acta ethologica
1998, Vol.I (1-2), pp. 43-48

Short-term social modulation of 11-ketotestosterone levels in males of the cichlid fish *Oreochromis mossambicus* during male-female interactions.

Rita A. Borges [1], Rui F. Oliveira [1*], Vitor C. Almada [1] & Adelino V. M. Canário [2].

[1] *Unidade de Investigação em Eco-Etologia, Instituto Superior de Psicologia Aplicada, Rua Jardim do Tabaco, 44, 1100 Lisboa, Portugal (ruiol@ispa.pt).*

[2] *Unidade de Ciências e Tecnologias dos Recursos Aquáticos, Universidade do Algarve, Campus de Gambelas, 8000 Faro, Portugal.*

After 48 hours of social isolation, male individuals of *Oreochromis mossambicus* were allowed to interact with a conspecific female in the same aquarium during a period of up to six hours. Colouration exhibited, and frequency of courting and agonistic behaviours performed by the males were recorded before and after introduction of the female stimulus. Urine samples were obtained after social isolation (T0) and six hours after the introduction of the female (T6). Male androgen (testosterone and 11-ketotestosterone, 11-KT) concentrations measured in the urine, at T0 were not correlated to subsequent levels of agonistic and sexual behaviours. However, behavioural interactions were correlated to 11-KT levels at T6. These results suggest a short-term social modulation of 11-KT levels by male-female interaction.

Key-words: androgens, sex steroids, sexual behaviour.

Introduction

In vertebrates, the relationship between hormones and behaviour is bi-directional. While the role of hormones in the causation of behaviour has been well established

* Author to whom correspondence should be addressed.

This study was funded by Praxis XXI and FEDER (Praxis/2/2.1/BIA/370/94). During part of this study Rita Borges was being supported by a M.Sc. research grant from FCT (Program Praxis XXI).

there is growing evidence that hormonal levels may themselves be modulated by behavioural interactions (e.g. Harding & Follett, 1979; Wingfield et al., 1987; Cardwell & Liley, 1991; Creel et al., 1993). In teleost fishes, androgens testosterone (T) and 11-ketotestosterone (l l-KT) are known to influence the expression of secondary sexual characters and several aspects of male reproductive behaviour, namely courtship and territorial aggression (for a review see Liley & Stacey, 1983 and Borg, 1994). Conversely, levels of these androgens have been shown to be socially modulated (Hannes et al. 1984; Hannes, 1986; Cardwell & Liley, 1991; Francis et al., 1993; Pankhurst & Barnett, 1993; Oliveira et al., 1996).

O. mossambicus is an African mouthbrooding cichlid fish in which males aggregate in arenas where they establish breeding territories. Males dig spawning pits in their territory, to which they attract females to mate (Brutton & Boltt, 1975). After spawning females leave the arena with the eggs in their mouths. Parental care is exclusively maternal.

Oliveira et al. (1996) showed that in males kept in isolation and subsequently exposed to other males, the androgen levels at the onset of the experiment were poor predictors of social dominance, while social status was a good predictor of the androgen levels measured after group formation, thus providing evidence of social modulation.

In the present paper we investigate the effects of male-female interactions on androgen levels of the male.

Methods

Fish used in the present study belonged to a strain kept in our laboratory (see Oliveira et al., 1996 for details of strain's previous history). Fish were kept at 25 ± 1°C temperature, under a 12D:12N photoperiod and were fed daily with commercial food for tropical aquarium fish.

At the beginning of the experiment, males (n=13) were individually isolated for 48 hrs in 122 l aquaria, in order to control for possible effects of prior experience. After this period of social isolation, a receptive female (primed with an intraperitoneal injection of 200µl saline containing 5µg des-Gly^{10}, [D-Ala^6]-LHRH ethylamide, Sigma) was introduced in the male's tank and the pair was allowed to interact for 6 hrs. Each female was used only once as a stimulus to avoid any sequence effect on the experimental design.

Urine was collected from each male after the period of social isolation and immediately before the introduction of the female at the tank (T_0) and at the end of 6 hrs male-female interaction (T_6) (for details of urine collection see Oliveira et al., 1996). Urine collection was successful for both T_0 and T_6 in only 8 out of the 13 males, and thus steroid analysis is restricted to these males. Behavioural observations were performed using focal sampling (Martin & Bateson, 1993) at 30 min, one hr, two hrs and six hrs after the introduction of the female.

At each 10 min focal sampling interval, the following variables were recorded:

a) frequency of sexual and agonistic behaviours, based on a description of the behavioural patterns given by Baerends and Baerends van Roon (1950);

b) the intensity of the male colour pattern measured in an ordinal scale, from zero to four, according to the degree of darkness (the minimal increment was 0.25): 0= Neutral; l= Dark l; 2= Dark2; 3= Dark3; 4= Black; the colouration patterns considered were described by Neil (1964);

c) the position of the fish was marked at 10 sec intervals during two min of sampling on a grid with 32 cells marked on the front wall of the aquaria (each cell having 8×4 cm). The degree of pair bonding was measured as the number of times the two individuals were seen in the same or contiguous grid cells over the total number of records (n=l 2).

For steroid analysis, urine samples were extracted for free, glucuronide and sulphate fractions as described by Oliveira et al. (1996). Androgen concentrations were measured using radioimmunoassays (RIA). Details of the T and 11-KT RIAs are described by Scott et al. (1984) and Scott & Sumpter (1988), respectively.

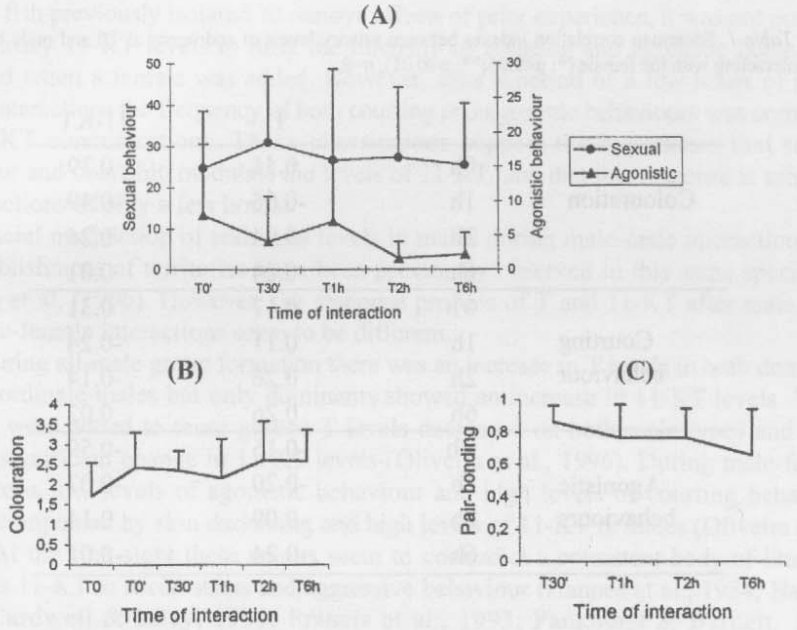

Figure 1. Behavioural variables sampled after the introduction of the female (N= 13 males): (a) frequency of sexual (●) and agonistic (▲) behaviours during each trial; (b) dark colouration of the male; (c) pair-bonding measured as a proximity measure (for further details see text); error bars represent the standard variation of the means.

Results

Results of behavioural observations are summarised in Fig. 1. In the presence of females, males adopted mainly sexual behaviours, while the agonistic behaviours were low and decreased during the 6 hrs period (Fig. la). Immediately after the introduction of a female ($T_{0'}$), males became darker (Wilcoxon Matched Pairs Test between T_0 and $T_{0'}$ scores: n = 13, z = 2.09, p = 0.04) and maintained on average a Dark 2 – Dark 3 colouration throughout the 6h of experiment (Fig. lb). Pair bond scores were consistently higher than 50%, beginning 30 min after the introduction of the female (Fig. lc). Taken together, these results indicate that males were sexually motivated and were actively courting the females.

In order to get an insight into the causal relationships between androgen levels and behaviours, correlation coefficients were calculated between: (a) androgen levels prior to the male-female interaction (T_0) and subsequent behavioural variables measured during the interactions (Table 1); (b) androgen levels after male-female interactions (T_6) and the behavioural variables measured during the interactions (Table 2). If baseline androgen levels play an effective role in the causation of reproductive behaviour we would predict that the androgen levels at T_0 would correlate well with the behavioural variables measured subsequently. Conversely, if male-female interactions modulate androgen levels we predict good correlations between the behavioural variables and

Table 1. Spearman correlation indexes between urinary levels of androgens at T0 and male behaviours when interacting with the female (*: $p<0.05$;**: $p<0.01$), n=8.

		T	11-KT
Colouration	0h	0.51	0.39
	1h	-0.05	-0.49
	2h	-0.04	-0.24
	6h	-0.04	0.01
Courting behaviour	0'h	0.17	0.31
	1h	0.11	-0.24
	2h	0.26	-0.14
	6h	0.26	0.02
Agonistic behaviours	0'h	-0.15	-0.59
	1h	-0.20	-0.07
	2h	-0.09	0.14
	6h	-0.24	-0.01

Table 2. Spearman correlation indexes between urinary levels of androgens at T6 and male behaviours when interacting with the female (*: $p<0.05$;**: $p<0.01$), n=8.

		T	11-KT
Colouration	0h	0.51	0.05
	1h	0.15	0.44
	2h	0.24	0.67
	6h	0.10	0.63
Courting behaviour	0'h	-0.71	-0.17
	1h	0.25	0.56
	2h	0.45	0.76*
	6h	0.45	0.71*
Agonistic Behaviour	0'h	0.11	-0.06
	1h	-0.32	-0.84**
	2h	-0.29	-0.76*
	6h	-0.44	-0.91**

androgen levels at T_6. From Tables 1 and 2 it can be shown that neither T nor 11-KT levels measured at T_0 were good predictors of behavioural variables and only the levels of 11-KT at T_6 were well correlated with the behavioural variables.

Owing to the high number of correlations computed it could be argued that some of them are significant by chance alone. However, the relevant point is not the value of each individual correlation coefficient but the consistency with which distinct measures of reproductive behaviour predict the level of 11-KT at T_6.

Discussion

In fish previously isolated, to remove effects of prior experience, it was not possible from urinary 11-KT levels to infer the intensity of courting and agonistic behaviours exhibited when a female was added. However, after a period of a few hours of male-female interactions the frequency of both courting and agonistic behaviours was correlated with ll-KT concentrations. These observations support the hypothesis that sexual behaviour and courtship modulate the levels of 11-KT, and that this response is sensitive to interactions of only a few hours.

Social modulation of androgen levels in males during male-male interactions and the establishment of territories have been previously observed in this same species by Oliveira et al. (1996). However, the response profiles of T and 11-KT after male-male and male-female interactions seem to be different.

During all-male group formation there was an increase in T levels in both dominant and subordinate males but only dominants showed an increase in 11-KT levels. When females were added to these groups T levels decreased on both male types and there was no significant change in 11-KT levels (Oliveira et al., 1996). During male-female interactions low levels of agonistic behaviour and high levels of courting behaviour were accompanied by skin darkening and high levels of 11-KT in males (Oliveira et al., 1996). At the first sight these results seem to contradict a consistent body of literature that links 11-KT to social status and aggressive behaviour (Hannes et al., 1984; Hannes, 1986; Cardwell & Liley, 1991; Francis et al., 1993; Pankhurst & Barnett, 1993; Oliveira et al., 1996). However, in *O. mossambicus* agonistic and sexual behaviours are part of the same behavioural axis (Oliveira & Almada, 1998).

We hypothesize that 11-KT is linked to this axis and that the direction of the response depends upon the context in which the individual is placed. When interacting with other males, high levels of agonistic encounters lead to high levels of 11-KT in dominant males (these individuals will also become reproductively mature) (Oliveira et al., 1996; Oliveira & Almada, unpublished data). Addition of reproductively mature females to an all male group lead to a decrease in aggressiveness and to an increase in sexual behaviours, maintaining in this way high levels of 11-KT in dominant males (Oliveira et al., 1996). Introduction of females to a male kept isolated stimulates sexual behaviour and causes an increase in 11-KT despite a decrease in aggressiveness (this study). In this way greater plasticity is allowed in the regulation of behaviour, with an economy of energy since the same hormone can be simultaneously associated with distinct male behaviours that need to be operational during reproduction. Thus, we suggest that the effects of 11-KT on reproductive behaviour can be seen as permissive. The presence of 11-KT is required (or permits) the production of reproductive behaviour, both agonism and courtship, but the specific type of behaviour produced may depend

on other hormones or neurotransmitters as shown for other species (e.g. Moore et al., 1994). In conclusion, this paper provides further evidence that sexual behaviour and courtship modulate the levels of 11-KT, and that this response is sensitive to interactions of only a few hours. It is also one of the first studies to use urinary steroids to analyse the interactions between steroids and behaviour, with the advantage that is a less intrusive method than blood collection.

References

Baerends, G. P. & Baerends van Roon, J. M. (1950). An introduction to the ethology of cichlid fishes. *Behaviour*, suppl. **1**, 1-242.

Borg, B. (1994). Androgens in teleost fishes. *Comp. Biochem. Physiol. C*, **109**, 219-245.

Brutton, M. N. & Boltt, R. E. (1975). Aspects of the biology of *Tilapia mossambica* Peters (Pisces: Cichlidae) in a natural freshwater lake (Lake Sibaya, South Africa). *J. Fish. Biol.*, 7, 423-445.

Cardwell, J. R. & Liley, N. R. (1991). Androgen control of social status in males of a wild population of stop-light parrotfish, *Sparisoma viride* (Scaridae). *Horm. Behav.*, **25**, 1-18.

Creel, S., Wildt, D. E. & Monfort, S. L. (1993). Aggression, reproduction, and androgens in wild dwarf mongooses: a test of the challenge hypothesis. *Am. Nat.*, **141**, 816-825.

Francis, R. C., Soma, K. & Fernald, R. D. (1993). Social regulation of the brain-pituitary-gonadal axis. *Proc. Natl. Acad. Sci. USA*, **90**, 7794-7798.

Hannes, R.-P. (1986). Blood and whole-body androgen concentrations of male swordtails correlated with aggression measures in a standard-opponent test. *Aggr. Behav.*, **12**, 249-254.

Hannes, R.-P., Franck, D. & Liemann, F. (1984). Effects of rank-order fights on whole-body and blood concentrations of androgens and corticosteroids in the male swordtail (*Xiphophorus helleri*). *Z. Tierpsychol.*, **65**, 53-65.

Harding, C. F. & B. K. Follett (1979). Hormone changes triggered by agression in a natural population of blackbirds. *Science*, **203**, 918-920.

Liley, N. R. & N. E. Stacey (1983). Hormones, pheromones, and reproductive behavior in fish. In W. S. Hoar, D. J. Randall, & E. M. Donaldson (Eds.), *Fish Physiology* (Vol. 9B, pp.1-63). New York: Academic Press.

Martin, P. & Bateson, P. (1993). *Measuring behaviour - an introduction guide*, 2nd Ed. Cambridge: Cambridge Univ. Press.

Moore, F. L., Lowry, C. A. & Rose, J. D. (1994). Steroid-neuropeptide interactions that control reproductive behaviors in an amphibian. *Psychoneuroendocrinol.*, **19**, 581- 592.

Neil, E. H. (1964). An analysis of color changes and social behavior of *Tilapia mossambica*. *Univ. Calif. Publ. Zool.*, **75**, 1-58.

Oliveira, R. F., Almada, V. C., Canário, A. V. M. (1996). Social modulation of sex steroid concentrations in the urine of male cichlid fish *Oreochromis mossambicus*. *Horm. Behav.*, **30**, 2-12.

Oliveira, R. F. & V. C. Almada (1998). Dynamics of social interactions during group formation in the cichlid fish *Oreochromis mossambicus*. *Acta Ethol.*, **1**, 57-70.

Pankhurst, N. W. & C. W. Barnett (1993). Relationship of population density, territorial interaction and plasma levels of gonadal steroids in spawning male demoiselles *Chromis dispilus* (Pisces: Pomacentridae). *Gen. Comp. Endocrinol.*, **90**, 168-176.

Scott, A. P. & Sumpter, J. P. (1988). Seasonal variations in testicular germ cell stages and in plasma concentrations of sex steroids in male rainbow trout (*Salmo gairdneri*) maturing at 2 years old. *Gen Comp. Endocrinol.*, **73**, 46-58.

Scott, A. P., Mackenzie, D. S. & Stacey, N. E. (1984). Endocrine changes during natural spawning in the white sucker, *Catostomus commersoni*. II. Steroid hormones. *Gen comp. Endocrinol.*, **56**, 349-359.

Wingfield, J. C., Ball, G. F., Dufty Jr., A. M., Hegner, R. E. & Ramenofsky, M. (1987). Testosterone and aggression in birds. *Am. Sci.*, **75**, 602-608.

Communicated by Manuel Eduardo dos Santos

(*Received* November 3, 1997. *Accepted* February 19, 1998)