of T-treatment all castrated males preferred the estrous female. Neonatally DHTP or oil treated males displayed significantly lower preference scores for the estrous female than the neonatally TP or control males.

The results of this experiment indicate that endogenous neonatal T, probably through E_2, organizes adult partner preference of male rats.

In other experiments male rats were pre- and/or neonatally treated with an aromatase inhibitor. For prenatal ATD treatment pregnant females were injected during days 11-22 of pregnancy, or were given on day 11 of pregnancy a silicone rubber implant, filled with ATD, which was removed immediately after birth. For neonatal treatment a small silicone rubber tube filled with ATD or atamestan was placed s.c. on the back between 5 and 9 h after birth, under ice anaesthesia. The implant was removed on day 10 or 21. In adulthood, the males were tested in a 3-compartment-box for preference for an estrous female or a sexually active male, while sexual interaction was possible in some tests but not in others.

In general, in the initial tests the males displayed no preference or a slight preference for the sexually active male, especially the ATD treated males. In consecutive tests, even when sexual

interaction was prevented, increasing preference scores for the estrous female emerged in aromatase inhibitor treated and control males. Males rats, neonatally treated with ATD or atamestan, had lower preference scores for an estrous female than controls. Prenatal ATD treatment had no effect on partner preference. Neonatally ATD treated males were less masculinized (i.e. had fewer ejaculations) and were less defeminized (i.e. showed more lordosis behavior) than controls. It is concluded that adult partner preference and sexual behavior of male rats is organized neonatally, presumably by endogenous E_2 derived from T through aromatization.

8-OH-DPAT and yohimbine

Administration of 8-OH-DPAT to ATD-treated or control males resulted in partner preference scores higher for the estrous female than for the sexually active male in all groups. However, 8-OH-DPAT was unable to undo the effect of neonatal ATD; neonatally ATD treated males still had lower preference scores for an estrous female than prenatal ATD treated and control males. After a high dose of 8-OH-DPAT all (including ATD-treated) males had one or more ejaculations; about 80% of these ejaculated during the first intromission. Treatment with yohimbine increased the percentage of males that ejaculated in the neonatally ATD treated group, but failed to enhance the ejaculation frequency.

In conclusion, rat-"aphrodisiacs" seem to be able to temporarily undo the detrimental organizational effects of perinatally administered aromatase inhibitors on adult partner preference and sexual behavior in male rats.

Hoofdstuk 9.

9.1 Conclusies

Perinatale androgenen en beklimgedrag

Door testosteron geactiveerd beklimgedrag (T-beklimgedrag) van volwassen vrouwelijke Wistar ratten lijkt niet geprogrammeerd te worden door perinataal aanwezige endogene androgenen. Immers, perinatale blootstelling aan anti-androgenen heeft op geen enkele wijze effect op volwassen T-beklimgedrag. Overigens, de broertjes van deze vrouwtjes zijn heel duidelijk geremd in hun genitale ontwikkeling, hetgeen een overtuigend bewijs is dat de anti-androgenen de foeten bereikten.

Literatuurgegevens suggereren dat prenatale blootstelling aan anti-androgenen een verlagend effect heeft op T-beklimgedrag van vrouwelijke ratten (Stewart, Pottier & Kaczender-Henrik, 1971; Ward & Renz, 1972; Clemens, Gladue & Coniglio, 1978). Deze experimenten werden echter met andere dan Wistar ratten uitgevoerd. Wellicht spelen stamverschillen in gevoeligheid voor anti-androgenen een rol. Ondersteuning voor deze mogelijkheid vonden we in het feit dat in Sprague Dawley vrouwtjes perinatale blootstelling aan anti-androgeen een remmend effect heeft op volwassen T-beklimgedrag. Overigens, stamverschillen in gevoeligheid voor de programmerende werking van perinataal aanwezige androgenen zijn eerder verondersteld en gevonden door Whalen en medewerkers (Whalen, Gladue & Olsen, 1986). Bij hen ging het niet om T-beklimgedrag van volwassen vrouwelijke ratten, doch om de programmering van lordosegedrag van volwassen mannelijke ratten.

Broertjes in utero en beklimgedrag

Uit het huidige onderzoek blijkt een masculiniserend effect van prenataal caudaal in de uterus gelegen broertjes op het volwassen T-beklimgedrag van Wistar wijfjes. Dit effect is slechts additief en geen 'conditio sine qua non', aangezien vrouwtjes zonder caudaal broertje ook hoge beklimfrequenties vertonen. Deze vondst is in overeenstemming met de literatuur. Ook daarin wordt een masculiniserend effect beschreven van caudaal gelegen broertjes op volwassen T-beklimgedrag van vrouwelijke (Sprague Dawley) ratten (Meisel & Ward, 1981). Onze vondsten wijken af van de studies waarin een masculiniserend gedragseffect gevonden wordt van naastgelegen broertjes (Clemens, 1974; Clemens et al., 1978). Wat dit laatste betreft, ook andere onderzoeken waren niet in staat het 'naastgelegen broertje effect' op volwassen gedragingen te repliceren (Meisel & Ward, 1981; Houtsmuller & Slob, 1990).

Partner preferentie van vrouwtjes

Partner preferentie van volwassen vrouwelijke Wistar ratten lijkt niet geprogrammeerd te worden door prenatale endogene androgenen. Immers, prenatale blootstelling aan anti-androgenen heeft geen effect op volwassen door T geactiveerd partner preferentie gedrag (T-partner preferentie).

Volwassen partner preferentiegedrag kán echter wel geprogrammeerd worden door neonatale hormonale manipulatie. Toediening vanaf de geboorte tot dag 10 van TP, maar niet van DHTP of olie, zorgt op volwassen leeftijd o.i.v. T of DHT in combinatie met E_2, voor een significant grotere voorkeur voor een bronstig vrouwtje, meer dan voor een seksueel aktief mannetje. Eerdere onderzoeken met exogeen T neonataal vinden vergelijkbare effecten op volwassen partner preferentie van vrouwelijke ratten (Meyerson, Eliasson & Hettta, 1979; de Jonge, Muntjewerff, Louwerse & van de Poll, 1988). Dit programmerend effect wordt waarschijnlijk veroorzaakt door de E_2 metaboliet van T, aangezien in het huidige onderzoek DHTP geen programmerend effect had.

Partner preferentie van mannetjes

Door testosteron geactiveerde partner preferentie voor een bronstig wijfje van volwassen mannelijke ratten wordt neonataal geprogrammeerd door T, waarschijnlijk via de E_2 metaboliet. Dit kan geconcludeerd worden, aangezien mannetjes alleen dan een duidelijke voorkeur vertonen voor een bronstig wijfje wanneer neonatale castratie gevolgd wordt door toediening van TP en op volwassen leeftijd DHT+E_2 of T wordt gegeven. Geheel in overeenstemming hiermee zijn ook de vondsten dat gonadaal intacte mannetjes, na neonatale blootstelling aan aromatase-remmers, een verminderde voorkeur vertonen voor een bronstig vrouwtje.

Deze gegevens wijken af van eerdere onderzoeken waarin geen of weinig effect op volwassen partner preferentie wordt gevonden van neonatale castratie (Eliasson & Meyerson, 1981; Merkx, 1984; Meyerson et al., 1979; Vega Matuszczyk, Fernandez-guasti & Larsson, 1988) of van neonatale toedienig van ATD, een aromatase-remmer (Davis, Chaptal & McEwen, 1979). Mogelijk is in sommige van deze onderzoeken neonataal te laat gecastreerd of is te laat begonnen met ATD behandeling.

In de huidige experimenten blijkt ook dat het seksueel gedrag van mannelijke ratten door perinataal endogeen T, waarschijnlijk via de E_2 metaboliet daarvan, wordt geprogrammeerd. Immers, neonataal met aromatase-remmers (ATD of atamestan) behandelde mannetjes zijn zowel minder gemasculiniseerd, d.w.z. ze vertonen minder ejaculaties, als minder gedefeminiseerd, d.w.z. ze vertonen meer lordosegedrag. De effecten zijn in overeenstem-

ming met de literatuur: een verhoogd lordosegedrag van pre- of neonataal met ATD behandelde mannetjes (McEwen, Lieberburg, Chaptal & Krey, 1977; Vreeburg, van der Vaart & van der Schoot, 1977; Clemens & Gladue, 1978; Davis et al., 1979; Whalen & Olsen, 1981; Whalen et al., 1986). De lagere ejaculatie-frequentie van neonataal met ATD of atamestan behandelde mannen wijkt af van onderzoek waarin geen remmend effect gevonden wordt na neonataal toediening van ATD (Vreeburg et al., 1977). Vermoedelijk begonnen deze onderzoekers te laat met ATD toediening. Slechts in één studie werd een remmend effect op de ejaculatie-frequentie gevonden van neonatale castratie plus toediening van T en ADT (een verwante aromatase remmer; Booth, 1978).

8-OH-DPAT en yohimbine

De serotonerge $5HT_{1A}$ receptor agonist 8-OH-DPAT stimuleert in mannelijke ratten de partner preferentie voor een bronstig vrouwtje. 8-OH-DPAT is echter niet in staat het bestaande verschil in partner preferentie (een lagere preferentie voor een bronstig vrouwtje) op te heffen tussen *peri*nataal met ATD behandelde mannetjes enerzijds en controles, of *pre*nataal met ATD behandelde mannetjes anderzijds.

8-OH-DPAT stimuleert het ejaculatiegedrag van zowel perinataal met ATD behandelde mannetjes als controles. Met een hoge dosis DPAT ejaculeren alle mannetjes, waarbij de eerste ejaculatie in circa 80% optreedt bij de eerste intromissie. De gevonden effecten bij controle dieren zijn in overenstemming met de literatuur (b.v. Ahlenius, Larsson, Svensson et al., 1981). De stimulerende effecten van 8-OH-DPAT op partner preferentiegedrag en ejaculatiegedrag van ATD mannetjes zijn niet eerder beschreven.

De α_2-adrenerge receptor antagonist yohimbine heeft een minder duidelijk stimulerend effect dan DPAT. Slechts het percentage dieren dat ejaculeert is hoger in de met ATD behandelde mannetjes. Er wordt geen effect gevonden op de ejaculatie-frequentie. De gevonden effecten bij de controledieren zijn minder duidelijk dan in de literatuur beschreven (Clark, Smith & Davidson, 1984, 1985a,b).

Modelvoorstelling

Uit de in dit proefschrift beschreven experimenten komt de volgende modelvoorstelling naar voren betreffende de mogelijke programmering van seksueel gedrag en partner preferentie van de Wistar rat.

T geactiveerd beklimgedrag van volwassen Wistar vrouwtjes lijkt enigszins gemasculiniseerd te worden door prenataal meer naar de cervix uteri gelegen broertjes. Op welke wijze dit effect

tot stand komt is niet duidelijk. Er zijn geen aanwijzingen gevonden dat het door T komt, afkomstig van de broertjes (zoals gesuggereerd in de literatuur). Immers, perinatale behandeling met anti-androgenen beïnvloedt (oftewel programmeert) op geen enkele wijze het volwassen T-beklimgedrag van Wistar vrouwtjes. Daarentegen lijkt bij Sprague Dawley vrouwtjes het perinatale T wel een rol te spelen in de programmering (masculinisering) van volwassen T-beklimgedrag.

Partner preferentie van vrouwelijke ratten voor een bronstig vrouwtje kan neonataal geprogrammeerd worden door exogeen TP. Dit gebeurt dan vermoedelijk via de E_2metaboliet, aangezien neonataal toegediend DHTP of olie dit effect niet heeft.

Seksueel gedrag en partner preferentie van mannelijke ratten wordt door neonataal aanwezig endogeen T, waarschijnlijk via de E_2metaboliet geprogrammeerd. Immers, neonataal met aromatase-remmers behandelde mannetjes zijn minder gemasculiniseerd, d.w.z. ze vertonen op volwassen leeftijd een duidelijk mindere voorkeur voor een bronstig vrouwtje en ze ejaculeren minder frequent. Tevens zijn ze minder gedefeminiseerd, aangezien ze zeer "gemakkelijk" lordosegedrag vertonen in de nabijheid van een seksueel aktief mannetje.

9.2 Conclusions

Perinatal androgens and mounting behavior

Testosterone activated mounting behavior (T-induced mounting behavior) of adult female Wistar rats does not seem to be programmed by perinatally circulating androgens. Perinatal exposure to antiandrogens had no effect on adult T-induced mounting behavior. The genital development of the brothers, however, was clearly affected, which demonstrates that the antiandrogens did reach the fetuses.

It has been found that prenatal exposure to antiandrogens lowered adult T-induced mounting behavior in female rats (Stewart, Pottier & Kaczender-Henrik, 1971; Ward & Renz, 1972; Clemens, Gladue & Coniglio, 1978). Those experiments were not carried out with Wistar rats, but Sprague-Dawley rats. Strain differences in sensitivity to antiandrogens may account for the difference into the response to antiandrogens. In fact we found that in Sprague Dawley females adult T-induced mounting behavior was reduced by perinatal antiandrogen exposure. Strain differences in sensitivity to the organizing action of perinatally circulating androgens have earlier been described by Whalen and co-workers (Whalen, Gladue & Olsen, 1986). Their study was not focussed on the organization of adult T-induced mounting in female rats, but on the organization of adult lordosis behavior in male rats.

Brothers in utero and mounting behavior

From the present experiments prenatal brothers located caudally in the uterus appeared to have a masculinizing effect on adult T-induced mounting of Wistar females. This effect was only additive, since females without a caudal brother also showed high mount frequencies. This finding corroborates earlier studies, in which a masculinizing effect was found of caudally located brothers on T-induced mounting behavior of female (Sprague Dawley) rats (Meisel & Ward, 1981). Our findings differ from studies in which a masculinizing behavioral effect was described of adjacent males (Clemens, 1974; Clemens et al., 1978). Others have also been unable to replicate the 'adjacent male effect' on adult T-induced mounting behavior (Meisel & Ward, 1981; Houtsmuller & Slob, 1990).

Partner preference in females

Adult partner preference of female Wistar rats does not seem not to be programmed by prenatal endogenous androgens. Prenatal exposure to antiandrogens had no effect on adult testosterone activated partner preference behavior.

However, adult partner preference can be programmed by neonatal hormone administration. Administration from birth through day 10 of TP, but not of DHTP or oil, and treatment in adulthood with T or DHT and E_2, resulted in a more pronounced preference for an estrous female over a sexually active male. Earlier studies, in which T was given neonatally, yielded similar effects on adult partner preference of female rats (Meyerson, Eliasson & Hetta, 1979; de Jonge, Muntjewerff, Louwerse & van de Poll, 1988). This organizing effect is presumably caused by E_2, since in the present study DHTP had no such effect.

Partner preference in males

In male rats adult partner preference is neonatally organized by T, probably through E_2. This can be concluded from the present studies, since males only showed preference for an estrous female when neonatal castration was followed by administration of TP and when in adulthood DHT and E_2 or T were given. Contrariwise, gonadally intact males exposed neonatally to aromatase inhibitors, showed a lower preference for an estrous female.

Some of our findings conflict with other studies in which neonatal castration (Eliasson & Meyerson, 1981; Merkx, 1984; Meyerson et al., 1979, Vega Matuszczyk, Fernandez-Guasti & Larsson, 1988) or neonatal administration of ATD (Davis, Chaptal & McEwen, 1979), appeared to have little or no effect on adult partner preference. In some of these latter studies neonatal castration was probably performed too long after birth or ATD treatment was started too late.

From the present experiments it is clear that sexual behavior of male rats is also organized perinatally by endogenous T, probably via E_2. Males treated neonatally with aromatase inhibitors (ATD or atamestan) were less masculinized (i.e. showed lower ejaculation frequencies), and were less defeminized (i.e. showed more lordoses). These effects are in accord with the published data: enhanced lordosis behavior of pre- and neonatally ATD treated males (McEwen, Lieberburg, Chaptal & Krey, 1977; Vreeburg, van der Vaart & van der Schoot, 1977; Clemens & Gladue, 1978; Davis et al., 1979; Whalen & Olsen, 1981; Whalen et al., 1986). A lower ejaculation frequency of neonatally ATD was not found by Vreeburg et al. (1977), who injected ATD within 4 h after birth and subsequently on days 3, 5, 10 and 15 days after birth. In one study a lowering effect on the ejaculation frequency was found after neonatal castration followed by combined administration of T and the aromatase inhibitor ADT (Booth, 1978).

8-OH-DPAT and yohimbine

The serotonergic $5HT_{1A}$ receptor agonist 8-OH-DPAT stimulated in male rats partner preference

for an estrous female. However, 8-OH-DPAT did not eliminate the difference in partner preference between *peri*natally ATD treated males (with a lower preference for an estrous female) and controls or *pre*natally ATD treated males.

8-OH-DPAT administration to intact male rats has been shown to reduce the number of intromissions and the latency to the first ejaculation (Ahlenius, Larsson, Svensson et al., 1981). In a number of studies a reduced postejaculatory interval was found after treatment with 8-OH-DPAT (Ahlenius et al., 1981; Schnur, Smith, Lee, Mas & Davidson, 1989). In the present study 8-OH-DPAT stimulated ejaculatory behavior of perinatally ATD treated males and controls. After a high dose of 8-OH-DPAT all males ejaculated, the first ejaculation coinciding with the first intromission in about 80% of the cases.

The α_2-adrenergic receptor antagonist yohimbine also stimulated sexual behavior, but less so than 8-OH-DPAT. Yohimbine increased the number of males that ejaculated; the number of intromissions till the first ejaculation was not reduced (Smith, Lee, Schnur, Davidson, 1987). In the present study the percentage of ATD treated males that ejaculated was increased by yohimbine. No effect was found on ejaculation frequency. The effects in our control animals were less marked than those reported by others (Clark, Smith, & Davidson, 1984, 1985a,b).

Organizational model

The experiments described in this thesis give rise to the following model of organizational action of gonadal steroids on sexual behavior and partner preference in the Wistar rat.

Adult T-induced mounting behavior of female Wistar rats seems to be somewhat masculinized by prenatal brothers, located more caudally in the uterus. The cause of this masculinizing effect is still unknown, but it has been suggested that andorgens of the caudal brothers may reach the young female. However, perinatally circulating androgens do not affect adult T-induced mounting behavior of Wistar females. In Sprague Dawley females prenatal as well as neonatal T is of some significance in programming adult T-induced mounting behavior.

Partner preference of female rats for an estrous female can be programmed neonatally through exogenous TP. E_2 is presumably responsible, since neonatally administered DHTP or oil had no such programming effect.

Sexual behavior and partner preference of male rats is organized neonatally by endogenous T, probably through E_2. Males, neonatally treated with aromatase inhibitors, were less masculinized, i.e. showed less preference for an estrous female and lower ejaculation frequencies. They were also less defeminized, i.e. they displayed lordosis behavior in the vicinity of sexually active males more easily and more frequently.

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Lijst van gebruikte afkortingen en triviaal namen

ACh	= acetylcholine
ADT	= androst-4-een-3,6,17-trion
Anandron	= 5,5-dimethyl-3-(4-nitro-3'-trifuloromethyl-isobutyraninlide)
Androsteendion	= 3α-hydroxy-5α-androstaan-17-on
Atamestan	= 1-methyl-1,4-androstariëen-3,17-dion
ATD	= 1,4,6-androstatrieen-3,17-dion
CA	= 1,2α-methyleen-6-chloor-4,6-pregnadiëeen-17α-ol-3,20-dion-17α-acetaat (cyproteron-acetaat)
DHT(P)	= 17ß-hydroxy-5α-androstaan-3-on [dihydrotestosteron (propionaat)]
DNA	= deoxyribonucleic acid
E_2(B)	= 1,3,5-estratriene-3,17ß-diol[oestradiol](benzoaat)
Flutamide	= 4'-nitro-3'-trifluoromethyl-isobutyranilide
GABA	= gamma-amino-boterzuur
5HT	= 5-hydroxy-tryptamine (serotonine)
MIF	= Müller inbitory factor
mRNA	= messenger ribonucleic acid
NADPH	= Nicotinamide adenine dinucleotide waterstof fosfaat
8-OH-DPAT	= 8-hydroxy-2-(di-n-propylamino) tetraline
P	= Δ^4pregneen-3,20-dion (progesteron)
R1881	= methyltrienolon
RU-486	= 17ß-hydroxy-11ß-[4-dimethyl-aminophenyl]-17α-[1-propynyl]-estra-4,9-diene-3-on (mefiproston)
T(P)	= 17ß-hydroxy-4-androsteen-3-on [testosteron (propionaat)]

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Curriculum vitae

De schrijver van dit proefschrift werd op 25 februari 1961 geboren te Dordrecht. In juni 1979 behaalde hij het VWO diploma aan het Erasmus College te Zoetermeer. In augustus 1979 werd een aanvang gemaakt met de studie geneeskunde aan de Erasmus Universiteit te Rotterdam. Het doctoraalexamen werd in oktober 1984 behaald en het artsexamen in maart 1986. Tijdens het derde studiejaar werd in het kader van het keuzepracticum bij het toenmalige Instituut Endocrinologie, Groei en Voortplanting (Faculteit der Geneeskunde, Erasmus Universiteit Rotterdam) onder leiding van Dr. A.K. Slob onderzoek verricht naar "Prenatale androgenen en (jong) volwassen TP-geïnduceerd beklim- en keuzegedrag van vrouwelijke ratten". Dit keuze-practicum is aanleiding geweest tot het huidige promotie-onderzoek, waarmee begonnen werd op 1 april 1986 binnen bovengenoemd instituut (vanaf 1990 opgenomen in de Vakgroep Endocrinologie & Voortplanting). De eerste anderhalf jaar was de schrijver van dit proefschrift aangesteld als wetenschappelijk assistent. Vanaf 1 oktober 1987 volgde een aanstelling als assistent in opleiding (op een AIO-poolplaats) op het project getiteld: "De invloed van prenatale androgenen op de seksuele differentiatie van de vrouwelijke rat" (projektleider: Dr. A.K. Slob). In de loop van het onderzoek zijn nieuwe aspecten toegevoegd, waarbij het onderzoek naar de invloed van perinatale androgenen op de volwassen partner preferentie van mannelijke en vrouwelijke ratten de belangrijkste plaats inneemt. Deze onderzoeken hebben geleid tot het verschijnen van dit proefschrift.