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Neuroendocrine Response to Stress In Tame Versus Untame Grasscutters (*Thryonomys Swinderianus*)

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Abstract*: Ninety three grasscutters (Thryonomys Swinderianus) were sampled from various agro ecological areas of Benin and subjected to docility test by a scoring method. They were then submitted to blood sample collection and assaying of plasma concentration of catecholamines (adrenalin, noradrenalin) by HPLC as well as glucose concentration using Trinder's method. Multivariate analysis (MCA) revealed three distinct categories of animals according to docility and levels of catecholamines and glucose concentration. These results suggest good prospects of using catecholamines from the sympathic nervous system as physiological indicator traits for selection on docility in grasscutter.*
Key words *- catecholamines, docility, glycaemia, grasscutter, stress*

I. Introduction

Grasscutter (Thryonomys Swinderianus) is a wild rodent which is widespread in Africa and particularly in Benin. The exposure to a strong hunting pressure represented for this species a threat of extinction. In fact, the meat of this rodent is highly demanded for human consummation because of it organoleptic quality. Grasscutter breeding in tied captivity was initiated in Benin [1] in order to prevent for extinction of this species and to ensure at the same time the request of glasscutters' meat consumers. Efforts have been made first to control breeding management including feeding, housing and health care and then directed to selection on docility as well as productive and reproductive traits such as body weight and litter size. Following Belyaev (1979) [2], selection in wild animals raised in captivity is likely to induce a destabilization of the endocrine regulation system of ontogenesis and reproduction. Stress and temperament control by endocrine system is well known. It's also thinkable that, catecholamines (adrenalin, noradrenalin, and dopamine) can be used as physiological indicators of docility. The purpose of this work is to assess variability in plasmatic blood concentrations of neurotransmitters in grasscutters and to establish a link with docility of grasscutters undergoing domestication process. Prospects of using neurotransmitters as physiological indicator traits for selection was also examined.

II. Materials And Methods

2.1. Animal material

Experimental animals consists of 93 male grasscutters aged three to five months originated from four departments of Benin i.e. Atacora, Atlantic, Mono and Littoral. The numbers of animals by locality and department are presented in Table 1. Sampling of animals took place from March 2012 to November 2013.

Table 1: Numbers of the animals sampled by locality and department

Department	Locality of source	
Atacora	Natitingou	4
	AV	5
	Ayimevo	3
	Dekoungbé	6
Atlantique	Pahou	8
	PK_14	38
	SEAG	2
	SO_AVA	4
Mono	Ahieme	9
	Agla	1
Littoral	Akpakpa	8
	Gbégamey	5
Total		93

2.2. Test on docility

The docility test were carried out early in the morning before feeding. A scoring method using a behavioural scale along the lines with Kukekova et al. (2008) [3; 4], Albert et al. (2009) [5] was performed. The experimenter presents himself in front of the cage of the animal. The score of the animal is 4, if it flees because of fear. It obtains score 3, if it flees when the experimenter moves towards him. It is given score 2 when it flees only by the attempt of the experimenter to touch him. It obtains score 1, if it lets itself gently stroke.

2.3. Blood sampling and biological analyses

Animals received first an anaesthetic dose of 0.1 ml per live weight. Anaesthetic was administered by intramuscular injection at the underside of the tail's base. It is a compound in equal proportion of Xylazine hydrochloride: ROMPUNND and Ketamine hydrochloride: KETAVETND. The Blood was taken from fasted animals, early in the morning (07 o'clock) and was directed by cardiac puncture [6] Blood samples was collected in EDTA tubes and centrifuged at 3000 revolutions per minute. From blood plasma samples, catecholamines were assayed by HPLC [7; 8] whereas glycaemia was determined by Trinder's method [9].

2.4 Statistical analysis

Statistical analysis was performed using the SAS package [10] and the procedures CORRESP for MCA and GLM for AOV. Variables studied were docility (DOC), glycaemia and plasmatic concentrations of adrenalin (ADR) and noradrenalin (NADR). Three levels referred to as low, medium and high were defined. For example, the classes ADR_S, ADR_M and ADR_L correspond respectively to the levels low, medium and high of adrenalin concentration. The levels of noradrenalin concentration and glycaemia are derived analogously. As for the character docility, its levels are defined by the scores: low, medium and high, where the highest score correspond to the panicking animals, whereas the medium and low scores represent the moderately docile and very docile animals, respectively.

III. Results And Discussions

The mean values (±SEM) of physiologic parameters assayed, i.e. adrenalin, noradrenalin, those of glycaemia and docility scores and multiple correlations are summarized in table 2.

Table 2: Mean values of physiologic parameters assayed: plasmatic concentrations of catecholamine (adrenalin, noradrenalin), that of glycaemia, docility scores and multiple correlations.

	Adrenalin	Noradrenalin	Glycaemia	Docility
	ADR (ng/ml)	NADR (ng/ml)	GL (g/L)	DOC (score)
Mean ± SEM	0.029 ± 0.003	0.597 ± 0.072	1.468 ± 0.150	1.988 ± 0.137
Min.	0.010	0.020	0.233	1.000
Max.	0.080	1.74	3.873	3.800
Multiple Correlations				
ADR				
NADR	0.4735			
GL	- 0.2091	- 0.1641		
DOC	0.3743	0.1418	0.2996	

Mean values obtained in the present study for glycaemia compare well with those found by Farougou (1992) [6] and Akpona (1992) [11]. Values reported by the first author range from 1.47g/l to 2.32g/l and those documented by the second one vary from 1.13g/l to 2.6g/. Similar results (1.56g/l) were reported by Clark (1986) [12] in agouti (*Dasyprocta leporina*). Mean value of 1.6g/l were also obtained by Coudert (1978) [13] for glycaemia in rabbit (Oryctolagus cuniculus). Wirth-Dzięciołowska et al (2009) [14] meanwhile assayed an average blood sugar of 1.3g/l in laboratory mice (*Mus musculus*). However, Al-Qarawi (2004) [15] and Padilla et al (2000) [16] reported lower average values for blood glucose in ruminants, respectively 0.61g/l in lambs (*Ovis aries*) and 0.9162g/l in deer (*Cervus elaphus*).

Average plasma concentration of adrenalin (Table 2) obtained in this study are similar to those recorded by Unceta et al (2001) and Sabbioni et al (2004) in rat [17]. Values found by these authors ranged from 0.002 to 0.6 ng/ml. Average blood adrenalin concentrations recorded by Ambad et al (2009) [18] in depressive patients (0.036 ng/ml) compare also with those obtained in the present study. In contrast, blood adrenalin concentrations assayed by Helge et al (2003) [19] in people exposed to stress are comparatively higher (0.128 ng/ml). Comparative higher values (from 0.07 to 1.2 ng/ml) to those obtain in the present study were reported for blood adrenalin concentration by Künzl et al (1999; 2003) [20; 21] in guinea pigs (*Cavea aperea*) and by De Boer et al (1987; 1990) [27; 28] and Enigk et al (2014) [22] in rats as well as by Wang et al (1999) [17] in rat and mice. More or less high values of plasmatic adrenalin concentration are documented in sheep by Thompson et al (1978) [23], Paulick et al (1988) [24] and Coburn et al (2010) [25]. These are 0.07 ng/ml, 0.3 ng/ml and 1.2

ng/ml respectively. In the shark, Hight et al (2007) [26] reported values of up to 20.331 ng/ml, for blood adrenalin concentration.

The mean value found for plasmatic concentration of noradrenalin in the present work compare well with those reported by Künzl et al (1999) [20] in non-aggressive guinea pigs (0.4 ng/ml) and Coburn et al (2010) [25] in non-aggressive sheep (0.6 ng/ml). But it is higher than those reported by Wang et al (1999) [17] in mice (0.38ng/ml), by De Boer et al (1987, 1990) [27; 28] in stressed rat (0.349 ng/ml and 0.409 ng/ml respectively), by Thompson et al (1978) in non-stressed sheep (0.24 ng/ml), by Helge et al (2002) [19] in inactive persons (0.219 ng/ml) and by Ambad et al (2009) [19] in depressed people (0.386 ng/ml). Künzl et al (1999) [20], found in contrast a higher mean value (1.7 ng/ml) for noradrenalin plasmatic concentration in aggressive guinea pigs. Unceta et al (2001) and Sabbioni et al (2004) [17] quoted however reference values from 0.07 to 0.94 ng/ml for plasmatic noradrenalin concentration. Higher plasma or serum concentrations of noradrenalin (2.979 ng/ml and 4.453 ng/ml respectively), were also reported by Enigk et al (2014) [22] in rats, also by Paulick et al (1988) (0.66 ng/ml) [24] in sheep, and by Helge et al (2002) [19] in very active persons (0.743 ng/ml). Hight et al (2007) [26] reported a much higher noradrenalin concentration, 22.07 ng/ml in shark.

Multiple correlations found between the variables studied (Table 2) reflect the existence of more or less closer link between the neurotransmitters (adrenalin, noradrenalin), glycaemia and docility.

The two-dimensional multiple correspondence analysis plot explaining about 75% of the total inertia is displayed in Fig 1. The first axis (Dim 1) opposes fearful grasscutters to very docile ones. Fearful animals also referred to as cowards are characterised by a high level of plasmatic adrenalin concentration whereas very docile ones exhibit a low adrenalin release and medium glycaemia level. The second axis (Dim 2) opposes meanwhile, grasscutter with low level of plasmatic noradrenalin to those with medium to high level of blood noradrenalin concentration. The first axis seems to be determined by the catecholamines released by the adrenal medulla (adrenalin) whereas the second axis is determined by the sympathetic noradrenalin.

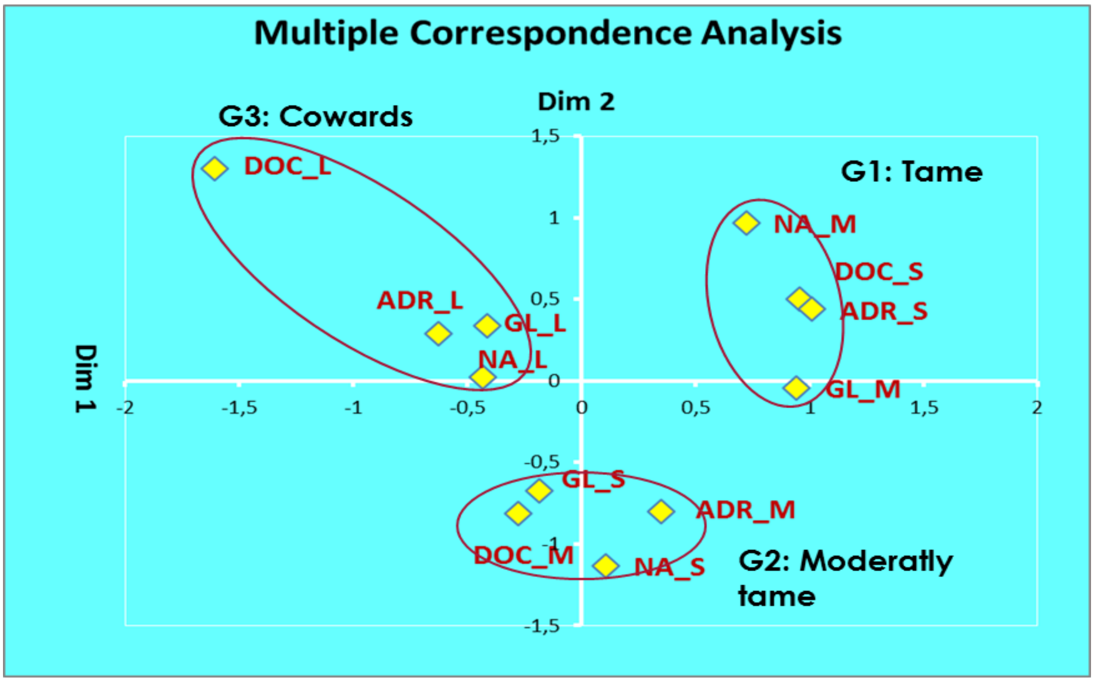

Figure 1: Results of the MCA and regrouping of the animals by category

As shown in Fig 1, MCA results reveal three groups of animals (G1, G2 and G3) with regard to levels of plasma adrenaline concentration and docility. The first group (G1) is characterised by the association of a low score of docility (<1.5) with a low adrenaline level (<0.02 ng/ml) and medium blood noradrenaline (from 0.32 to 0.465 ng/ml) and glucose (from 0.8 to 1.365 g/L) concentrations. This group can be called the docile or tame group. The second group, that of the moderately tame grasscutters show the association of having a medium score of docility (from 1.5 to 3), a moderate concentration of adrenalin (from 0.02 to 0.025 ng/ml) but week concentrations of blood noradrenalin (< 0.32 ng/ml) and glucose (< 0.8 g/L). As for the third group (G3), here referred to as that of the cowards, it associates a high score of docility (≥ 3) with very high plasmatic concentrations of adrenalin (≥ 0.025 ng/ml) and noradrenalin (≥ 0.465 ng/ml) and a high level of glycaemia (≥ 0.8 g/l).

Results of MCA provide evidence, that neurotransmitters profile can be used as physiologic marker of docility in grasscutter, as docile animals exhibit a low level of neurotransmitters release whereas non-docile animals are associated with a high level of neurotransmitters coupled with a high glycaemia.

These results are consistent with those of Thompson et al (1978) [23], De Boer et al (1987; 1990) [27; 28], Künzl et al (1999; 2003) [20; 21], Ambad et al (2009) [18], Coburn et al (2010) [25], Enigk et al (2014) [22], Hight et al (2007) [26], Ndlovu et al (2008) [29] and Higashiyama et al (2009) [30], who showed a significant difference in behaviour of animals with respect to their catecholamine release. Accordingly, fearing animals are subject to a significant higher rise of catecholamine and glucose, compared to their tame ones. Blood plasma concentrations of adrenalin and noradrenalin reflect the reactivity of the autonomic nervous system [21] and blood glucose concentration, the energetic metabolism of living things.

Indeed, the increase in the concentrations of catecholamines and glucose observed in the stressed animals can be explained by the existence of a psychological activity as a response to environmental changes [27]. This activity may be caused by the stimulation of the sympathetic system and the adrenal medulla leading to the release of noradrenalin and adrenalin. Adrenalin might in tern accelerate heart and respiratory rhythm in order to supply muscles with oxygen required. Adrenalin might also be responsible for glycogenolysis and consequently for the rise of glycaemia, as glucose is needed by fearing animals for escape. On the other hand, the low concentration of catecholamines recorded in docile animals reflects a weak reactivity of autonomous nervous system to the stress caused by human contact [20]. This weak reactivity might result in a weak alertness, a low irritability and a poor sensitivity of tame grasscutters, compared to their fearful counterparts. This is consistent with Künzl's results from his experiment on aggressive versus non-aggressive guinea pigs [20]. Reduced reactivity is also required by captive animals in order to adapt to the human environment.

IV. Conclusion

Results reveal that the docility status of animals is significant for variation in the physiological traits assayed. Inversely physiological parameters such as plasmatic concentrations of catecholamines (adrenalin and noradrenalin) coupled with blood glucose level can be viewed as indicator traits for selection on docility. To gain more understanding of the genetic basis of tameness, molecular genetic analysis are highly desirable in order to identify genes coding for tameness.

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