Pharmacology Biochemistry and Behavior, Vol. 65, No. 4, pp. 731–736, 2000

0091-3057/00/$–see front matter

ELSEVIER

PII S0091-3057(99)00266-X

Gonadectomy Enhances Shock-Induced Behavioral Inhibition in Adult Male Rats: Implications for Impulsive Behavior

ANDERS I. SVENSSON, BO SÖDERPALM AND JÖRGEN A. ENGEL

Institute of Physiology and Pharmacology, Department of Pharmacology, Box 431, SE 405 30 Göteborg, Sweden

Received 15 July 1999; Revised 15 November 1999; Accepted 30 November 1999

SVENSSON, A. I., B. SÖDERPALM AND J. A. ENGEL. *Gonadectomy enhances shock-induced behavioral inhibition in adult male rats: Implications for impulsive behavior.* PHARMACOL BIOCHEM BEHAV **65**(4) 731–736, 2000.—The effects of gonadectomy on shock-induced behavioral inhibition in a modified Vogel's drinking conflict model and on diazepam-induced disinhibition and sedation were investigated in adult male rats. Gonadectomy enhanced shock-induced behavioral inhibition when determined 9, 21, 45, and 65 days, but not 3 days, after operation, without affecting shock sensitivity or drinking motivation. Testosterone-substitution for 21 days following gonadectomy prevented this enhanced inhibition without significantly affecting the behavior in sham-operated rats. Diazepam produced behavioral disinhibition both in sham-operated and gonadectomized rats. However, after the highest dose (16 mg/kg, IP) the disinhibited behavior decreased only in sham-operated animals, most likely due to sedation. Moreover, whereas there was no difference in basal rotarod-performance between controls and gonadectomized rats, the latter animals were less sensitive to diazepam-induced disruption of rotarod walking ability. Sham-operated or gonadectomized animals did not differ with respect to serum diazepam levels at the postinjection times used in the behavioral tests. Taken together, gonadectomized rats were less sensitive towards diazepam-induced sedation, possibly due to a subsensitivity at or beyond $GABA_A$/benzodiazepine receptors. Furthermore, the finding that lack of testosterone enhanced shock-induced inhibition could be interpreted to reflect increased impulse control and may involve an altered activation of $GABA_A$/benzodiazepine receptors. © 2000 Elsevier Science Inc.

Conflict behavior Diazepam $GABA_A$-receptor Impulsivity Serotonin Testosterone

EVIDENCE has been presented for a link between testosterone and impulsive behavior in humans. Clinical studies and case reports indicate a connection between the use of anabolic steroids and impulsive and aggressive behavior (5,13,25–27,44). Furthermore, women with bulimia nervosa, a disorder possibly involving poor impulse control, display significantly higher serum levels of free testosterone compared to controls (38).

Evidence also indicates a connection between testosterone and aggressive and impulsive behavior in animals. Thus, aggressive behavior seems to be positively correlated to blood testosterone levels in several species, for example, in the rat (9) and in the squirrel monkey (43). Furthermore, in the rat, supraphysiological levels of testosterone have been demonstrated to alleviate behavioral inhibition in the elevated plus-maze (3) and shock-induced behavioral inhibition in Vogel's conflict model (2).

Disinhibited behaviors in these latter models have been interpreted to reflect anxiolytic-like effects. However, these models could just as well reflect the ability of the animal to wait before it acts. Disruption of such an ability, i.e., disinhibited behavior, could in turn be interpreted as impulsive behavior. Thus, enhanced behavioral inhibition could be linked to an increased impulse control. Indeed, it has earlier been suggested (37) that disinhibition in various conflict models may, at least after certain manipulations, reflect impulsive behavior rather than anxiolysis.

Interestingly, earlier studies indicate that testosterone may influence both brain serotonin (5-HT) and $GABA_A$/benzodiazepine receptor activity (see Discussion). Both 5-HT [cf. (12,20–22,37,39)] and GABAergic (18,24) mechanisms have been implicated—and may be intimately connected (32)—in the regulation of conflict behavior and impulse control.

Here we studied the effect of gonadectomy, with or without testosterone substitution, on shock-induced behavioral inhibition in a modified Vogel's drinking conflict model, as well

Requests for reprints should be addressed to A. I. Svensson, Department of Pharmacology, Box 431, SE 405 30, Göteborg, Sweden.

as the effect of gonadectomy on diazepam-induced disinhibition and sedation/muscle relaxation.

METHOD

Animals

Male Sprague–Dawley rats (B&K, Universal AB, Sollentuna, Sweden) weighing 275–350 g were used. The animals were living in colonies and were kept under controlled light–dark conditions (light on at 0500 h and off at 1900 h) and at constant cage temperature (25°C) and humidity (65%). A 7-day adaptation period to the animal maintenance facilities of the department was allowed prior to the start of the experiments. The animals had free access to standard laboratory chow and water when not participating in Vogel's drinking conflict test, drinking motivation, or shock threshold experiments. All animal procedures were approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

Surgery

The rats were anaesthetized with ketamine 50 mg/ml and xylazine 20 mg/ml in a mixture of 2:1, that was injected in a volume of 2 ml/kg (IP). Scrotal incisions were performed, and the main arteries and veins as well as the ductus deferens were located and ligated, after which the testes were removed. Sham-operated rats were exposed to similar scrotal incisions. All the rats weighed at least 275 g when operated upon.

Shock-Induced Behavioral Inhibition

A modified Vogel's drinking conflict model was used (32). Briefly, on the first day of the experiment, the animals were adapted for 10 min to the test box in which they had access to a 5.5% (w/v) glucose solution. Thereafter, the rats were not allowed to drink for 24 h, after which they were again adapted to the test chamber for 10 min, as above. Following a further 24-h period of water deprivation, the animals were returned to the test box and subjected a 30-s period of glucose solution drinking. Upon every further attempt to drink, an electric shock (0.16 mA for 2 s) was administered between the spout of the drinking bottle and the grid floor. The number of shocks accepted during a 10-min session was recorded. All experiments were carried out between 1000 and 1600 h.

Shock Threshold Test and the Drinking Motivation Test

In these tests, the rats were deprived as above and adapted to the test box in a manner identical to that used in Vogel's conflict test. The shock threshold was determined by manually increasing the current delivered through the grid floor. This was done step-wise (12 levels from 0.05 to 0.6 mA) until the rat showed an avoiding reaction to the electrical stimulus (jump, jerk, or similar) as judged by an investigator blind to the treatment and shock level applied. There was a 15-s shock-free interval between each step. Immediately after the shock threshold had been determined each rat was placed in an individual cage that was equipped with a drinking bottle containing 5.5% (w/v) glucose solution. The total amount of liquid (g) consumed during 2 h was recorded for every rat.

Rotarod Performance

For 10 days prior to the experiment the animals were trained to maintain themselves on a rotating treadmill (6 cm in diameter; eight rounds/min) for at least 5 min. Animals unable to sustain 5 min on the rotarod were not included in the study (approximately 10% of the rats). The animals were allowed three consecutive falls from the rotarod, and the cumulated walking time (seconds) during 5 min on the rotarod was recorded. Drugs were administered 30 min prior to the experiment.

Pharmacokinetics

The serum concentration of diazepam in trunk blood was estimated in gonadectomized and sham-operated rats 40 min after injection of diazepam 2 mg/kg IP and after vehicle injections (as a control), by using a reagent system for the detection of diazepam (TDx/TDxFLx Benzodiazepines Serum assay, Abbott Laboratories). The rats were injected 2 months after operation.

Experimental Designs

Shock-induced behavioral inhibition. Three separate experiments were performed. Experiment 1: shock-induced behavioral inhibition was estimated in gonadectomized and sham-operated rats 3, 9, 21, 45, and 65 days after the operation. Each rats was used in only one experiment. Two rats from each treatment group were housed together in a single cage (four rats per cage). Experiment 2: gonadectomized rats were substituted with testosterone following the operation. One group of gonadectomized rats was implanted subcutaneously in the flank with a silastic capsule containing testosterone, and another group was implanted with an empty capsule. The sham-operated rats were also divided into two groups—one receiving testosterone-containing capsules, and one receiving empty capsules. Shock-induced behavioral inhibition was evaluated 21 days after operation. Each rat was used in only one experiment. One rat from each treatment group was housed together in a single cage (four rats per cage). Experiment 3: the effect of diazepam (0, 0.5, 2, 4, 8, and 16 mg/kg IP, injected 30 min prior to the test) on shock-induced behavioral inhibition was studied in sham-operated rats and in gonadectomized nonsubstituted rats, 21 days after the operation. Each rat was used in only one experiment. Two rats from each treatment group were housed together in a single cage (four rats per cage).

Rotarod performance. Twenty-one days after the operation gonadectomized rats and sham-operated rats were compared with respect to rotarod performance after injection of diazepam (0, 2, and 4 mg/kg IP) 30 min prior to placement on the rotarod. Each rat was used in only one experiment. Two rats from each treatment group were housed together in a single cage (four rats per cage).

Drugs

Diazepam (courtesy Hoffmann–LaRoche) was dissolved in a drop of 2 N HCl, diluted with 0.9% NaCl, neutralized with NaOH and administered IP. Silastic capsules (inner diameter 3.18 mm, Dow Corning Corp.) were filled with testosterone (4-androsten-17β-ol-3-one, Sigma Chemical Company, St. Louis, MO) according to an earlier protocol (17). All capsules were incubated in 0.9% NaCl at least 1 day before use, and the saline was exchanged 30 min before implantation. The capsules were 15 mm long, and implants of this size will produce plasma testosterone concentrations of about 1.0 ng/ml (7,36). Ketamine hydrochloride (Parke-Davies, Barcelona,

Spain) and xylazine hydrochloride (Bayer, Leverkusen, Germany) were used for anesthesia.

Statistics

Differences between treatment groups in the shock-induced behavioral inhibition and rotarod performance test were statistically evaluated by means of ANOVA followed by Fisher PLSD (8,42) implemented for comparisons with unequal n:s (1). Student's *t*-test was used to evaluate the difference between treatment groups in serum concentrations of diazepam and to analyze drinking motivation data. The shock threshold data were analyzed using the Mann–Whitney *U*-test. A *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Shock-Induced Behavioral Inhibition

Gonadectomy decreased the number of shocks accepted during a 10-min test session in the Vogel test, and thus enhanced shock-induced behavioral inhibition, when determined 9 and 21, but not 3, 45, and 65 days, after operation (Fig. 1).

In another experiment gonadectomy again enhanced shock-induced behavioral inhibition 21 days after gonadectomy. Substitution with testosterone prevented this enhanced inhibition. The testosterone implants significantly enhanced shock-induced behavioral inhibition in the sham-operated rats (Fig. 2).

Diazepam dose dependently increased the number of shocks accepted in the conflict task, and thus alleviated shock-induced behavioral inhibition almost significantly ($p = 0.05$) in sham-operated (significantly in the dose 8 mg/kg) and significantly in gonadectomized rats. The maximal behavioral response, in relation to baseline responding of the respective groups, was similar in both groups. Furthermore, after the highest dose of diazepam (16 mg/kg, IP) the disinhibited behavior decreased statistically significantly in sham-operated animals, most likely due to sedation (gross observation). No such effect was observed in gonadectomized rats (Fig. 3).

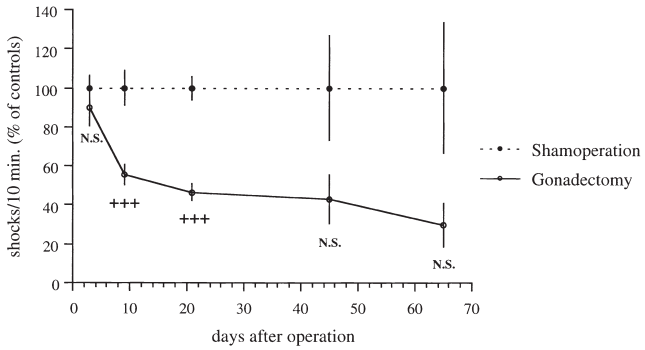

FIG. 1. Effect of gonadectomy on shock-induced behavioral inhibition 3 (sham-operation = sham: $n = 8$, gonadectomy = gon: $n = 8$), 9 (sham: $n = 10$, gon: $n = 10$), 21 (sham: $n = 27$, gon: $n = 21$), 45 (sham: $n = 8$, gon: $n = 7$), and 65 days (sham: $n = 9$, gon: $n = 5$) after operation. Shown are the means ± SEM. Statistics: ANOVA followed by Fisher's PLSD. $+++p < 0.001$ and N.S. = not significant ($p > 0.05$), compared to sham-operated rats at each day after operation.

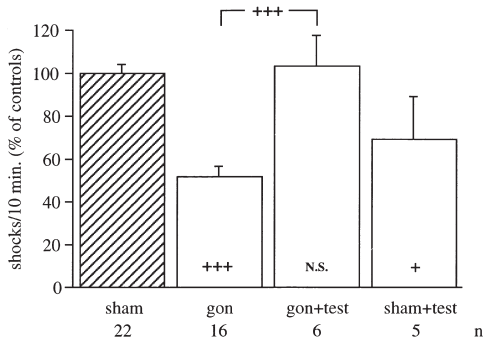

FIG. 2. Effect of substitution with testosterone for 21 days after gonadectomy on shock-induced behavioral inhibition. Shown are the means ± SEM of 5–22 observations. Statistics: ANOVA, $F(3, 45) = 13.77$, $p < 0.001$, followed by Fisher's PLSD. $+p < 0.05$, $+++p < 0.001$, and N.S. = not significant ($p > 0.05$), compared to sham-operated rats, or as indicated.

Shock Threshold and Drinking Motivation Tests

There were no statistically significant differences in shock threshold or drinking motivation between gonadectomized and sham-operated rats (Table 1).

Rotarod Performance

There was no difference in basal rotarod performance between controls and gonadectomized rats (Fig. 4). Diazepam disrupted the rotarod performance in both gonadectomized and sham-operated rats, but the gonadectomized rats were less sensitive to this effect of diazepam than sham-operated rats.

Pharmacokinetics

There was no difference between gonadectomized and sham-operated rats in serum concentrations of diazepam 40 min after diazepam 2 mg/kg IP (gon: 0.186 ± 0.014 μM vs. sham: 0.170 ± 0.031 μM, $p = 0.652$, $n = 9$, Student's *t*-test).

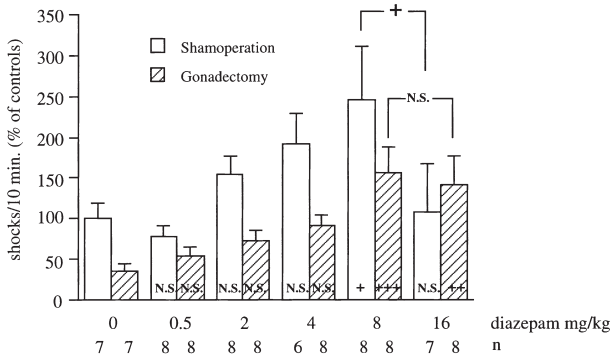

FIG. 3. Effect of diazepam on shock-induced behavioral inhibition in sham-operated and gonadectomized rats. Shown are the means ± SEM of six to eight observations. Statistics: ANOVA [sham-operated: $F(5, 38) = 2.44$, $p = 0.05$; gonadectomized: $F(5, 41) = 4.74$, $p < 0.01$] followed by Fisher's PLSD. $+p < 0.05$, $++p < 0.01$, $+++p < 0.001$, and N.S. = not significant ($p > 0.05$), compared to vehicle-treated rats for sham-operated and gonadectomized rats, respectively, or as indicated.

TABLE 1

EFFECT OF GONADECTOMY ON THE SHOCK THRESHOLD AND ON THE AMOUNT OF 5.5% GLUCOSE SOLUTION CONSUMED DURING 2 H AFTER A 48-H PERIOD OF WATER DEPRIVATION

Treatment	Shock Threshold (mA) (Medians)	Liquid Consumption (ml) (means ± SEM)
Sham operation	0.13	28.7 ± 1.2
Gonadectomy	0.13	29.0 ± 1.2

Shown are the shock threshold and drinking motivation in sham-operated (=sham) and gonadectomized (=gon) rats. Statistics: Shock threshold data: Mann–Whitney *U*-test (sham: $n = 11$, gon: $n = 12$). Drinking motivation data: Student's *t*-test (sham: $n = 6$, gon: $n = 6$). There were no statistically significant differences ($p > 0.05$).

DISCUSSION

In the present study, gonadectomy enhanced shock-induced behavioral inhibition, and this effect could not be explained by an altered shock sensitivity or drinking motivation. Furthermore, the enhancement of shock-induced behavioral inhibition was prevented in rats receiving testosterone substitution after gonadectomy, whereas testosterone in the dose used enhanced shock-induced behavioral inhibition in sham-operated rats. The mechanism underlying this latter effect of testosterone is unclear. However, it is known that testosterone inhibits its own secretion indirectly (28), and hence, endogenous testosterone levels may decrease in testosterone-treated sham-operated rats. The enhanced shock-induced behavioral inhibition induced by gonadectomy was manifest 9 and 21 but not 3, 45, or 65 days after operation.

Interestingly, testosterone levels, measured by using a radioimmunoassay technique, have been reported to be already markedly reduced a few hours after gonadectomy (6,29). Thus, even though testosterone levels probably are markedly reduced within a few hours of gonadectomy, no behavioral consequence of gonadectomy could be observed after 3 days in the present study, whereas 9 days and later postgonadectomy, a marked enhancement of shock-induced behavioral inhibition was observed. This finding is supported by the notion that the half-life of a testosterone-dependent (15) prostate receptor protein for dihydrotestosterone is about 3 to 4 days after gonadectomy (19). Thus, the time lag for the effect of gonadectomy on shock-induced behavioral inhibition could point to an involvement of genomic effects exerted by testosterone. It is interesting to note that effects of castration on sexual behavior and monoamine synthesis (11), as well as on ethanol-induced locomotor stimulation (10), are observed after a similar delay.

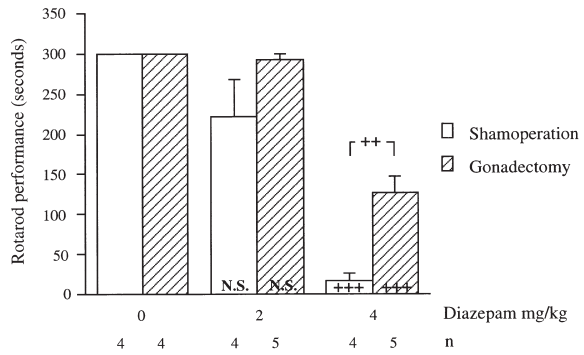

FIG. 4. Effect of diazepam on rotarod performance in sham-operated and gonadectomized rats. Shown are the means ± SEM of four to five observations. Statistics: ANOVA followed by Fisher's PLSD. $++p < 0.01$ and N.S. = not significant ($p > 0.05$), compared to basal rotarod performance in sham-operated and gonadectomized rats, respectively, or as indicated.

Hence, gonadectomy enhanced shock-induced behavioral inhibition in the present experiments. As mentioned in the introduction, enhanced shock-induced behavioral inhibition may reflect an increased impulse control (37). An alternative explanation would be that gonadectomy produces an anxiogenic-like effect and thereby suppresses the behavior in this type of behavioral model that traditionally has been used as an animal anxiety model. However, conclusive evidence for a link between gonadectomy and anxiety in animals or humans is lacking. On the contrary, gonadectomized patients seem to be less restless, and do not demand, for example, increased medication with anxiolytic drugs (16).

As to possible neurochemical mechanisms involved in the above behavioral effects, it is notable that testosterone may be involved in regulating brain 5-HT status. Thus, an increase of 5-HT synthesis rate has been demonstrated earlier 20 to 40 days after gonadectomy in adult male rats (11). This increase was prevented in rats substituted with testosterone. In another study (23), testosterone propionate given every 7 days to male rats produced a decrease in diencephalic 5-HT levels, whereas gonadectomy, instead, increased 5-HT levels in the same area. Hence, a lack of testosterone may promote the synthesis of brain 5-HT, whereas supraphysiological levels of testosterone may instead decrease 5-HT levels. According to earlier investigations, manipulations increasing 5-HT neurotransmission may produce enhanced shock-induced behavioral inhibition [cf. (33)]. This hypothesis, that enhanced behavioral inhibition after gonadectomy involves increased 5-HT neurotransmission has, however, to await neurochemical confirmation, for example, by means of in vivo microdialysis in awake, freely moving rats.

Also, other neurochemical mechanisms possibly involved in the enhancement of shock-induced behavioral inhibition after gonadectomy should, however, be considered. Androsterone (5α-androstan-3α-ol-17-one) and 3α-androstanediol (5α-androstan-3α,17β-diol) are two testosterone metabolites with agonistic properties at the $GABA_A$/benzodiazepine receptor (4,14,30,40). $GABA_A$/benzodiazepine receptor agonists of various kinds are known to induce behavioral disinhibition in Vogel's drinking conflict model [cf. (41)]. Thus, it could be speculated that gonadectomy decreases the levels of these $GABA_A$/benzodiazepine receptor agonistic testosterone metabolites, which may result in enhanced behavioral inhibition.

To investigate a possible involvement of $GABA_A$/benzodiazepine receptor mechanisms in the behavioral effects induced by gonadectomy, the $GABA_A$/benzodiazepine receptor agonist diazepam was applied. Diazepam produced behavioral disinhibition both in gonadectomized and sham-operated rats. It should be noted, however, that in sham-operated rats the disinhibited behavior decreased after the highest dose tested (16 mg/kg), most likely due to sedation (gross observation), compared to that observed after 8 mg/kg diazepam. No such effect was observed in gonadectomized rats. Moreover, gonadectomized rats were less sensitive to diazepam-induced disruption of rotarod performance (muscle relaxation/sedation) compared to sham-operated rats, without displaying any

difference in the basal rotarod performance. These differences in behavioral sensitivity to diazepam probably cannot be explained by pharmacokinetics, because there was no difference in serum concentrations of diazepam between gonadectomized and sham-operated rats. It is, thus, possible that the lower baseline behavior and the lower maximal effect of diazepam observed in the conflict model as well as the decreased sedative and/or muscle-relaxant effects of diazepam in gonadectomized vs. sham-operated rats derive from a lower $GABA_A$/benzodiazepine receptor sensitivity in vivo in gonadectomized animals. To speculate further, this lower sensitivity could depend on a decreased activity of, for example, testosterone metabolites at the receptor complexes. Interestingly, indirect support for this hypothesis has previously been provided (3), demonstrating that supraphysiological levels of testosterone in vivo increase $GABA_A$/benzodiazepine receptor function when examined post mortem in vitro.

As speculated above, both serotonergic and GABAergic mechanisms may thus be involved in mediating the shock-induced behavioral inhibition observed after gonadectomy. In this context it should be noted that it previously has been suggested that GABAergic and serotonergic systems are intimately connected in the regulation of shock-induced behavioral inhibition (31,34,35).

In conclusion, gonadectomy enhanced shock-induced behavioral inhibition, and resulted in a decreased sensitivity to the $GABA_A$/benzodiazepine receptor agonist diazepam, regarding its sedative and/or muscle-relaxant effects in adult male rats. On the basis of previous comments (vide supra), it may be suggested the enhanced shock-induced behavioral inhibition after gonadectomy reflects an increased impulse control. Further investigations are needed to explore the neurochemical mechanisms underlying the enhanced shock-induced behavioral inhibition after gonadectomy.

ACKNOWLEDGEMENTS

This study was financially supported by grants from The Swedish Medical Research Council (No. 4247 and No. 11583), Gothenburg Medical Society, Fonden för studerande av Läkarvetenskapen vid Sahlgrenska Sjukhuset, Magnus Bergvalls Stiftelse, Lundbecks Fond för Psykofarmakologisk Forskning, Novo Nordisk Forskningsfond, Svenska Lundbeckstiftelsen, Åke Wibergs stiftelse, Åhlén-stiftelsen, and Wilhelm och Martina Lundgrens vetenskapsfond. The expert technical assistance of Margit Englund is gratefully acknowledged.

REFERENCES

1. Abacus Concepts: Stat View. Berkeley, CA: Abacus Concepts Inc.; 1992:319–332.
2. Bing, O.; Heilig, M.; Kakoulidis, P.; Sundblad, C.; Wiklund, L.; Eriksson, E.: High doses of testosterone increase anticonflict behaviour in rat. Eur. Neuropsychopharmacol. 8:321–323; 1998.
3. Bitran, D.; Kellogg, C. K.; Hilvers, R. J.: Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical $GABA_A$ receptors in the rat. Horm. Behav. 27:568–583; 1993.
4. Bitran, D.; Hilvers, R. J.; Frye, C. A.; Erskine, M. S.: Chronic anabolic-androgenic steroid treatment affects brain $GABA_A$ receptor-gated chloride ion transport. Life Sci. 7:573–583; 1996.
5. Conacher, G. N.; Workman, D. G.: Violent crime possibly associated with anabolic steroid use. Am. J. Psychiatry 146:679; 1989.
6. Coyotupa, J.; Parlow, A. F.; Kovacic, N.: Serum testosterone and dihydrotestosterone levels following orchiectomy in the adult rat. Endocrinology 92:1579–1581; 1973.
7. Damassa, D. A.; Kobashigawa, D.; Smith, E. R.; Davidson, J. M.: Negative feedback control of LH by testosterone: A quantitative study in male rats. Endocrinology 99:736–742; 1976.
8. Davies, O. L.: Statistical methods in research and production. London: Oliver & Boyd; 1949.
9. DeBold, J. F.; Miczek, K. A.: Sexual dimorphism in the hormonal control of aggressive behavior of rats. Pharmacol. Biochem. Behav. 14(Suppl. 1):89–93; 1981.
10. Engel, J. A.: Influence of age and hormones on the stimulatory and sedative effects of ethanol. In: Rydberg, U.; Alling, C.; Engel, J., eds. Alcohol and the developing brain. New York: Raven Press; 1985:57–67.
11. Engel, J.; Ahlenius, S.; Almgren, O.; Carlsson, A.; Larsson, K.; Södersten, P.: Effects of gonadectomy and hormone replacement on brain monoamine synthesis in male rats. Pharmacol. Biochem. Behav. 10:149–154; 1979.
12. Engel, J. A.; Hjorth, S.; Svensson, K.; Carlsson, A.; Liljequist, S.: Anticonflict effect of the putative serotonin receptor agonist 8-hydroxy-2-(DI-n-propylamino)tetralin (8-OH-DPAT). Eur. J. Pharmacol. 105:365–368; 1984.
13. Galligani, N.; Renck, A.; Hansen, S.: Personality profile of men using anabolic androgenic steroids. Horm. Behav. 30:170–175; 1996.
14. Gee, K. W.; Bolger, M. B.; Brinton, R. E.; Coirini, H.; McEwen, B. S.: Steroid modulation of the chloride ionophore in the rat brain: Structure–activity requirements, regional dependence an mechanism of action. J. Pharmacol. Exp. Ther. 246:803–812; 1988.
15. Guthrie, P. D.; Freeman, M. R.; Liao, S. T., Chung, L. W.: Regulation of gene expression in rat prostate by androgen and beta-adrenergic receptor pathways. Mol. Endocrinol. 4:1343–1353; 1990.
16. Hedelin, H.; personal communication; 1997.
17. Legan, S. J.; Coon, G. A.; Karsch, F. J.: Role of estrogen as initiator of daily LH surges in the ovariectomized rat. Endocrinology 96:50–56; 1975.
18. Liljequist, S.; Engel, J. A.: The effects of GABA and benzodiazepine receptor antagonists on the anti-conflict actions of diazepam or ethanol. Pharmacol. Biochem. Behav. 21:521–525; 1984.
19. Liao, S.: Receptors and the mechanism of action of androgenes. In: Pasqualani, J. R., ed. Receptors and mechanism of action of steroid hormones, Part 1. New York: Decker; 1977: 159–214.
20. Lidberg, L.; Tuck, J. R.; Åsberg, M.; Scalia-Tomba, G. P.; Bertilsson, L.: Homicide, suicide and CSF 5-HIAA. Acta Psychiatr. Scand. 71:230–236; 1985.
21. Linnoila, M.; Virkkunen, M.; Scheinin, M.; Nuutila, A.; Rimon, R.; Goodwin, F. K.: Low cerebrospinal fluid 5-hydroxyindoleacetic acid concentration differentiates impulsive from nonimpulsive violent behavior. Life Sci. 33:2609–2614; 1983.
22. Linnoila, M.; Virkkunen, M.: Aggression, suicidality and serotonin. J. Clin. Psychiatry Suppl. 53:10:46–51; 1992.
23. Martinez-Conde, E.; Leret, M. L.; Diaz, S.: The influence of testosterone in the brain of the male rat on levels of serotonin (5-HT) and hydroxyindole-acetic acid (5-HIAA). Comp. Biochem. Physiol. 80C:411–414; 1985.
24. Miczek, K. A.; Weerts, E. M.; Vivian, J. A.; Barros, H. M.: Aggression, anxiety and vocalizations in animals: GABAA and 5-HT anxiolytics. Psychopharmacology (Berlin) 121:38–56; 1995.
25. Pope, H. G.; Katz, D. L.: Affective and psychotic symptoms associated with anabolic steroid use. Am. J. Psychiatry 145:487–490; 1988.
26. Pope, H. G.; Katz, D. L.: Homicide and near-homicide by anabolic steroid users. J. Clin. Psychiatry 51:28–31; 1990.
27. Pope, H. G.; Katz, D. L.: Psychiatric and medical effects of anabolic-androgenic steroid use. Arch. Gen. Psychiatry 51:375–382; 1994.
28. Rang, H. P.; Dale, M. M.: In: Pharmacology. Edinburgh: Churchill Livingstone; 1991:542.
29. Schwartz, N. B.; Justo, S. N.: Acute changes in serum gonadotrophins and steroids following orchidectomy in the rat: Role of the adrenal gland. Endocrinology 100:1550–1556; 1977.
30. Simmonds, M. A.; Turner, J. P.; Harrison, N. L.: Interactions of steroid with the GABA-A receptor complex. Neuropharmacology 23:877–878; 1984.

31. Söderpalm, B.; Engel, J. A.: Does the PCPA induced anticonflict effect involve activation of the $GABA_A$/benzodiazepine chloride ionophore receptor complex? J. Neural Transm. 76:145–153; 1989.
32. Söderpalm, B.: On the neuropharmacology of conflict behaviour. Studies on noradrenergic, serotonergic and GABAergic mechanisms in experimental anxiety in the rat. Thesis. ISBN 91-626-0175-9; 1990.
33. Söderpalm, B.; Engel, J. A.: Serotonergic involvement in conflict behaviour. Eur. Neuropsychopharmacol. 1:7–13; 1990.
34. Söderpalm, B.; Engel, J. A.: Involvement of the $GABA_A$/benzodiazepine chloride ionophore receptor complex in the 5,7-DHT-induced anticonflict effect. Life Sci. 49:139–153; 1991.
35. Söderpalm, B.; Engel, J. A.: The 5,7-DHT-induced anticonflict effect is dependent on intact adrenocortical function. Life Sci. 51:315–326; 1992.
36. Södersten, P.; Damassa, D. A.; Smith, E. R.: Sexual behavior in developing male rats. Horm. Behav. 8:320–341; 1977.
37. Soubrié, Ph.: Reconciling the role of central serotonin neurons in human and animal behaviour. Behav. Brain Sci. 9:319–364; 1986.
38. Sundblad, C.; Bergman, L.; Eriksson, E.: High levels of free testosterone in women with bulimia nervosa. Acta. Psychiatr. Scand. 90:397–398; 1994.
39. Träskman-Bendz, L.; Åsberg, M.; Schalling, D.: Serotonergic function and suicidal behavior in personality disorders. Ann. NY Acad. Sci. 487:168–174; 1986.
40. Turner, D. M.; Ransom, R. W.; Yang, J. S.-J.; Olsen, R. W.: Steroid anesthetics and naturally occurring analogs modulate the (gamma)-aminobutyric acid receptor complex at a site distinct from barbituates. J. Pharmacol. Exp. Ther. 248:960–966; 1989.
41. Vogel, J. R.; Beer, B.; Clody, D. E.: A simple and reliable conflict procedure for testing anti-anxiety agents. Psychopharmacologia 21:1–7; 1971.
42. Winer, B. S.: Statistical principles in experimental design. New York: McGraw-Hill; 1971.
43. Winslow, J. T.; Miczek, K. A.: Androgen dependency of alcohol effects on aggressive behavior: A seasonal rhythm in high-ranking squirrel monkeys. Psychopharmacology (Berlin) 95:92–98; 1988.
44. Yates, W. R.; Perry, P.; Murray, S.: Aggression and hostility in anabolic steroid users. Biol. Psychiatry 31:1232–1234; 1992.