ANIMAL SEXOLOGY

Francien de Jonge[1], Pieter van der Schoot[2] and Jacques Balthazart[3]

Animal sexology investigations in Belgium and The Netherlands can boast two research traditions. The ethological tradition is concerned with observations on mating strategies under natural or seminatural conditions. This approach is followed by the University of Utrecht, and observations are taking place at Burgers Zoo and Safari Park in a variety of species. The second research tradition is directed at the experimental analysis of sexual behaviour under controlled laboratory conditions. The research efforts concentrate on brain mechanisms involved in hormonal control of sexual behaviour. This approach is followed by three research groups at the universities of Liège, Amsterdam and Rotterdam. These investigations concern the analysis of copulatory behaviour in the quail (Liège) and in the laboratory rat (Amsterdam and Rotterdam) and the stumptailed monkey (Rotterdam).

Burgers Zoo and Safari Park in The Netherlands, offer a unique possibility for the analysis of animal sexology in a seminatural environment. In this setting, mating strategies of such different species as wolves [37], plains zebras [29] and chimpanzees [21], are being investigated by van Hooff and collaborators. Mating strategies are typically analysed within the species-specific social context. In collaborative projects, investigations have been extended to include wild populations of long-tailed macaques [40] and orang-utans [31, 32] in the Ketambe Gunung Leuser Reserve (Sumatra) and of savanna baboons in the Amboseli National Park (Kenya) [27].

Mating behaviour of a colony of stumptail macaques in relation to the gonadal hormonal environment of the individuals, has been investigated by Slob and co-workers under both seminatural and laboratory conditions. Regrettably, this colony has now been dispersed and further research has been discontinued.

In addition to the above mentioned investigations on animal sexology in natural or seminatural environments, three major research groups in Belgium and The Netherlands concentrate on the experimental analysis of sexual behaviour under strictly controlled laboratory conditions in the rat (van de Poll and co-workers and Slob and co-workers) and in the quail (Balthazart and collaborators). The common research interests of these groups concentrate on brain mechanisms underlying hormonal control (both organizing and activating effects) of sexual behaviour. Research in the

[1] F.H. de Jonge, PhD. Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam.

[2] P. van der Schoot, PhD. Department of Endocrinology and Reproduction, Faculty of Medicine and Health Sciences, Erasmus University, Postbox 1738, 3000 DR Rotterdam.

[3] J. Balthazart, PhD. Laboratoire de Biochimie Générale et Comparée, 17 Place Delcour (Bat. L1) B-4020 Liège, Belgique.

quail occupies a special place, since gonadal hormonal mechanisms involved in sexual differentiation in a typical avain species vary in some aspects from those traditionally described in mammalian species. The research is focused on the sexually dimorphic part of the medial preoptic area as a target site for gonadal hormones and their metabolites.

The groups of van de Poll and Slob are basically following the same lines of research in the rat. Their research has taken a distinct direction by the development of tests that differentiate between sexual arousal, sexual orientation, sexual performance and sexual reward. These tests have been developed since accumulating evidence indicates that different physiological mechanisms are associated with these different aspects of sexual behaviour.

In the following pages, recent scientific developments made by these Belgian and Dutch research groups will be summarized.

Courtship and mating of wild orang-utans and captive chimpanzees

Ethological observations on mating behaviour typically derive their significance when analysed in relation to the social structure of the species, an approach that does not easily allow to be summarized. The unique observations on mating strategies of orang-utans [28, 31, 32] and chimpanzees [21] were therefore selected as examples.

The Sumatran orang-utan is an arboreal animal with a predominantly solitary life style. "Subadult" males, which are sexually mature but without fully developed secondary sexual characteristics, tend to take the initiative in copulation, and often enforce copulation ("rapes"). Only when "fully adult" (between the 10th and 20th year), males become attractive to females. With these males, according to Schürmann, most copulations occurred cooperatively, in a consort relationship in which males and females travel together for several days [31, 32].

During 40 observed copulations between female "Yet" and the unquestionably most dominant alpha male "Jon", Schürmann described how Yet not only took the sexual initiative in most copulations, she was also the active partner and did all the pelvic thrusting, most frequently in ventro-ventral position, while Jon remained completely motionless. According to previous observations by Rijksen [28], female Yet had been showing sexual interest in Jon for at least 5 years before Jon started to tolerate her initiative, starting to react with male presenting (bending backwards until he lays on his back). Ultimately however, Yet's interest and initiative culminated in consort relationship, copulations and delivery of an infant. The most remarkable observation, is that female Yet during these 5 years had already been menstruating and had been copulating dozens of times with subadult males without conceiving. These observations suggest that reproductive success of the male orang-utan may probably be determined by the female's sexual "preference".

The observation on copulatory behaviour of the orang-utan with its typical solitary life style, more or less contrasts with the now famous social and sexual interactions in the chimpanzee colony of Burgers Zoo, as described by De Waal [21].

Although alpha-males are, according to De Waal, involved in the majority of sexual interactions, other copulations may be tolerated as a result of extensive negotiations, may be enforced by coalitions or subversive activities, undermining the alpha-male's influence, or copulations may take place surreptitiously outside the sphere of influence of the alpha male. Ultimately however, the female chimpanzee decides whether or not copulation will take place. Unlike the outspoken sexual preference observed in the orang-utan female Yet, exclusive sexual preferences in the chimpanzee colony were only occasionally observed, and the chimpanzee may therefore be considered as a fully promiscuous species.

Ethology and physiology of captive stumptail macaques

The stumptail macaque is a highly social primate species with remarkably abundant copulatory activity: in pair tests in the laboratory, tens of ejaculations were observed in 24 hr observation periods, apparently without a sign of exhaustion of either partner. In the colony that was studied during 3 years by Nieuwenhuijsen, Slob and others, overt copulation was shown to be privileged to the highest ranking male, while other males copulated surreptitiously. The lowest ranking adult males on the other hand, were never seen to be engaged in copulations [9, 13, 22, 25, 26, 34, 35].

Several observations indicate that the large interindividual differences in copulatory behaviour of male stumptail macaques are socially determined phenomena, that can easily alter after changes in the social structure of the colony. This may be illustrated by masturbatory behaviour that occurs in males of all ranks: temporary removal of the alpha-male resulted in an immediate shift from masturbatory to copulatory behaviour in the sub-alpha-male, which showed low levels of copulation when the alpha-male was present [22, 25].

Ejaculation by the male is accompanied by the display of a typical facial expression ("ejaculation face"). Female stumptail macaques occasionally show such facial expressions as well. In the female, this expression is associated with specific changes in uterine muscular activity and sudden changes in heart rate. In view of the known features of human female orgasm, it was concluded that stumptail females may show orgasm during sexual interactions [13, 34].

Copulatory behaviours of male and female stumptail macaques are hardly affected by steroid hormones. Females allow copulations throughout their ovarian cycle, but cease copulatory activity during pregnancy and lactation. In laboratory pair tests, neither ovariectomy, nor combined ovariectomy and adrenalectomy, affects copulatory activity [9, 26]. Although similar to the human, this situation is quite exceptional among primates and mammals in general. In addition, female stumptail macaques, as the second species besides humans, may experience orgasm. These observations suggest that stumptail macaques may serve as an animal model for physiological correlates of human sexual behaviour (especially the human female) in ways that other species may not allow.

Sexual arousal, copulatory performance and reward

According to observations in rat colonies, female sexual initiative plays a major role in accomplishing sexual interactions. Sexual behaviour in the laboratory rat, however, is typically studied in small test cages that do not offer possibilities for escape or shelter, thys depriving the female rat of possibilities to reject mount attempts, to pace sexual contacts, or to take sexual initiative. Although male sexual performance and female receptivity (lordosis posture in reaction to male mounting) may be adequately investigated in such tests, they definitely fall short when motivational aspects of sexual behaviour are to be investigated.

Female approach behaviour to a sexual stimulus was measured in an open field test with sexually active and sexually inactive partners located opposite to each other behind a wire mesh. Female sexual initiative was measured in a different situation, in which male rats were tethered by means of a harness, thus restricting their movements to half of the test arena. Estrous female rats then show high levels of proceptive behaviour, provoking the male by incessant hopping, darting and ear quivering just in front of him, but out of his reach. Occasionally, and at regular intervals, the female allows intromissions [12, 14-18].

It is remarkable that gonadal hormones which fluctuate during the estrous cycle, differentially affect the above aspects of female sexual activity. Whereas a single injection with estradiol (E) sufficiently activates lordosis behaviour in the ovariectomized rat, additional progesterone (P) is necessary to further facilitate her proceptive behaviour. Motivation to approach a sexually active male on the other hand was in particular stimulated by androgens. These differential effects further depend upon duration of exposure and dosage of the hormone. Thus, chronic exposure to E, or single injections with supraphysiological doses of E, elicit the full receptive and proceptive behavioural repertoire of the female rat, even without additional P or T [14-18].

The finding that androgens may in particular stimulate appetitive (i.e. motivational) aspects of female sexual behaviour was nicely illustrated in females treated with the non-aromatizable androgen R1881, that can not be converted to E [16]. R1881-treated females actively approach male partners, but do not show receptive behaviour when mounted. These results corroborate preliminary data in human females with excessive endogenous androgen production which indicate that these females have higher libido than controls [33].

That heterosexual copulation has both aversive and rewarding components for the female rat, was shown in a variety of tests indicating that both gonadal hormonal priming and the female's opportunities to pace her sexual contacts, determine whether heterosexual copulation is rewarding or not [12, 17,18]. Rewarding aspects of heterosexual experience were demonstrated in a "place preference paradigm", indicating that female rats prefer distinct environments previously associated with sexual interaction.

Aversive aspects of copulation were demonstrated when the female's motivation to approach a sexually active male was decreased after prior heterosexual interactions in small test cages. Her interest in sexually active male partners was actually

changed into a preference for female partners after she had had prior sexual experience with females [18]. Penile insertions in particular, were shown to evoke ambivalent responses. This was nicely illustrated in a 3-compartment cage, in which active males were tethered to the posterior wall and non-intromitting conspecifics were given as an alternative: soon after an intromission, the female escaped from the male compartment, but reentered after a while. When offered a choice between a sexually active male and a Testosterone (T)-treated female – which shows mounting without genital insertion – the female is preferred to the male [12]. In fact, the period of heat becomes reduced through genital sensory stimuli associated with penile insertions, which possibly reach the central nervous system through the pelvic nerves [23].

The picture that emerges from studies on male sexual motivation and reward, is less complicated than that in females: gonadal hormones that facilitate copulatory performance, also stimulate the male's motivation to approach an estrous female, and heterosexual interaction in males was invariably found to be rewarding. In a residential 4-arms plus-maze, intact male rats spend virtually the whole night in close proximity to the female when she is in estrus. After castration, his interest gradually declines but can be reinstated by T-treatment [24, 39].

Ongoing research concentrates upon the neural substrate associated with various aspects of sexual behaviour. Previous observations with electrodes in the medial preoptic area (MPOA) that elicit self-stimulation behaviour in a hormone dependent way, suggest that this area may play a role in sexual reward, but future experiments should further elucidate this finding [38].

Sexual differentiation of brain and behaviour in rat

It has been well established now, that sexual differentiation of the developing brain is controlled by gonadal hormones which permanently organize brain and behaviour during early development [4, 10, 11, 14, 20, 36]. Thus, neonatally castrated male rats may show both increased levels of lordosis behaviour, and a sexually dimorphic nucleus of the preoptic area (SDN-POA) which is smaller than that observed in normally developed males [4, 6, 20]. From these observations, a theory emerged hypothesizing that human homosexuality might result from gonadal hormonal disturbances during early development. Recent evidence indicates, however, that the volume of a sexually dimorphic cell group within the hypothalamus of human homosexuals, is entirely within the range of that observed in heterosexual males, and significantly larger than that of human females, thus refuting the hypothesis of a "female differentiated hypothalamus" in homosexual males [20]. Also in animal studies, experiments now show that genetic males that are neonatally castrated, do not necessarily show a "homosexual orientation", even though female typical behaviours like lordosis are increased. It is concluded from these studies that sexual orientation of adult male and female rats is a function of previous sexual experience and of both neonatal and adult exposure to gonadal hormones [11].

With respect to the well-established concept of the organizing effects of gonadal hormones on differentiation of sexual behaviour, a number of questions emerge.

For instance, where do neonatal hormones act in order to organize brain and behaviour? Are decreases in masculine copulatory performance of neonatally castrated males caused by penile maldevelopment? Is mounting behaviour in normally developed female rats dependent upon androgen exposure in utero? And, a question particularly addressed by Balthazart et al., do androgens organize and activate sexual behaviour, or do their metabolites do this?

Findings obtained after implantation of testosterone propionate (TP) pellets in the MPOA of the neonatal female rat brain, indicate that right-sided TP implants masculine sexual behaviour, whereas left-sided implants do not. These results not only illustrate a considerable asymmetrical action of gonadal hormones on the neonatal rat brain, they also confirm that the MPOA is the major site of action in this respect [36].

It is known that androgens act after conversion to estrogens in order to masculinize the fetal rat brain, whereas penile development is dependent upon androgens. Treatment with the aromatization inhibitor ATD therefore results in male rats with testes and masculinized genitalia, but with a non-masculinized (i.e. female) differentiated hypothalamus. Behaviorally, these animals appeared to be completely bisexual, thus showing receptive behaviour when confronted with a male, and showing masculine copulatory responses when introduced to a female. In addition, neonatally ATD-treated males equally approached male and female partners when given the choice, thus showing a completely bisexual orientation that further developed depending on subsequent sexual experience. Preliminary results indicate that these animals have a female type SDN-POA. It is concluded that absence of masculine copulatory responses and a female type sexual orientation in neonatally castrated rats, may be partly attributed to penile maldevelopment. The results therefore suggest, that T in the neonatal brain defeminizes sexual behaviour in the rat, whereas absence of T in the brain does not necessarily demasculinize the neonatal rat brain.

This conclusion basically corroborates the observation that masculine copulatory responses do regularly occur in normally developed female rats. However, according to theories supposing that neonatal androgens are an absolute prerequisite for the development of masculine copulatory behaviour, normally developed female rats that mount must have been neonatally exposed to androgens derived from male siblings in utero. This theory could be refuted by showing that females born in all-female litters show similar mounting behaviour as those born from mixed female litters [10].

Sexual differentiation of brain and behaviour in quail

The Japanese quail (Coturnix coturnix japonica), is a domestic bird species which is studied in Liège. Within seconds after a sexually mature male has been presented to an egg-laying female in the laboratory, the pair displays sexual behaviour. The copulatory sequence is very brief: the female grabs with its beak in the neck region of the female, he mounts her and then opens his wings, fall backward and achieves cloacal contact during which sperm is transferred to the female cloaca. Two other behaviours

are also seen in standardized test conditions: the strutting, a pre- and post-copulatory display and the crowing, a contact vocalization. The copulatory behaviour sensu stricto is only activated by aromatizable androgens and abundant evidence demonstrates that estrogens alone or in combination with androgens are, at the cellular level, the active hormones which stimulate the behaviour. By contrast, the activation of crowing and strutting as well as the growth of the cloacal gland are strictly androgen dependent and not influenced by oestrogens [1-8,30].

Many aspects of brain morphology and physiology and reproductive behaviour are sexually differentiated in quail as in mammals. However, normally developing male rats are typically defeminized after early exposure to endogenous T, while in contrast, sexual differentiation in quail behaviour concerns male copulatory behaviour: normally developing female quails are demasculinized by exposure to their ovarian secretion of estrogens. The latter was proposed, since it had been known for some time, that early exposure of male quail embryos to exogenous oestrogens suppress their capacity to show male sexual behaviour as adults in response to T. By ultra-sensitive radioimmunoassays, Balthazart et al. showed that the circulating oestradiol levels are indeed higher in female than in male embryos during the critical period of sexual differentiation. In addition, injection into the eggs of the aromatase inhibitor R76713 is able to prevent the female demasculinization. Birds born from female embryos injected with R76713 show a complete male pattern of copulatory behaviour in response to exogenous T [2, 4, 6, 30].

Activation of sexual behaviour in quail and rat

The sexually dimorphic nucleus of the preoptic area is referred to as SDN-POA in rat, and as POM (medial preoptic nucleus) in quail. Both in rat, quail and several other species, the POA and in particular the sexually dimorphic part, is a major target site for gonadal hormonal activation of masculine copulatory behaviour. The volume of the POM in quail for instance, decreases with castration and increases following testosterone treatment. Lesions of the SDN-POA inhibited masculine copulatory responses in male and female rats, and POA lesions in the quail only reduced masculine copulatory behaviour if the lesions involved part of the sexually dimorphic POM [6, 19, 20].

Enzymes in the nervous system can metabolize steroid hormones. Depending on the species, metabolites of T, like dihydrotestosterone (DHT) and oestradiol (E2, produced by aromatization) are able to mimic alone or in combination most of the effects of T. Stereotaxic implantation of hormones, anti-hormones and metabolism inhibitors were used in recent years to study the sites of androgen and estrogen action on sexual behaviour in the POA of castrated male quail. It could first be shown that T-implants in the POA activate copulatory behaviour only when they are located within the boundaries of the sexually dimorphic POM. Additional studies demonstrate that the androgen acts in the POM through its conversion to estrogens: stereotaxic implantation of a synthetic estrogen (DES: diethylstil-boestradiol), but not of the

synthetic nonaromatizable androgen (R1881: methyltrienolone), could restore copulatory behaviour in castrated male rats. On the other hand, activating effects of systemic T were blocked by stereotaxic implants of aromatase inhibitors (ATD or R76713) or an anti-estrogen (tamoxifen). Surprisingly, activating effects of systemic T on copulatory behaviour were also (partly) blocked by an anti-androgen (flutamide).

As indicated by particular effectiveness of DES and ATD within the boundaries of the POM, these results demonstrate that the sexually dimorphic POM is the area where the behaviourally active estrogenic metabolites of T have to be produced. Androgens could also have a direct action on sexual behaviour as suggested by the partial inhibition observed in flutamide-treated birds [1-8,30].

In the Japanese quail, the aromatization of T into E appears to be a limiting step in the activation of copulatory behaviour. Assays of the enzyme activity which metabolize steroid hormones, demonstrate that aromatase activity (AA) in the POA is induced by T in a dose-dependent fashion. Precise information on the neuroanatomical localization of the enzyme was recently obtained by an immunocytochemical method using a polyclonal antibody against human placental aromatase. Balthazart et al. thus identified aromatase immunoreactive neurons (AR-ir) in all quail brain areas that contain AA. Within the POA, AR-ir cells are a specific neurochemical marker for the POM. In this nucleus, the number of immunoreactive cells is increased by T in parallel with the increase in AA which strongly suggests that the increased enzyme activity reflects a real change in enzyme concentration. At the electron microscope level, immunoreactive material is found in the perikarya and fills cellular processes including the full length of dendrites and axons, and the synaptic boutons. Double label immunocytochemical experiments indicate that very few AR-ir neurons in POM also contain estrogen receptors. These findings raise questions concerning the mode of action on behaviour of the estrogens formed by aromatization of T and suggest that estradiol might act as a neuromodulator at the level of the synapse [7, 8].

Dutch and Belgian research groups in animal sexology:
Head: Prof. dr N.E. van de Poll, Netherlands Institute for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.
Head: Dr A.K. Slob, Dept. Endocrinology and Reproduction, Faculty of Medicine and Health Sciences, Erasmus University, Postbox 1738, 3000 DR Rotterdam, The Netherlands.
Head: Dr J. Balthazart, Laboratoire de Biochimie Générale et Comparée, 17 Place Delcour (Bat. L1) B-4020 Liège, Belgique.
Head: Prof. dr J.A.R.A.M. van Hooff, Dept. of Comparative Physiology, Ethology and Socio-oecology group, University of Utrecht, Padualaan 14, 3584 CH Utrecht, The Netherlands.

References

1) Balthazart, J. (1983). Hormonal correlates of behavior. In: D.S. Farner, J.R. King and K.C. Parkes (eds.). *Avian biology,* Academic Press, New York.

2) Balthazart, J., M. Schumacher and M.A. Ottinger (1983). Sexual differences in the Japanese quail: behavior, morphology and intracellular metabolism of testosterone. *General and Comparative Endocrinology, 51:* 191-207.

3) Balthazart, J. and M. Schumacher (1985). Role of testosterone metabolism in the activation of sexual behaviour in birds. In. R. Gilles and J. Balthazart (eds.), *Neurobiology.* Springer Verlag.

4) Balthazart, J. and M. Schumacher (1987). A two-step model for sexual differentiation. In: B.R. Komisaruk, H.I. Siegel, M.F. Cheng and H.H. Feder (eds). *Reproduction: a behavioral and neuroendocrine perspective.* Annals of the New York Academy of Sciences.

5) Balthazart, J. (1988). Steroid metabolism and the activation of social behavior. In: J. Balthazart (ed.). *Advances in Environmental and Comparative Physiology: Molecular and Cellular Basis of Social Behavior in Vertebrates.* Springer Verlag, Berlin.

6) Balthazart, J. and C. Surlemont (1990). Copulatory behavior is controlled by the sexually dimorphic nucleus of the quail preoptic area. *Brain Research Bulletin, 25:* 7-14.

7) Balthazart, J., A. Foidart, C. Surlemont and N. Harada (1990). The preoptic aromatase in quail: behavioral, biochemical and immunocytochemical studies. In: J. Balthazart (ed.). *Hormones, Brain and Behavior in Vertebrates. 2. Behavioral activation in males and females - Social interactions and reproductive endocrinology.* Comparative Physiology, Karger, Basel.

8) Balthazart, J., A. Foidart, C. Surlemont, A. Vockel and N. Harada (1990). Distribution of aromatase in the brain of the Japanese quail, ring dove and zebra finch: an immunocytochemical study. *Journal of Comparative Neurology, 301:* 276-288.

9) Baum, M.J., A.K. Slob, F.H. de Jong, and D.L. Westbroek (1978). Persistence of sexual behavior in ovariectomized stumptail macaques following dexamethasone treatment or adrenalectomy. *Hormones and Behavior, 11:* 323-347.

10) Brand, T., E.J. Houtsmuller and A.K. Slob (1990). Androgens and the propensity for adult mounting behavior in the female Wistar rat. In: J. Balthazart (ed.). *Hormones, Brain and Behavior in Vertebrates. 1. Sexual differentiation, neuroanatomical aspects, neurotransmitters and neuropeptides.* Comparative Physiology, Karger, Basel.

11) Brand, T. and A.K. Slob (1991). Neonatal organization of adult partner preference behavior in male rats. *Physiology and Behavior, 49:* 107-111.

12) Broekman, M., M. de Bruin, J. Smeenk, A.K. Slob and P. van der Schoot (1988). Partner preference behavior of estrous female rats affected by castration of tethered male incentives. *Hormones and Behavior, 22:* 324-337.

13) Goldfoot, D.A., H. Westerborg-van Loon, W. Groeneveld and A.K. Slob (1980). Behavioral and physiological evidence of sexual climax in the female stumptailed macaque. *Science, 208:* 1477-1479.

14) de Jonge, F.H. (1986). *Sexual and aggressive behavior in female rats: Psychological and endocrine factors.* PhD. thesis, Netherlands Institute for Brain Research, Amsterdam.

15) de Jonge, F.H., E.M.J. Eerland and N.E. van de Poll (1986). The influence of estrogen, testosterone and progesterone on partner preference, receptivity and proceptivity. *Physiology and Behavior, 37:* 885-891.

16) de Jonge, F.H., E.H. Kalverdijk and N.E. van de Poll (1986). Androgens are specifically implicated in female rat sexual motivation. The influence of methyltrienelone (R1881) on sexual orientation. *Pharmacology, Biochemistry and Behavior, 24:* 285-289.

17) de Jonge, F.H. and N.E. van de Poll (1986). On the involvement of progesterone in sexually rewarded choice behavior of the female rat. *Physiology and Behavior, 37:* 93-98.

18) de Jonge, F.H., J. Burger, F. van Haaren, J. Overdijk and N.E. van de Poll (1987). Sexual experience and preference for males and females in the female rat. *Behavioral Neurobiology, 47:* 369-383.

19) de Jonge, F.H., A.L. Louwerse, M.P. Ooms, P. Evers, E. Endert and N.E. van de Poll (1989). Lesions of the SDN-POA inhibit sexual behavior of male Wistar rats. *Brain Research Bulletin, 23:* 483-492.

20) de Jonge, F.H., D.F. Swaab, M.P. Ooms, E. Endert and N.E. van de Poll (1990). Developmental and functional aspects of the human and rat sexually dimorphic nucleus of the preoptic area

(SDN-POA). In: J. Balthazart (ed.). *Hormones, Brain and Behavior in Vertebrates. 1. Sexual differentiation, neuroanatomical aspects, neurotransmitters and neuropeptides.* Comparative Physiology, Karger, Basel.

21) de Waal, F.B.H. (1982). *Chimpanzee Politics: power and sex among apes.* Jonathan Cape, London.

22) Estep, D.Q., K. Nieuwenhuijsen, K.E.M. Bruce, K. de Neef, P.A. Walters, S.C. Baker and A.K. Slob (1988). Inhibition of sexual behaviour among subordinate stumptail macaques (Macaca arctoides). *Animal Behaviour, 36:* 854-864.

23) Lodder, J. and G.H. Zeilmaker (1976). Role of pelvic nerves in the postcopulatory abbreviation of behavioral estrus in female rats. *Journal of Comparative and Physiological Psychology, 90:* 925-929.

24) Merkx, J. (1984). Effect of castration and subsequent substitution with testosterone, dihydrotestosterone and oestradiol on sexual preference behavior in the male rat. *Behavioral Brain Research, 11:* 59-65.

25) Nieuwenhuijsen, K., A.K. Slob and J.J. van der Werff ten Bosch (1988). Gender-related behaviors in group living stumptail macaques. *Psychobiology, 16:* 357-371.

26) Nieuwenhuijsen, K., K.J. de Neef and A.K. Slob (1986). Sexual behavior during ovarian cycles, pregnancy, and lactation in group-living stumptail macaques (Macaca arctoides). *Human Reproduction, 1:* 159-169.

27) Noë, R. and A.A. Sluijter (1990). Reproductive tactics of male savanna baboons. *Behavior, 113:* 117-170.

28) Rijksen, H.D. (1978). *A field study on Sumatran Orangutans (Pongo pygmaeus abelii Lesson 1827). Ecology, behavior and conservation.* PhD Thesis, Wageningen.

29) Schilder, M.B.H. (1990). Interventions in a herd of semi-captive plains zebras. *Behavior, 112:* 53-83.

30) Schumacher, M., J.C. Hendrick and J. Balthazart (1989). Sexual differentiation in quail: critical period and hormonal specificity. *Hormones and Behavior, 23:* 130-149.

31) Schürmann, C.L. (1981). In: A.B. Chiarelli and R.S. Corruccini (eds.). *Primate Behavior and Sociobiology.* Springer-Verlag, Berlin.

32) Schürmann, C.L. and J.A.R.A.M. van Hooff (1986). Reproductive strategies of the orang-utan: New data and a reconsideration of existing sociosexual models. *International Journal of Primatology, 7:* 265-287.

33) Sitters-Zwolsman, C.M., K. Boer, P. Cohen-Kettenis, F.H. de Jonge, J.V. Hamerlynck, H.V. Hogerzeil and N.E. van de Poll (1987). Aggressive and sexual behavior in adult women with high levels of testosterone. In: J.M. Ramirez (ed.). *Research on Aggression.* Univ. of Seville Press, Spain.

34) Slob, A.K., W.H. Groeneveld and J.J. van der Werff ten Bosch (1986). Physiological changes during copulation in male and female stumptail macaques (Macaca arctoides). *Physiology and Behavior, 38:* 891-895.

35) Slob, A.K. and K. Nieuwenhuijsen (1980). Heterosexual interactions of pairs of laboratory-housed stumptail macaques (Macaca arctoides) under continuous observation with closed-circuit video recording. *International Journal of Primatology, 1:* 63-80.

36) Swanson, H.H., E.J. Houtsmuller, R. Diaz, A.L. Louwerse, and N.E. van de Poll. The preoptic area and sexual differentiation. In: J. Balthazart (ed). *Hormones, Brain and Behavior in Vertebrates. 1. Sexual differentiation, neuroanatomical aspects, neurotransmitters and neuropeptides.* Comparative Physiology, Karger, Basel.

37) Van Hooff, J.A.R.A.M. and J.A.B. Wensing (1987). Dominance and its behavioral measures in a captive wolf pack. In: H. Frank (ed). *Man and wolf, Advances, issues, and problems in captive wolf research.* Dr W. Junk Publishers, Dordrecht, Boston, Lancaster.

38) Van de Poll, N.E. and H. van Dis (1971). Sexual motivation and medial preoptic self-stimulation in male rats. *Psychonomic Science, 25:* 137-138.

39) Van der Schoot, P. and A. Kooy (1988). Current topics in the study of sexual behavior in rats. In: J.M.A. Stitsen (ed). *Handbook of Sexology.* Elsevier, Amsterdam.

40) Van Noordwijk, M.A. (1985). *The socio-ecology of Sumatran long-tailed macaques (macaca fascicularis) II. The behavior of individuals.* PhD-thesis, University of Utrecht.