Available online at www.sciencedirect.com
ScienceDirect
Hormones and Behavior 53 (2008) 413–421
Hormones and Behavior
www.elsevier.com/locate/yhbeh

Phenotypic differences in behavior, physiology and neurochemistry between rats selected for tameness and for defensive aggression towards humans

Frank W. Albert [a,*], Olesya Shchepina [b], Christine Winter [c], Holger Römpler [d,e], Daniel Teupser [f], Rupert Palme [g], Uta Ceglarek [f], Jürgen Kratzsch [f], Reinhard Sohr [h], Lyudmila N. Trut [b], Joachim Thiery [f], Rudolf Morgenstern [h], Irina Z. Plyusnina [b], Torsten Schöneberg [d], Svante Pääbo [a,i]

[a] *Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany*
[b] *Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia*
[c] *Department of Psychiatry and Psychotherapy, University Medicine Berlin, Charité Campus Mitte, Berlin, Germany*
[d] *Institute of Biochemistry, Molecular Biochemistry, Medical Faculty, University of Leipzig, Leipzig, Germany*
[e] *Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, USA*
[f] *Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Hospital Leipzig, Leipzig, Germany*
[g] *Institute of Biochemistry, Department of Natural Sciences, University of Veterinary Medicine, Vienna, Austria*
[h] *Institute of Pharmacology and Toxicology, University Medicine Berlin, Charité Campus Mitte, Berlin, Germany*
[i] *Department of Genetics and Pathology, Rudbeck Laboratory, Uppsala University, Uppsala, Sweden*

Received 12 March 2007; revised 14 November 2007; accepted 15 November 2007
Available online 4 January 2008

Abstract

To better understand the biology of tameness, i.e. tolerance of human presence and handling, we analyzed two lines of wild-derived rats (*Rattus norvegicus*) artificially selected for tameness and defensive aggression towards humans. In response to a gloved human hand, tame rats tolerated handling, whereas aggressive rats attacked. Cross-fostering showed that these behavioral differences are not caused by postnatal maternal effects. Tame rats were more active and explorative and exhibited fewer anxiety-related behaviors. They also had smaller adrenal glands, larger spleens and lower levels of serum corticosterone. Blood glucose levels were lower in tame rats, whereas the concentrations of nine amino acids were higher. In the brain, tame rats had lower serotonin and higher taurine levels than aggressive rats. Our findings reinforce the notion that tameness is correlated with differences in stress response and will facilitate future efforts to uncover the genetic basis for animal tameness.

Keywords: Domestication; Behavior; Corticosterone; Anxiety; Taurine; Serotonin

Introduction

Domesticated animals played important roles in the development of large-scale human agricultural societies (Jobling et al., 2004; Price, 2002). Recently, the determination of DNA sequences from some domesticated animals, such as pigs, cattle, horses and dogs, and their extant wild relatives have allowed for a better understanding of the time and process of their domestication (Larson et al., 2005; Leonard et al., 2002; Savolainen et al., 2002; Troy et al., 2001; Vila et al., 2001). Other genetic studies have shed light on aspects of the physiology of domesticated animals such as pigs and dogs (Andersson and Georges, 2004; Sutter et al., 2007; Van Laere et al., 2003).

However, one general precondition necessary for humans to impose selection on any animal is that it tolerates human presence and handling. Thus, a first or early step presumably common to all domestication events is that the wild animal must be "tamed"—i.e. its behavior must be modified in order to avoid constant attempts to escape or attack humans. This aspect of

* Corresponding author. Max Planck Institute for Evolutionary Anthropology, Department of Evolutionary Genetics, Deutscher Platz 6, 04103 Leipzig, Germany. Fax: +49 341 35 50 555.
E-mail address: falbert@eva.mpg.de (F.W. Albert).

0018-506X/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.yhbeh.2007.11.010

domestication and its genetic basis has hitherto received little attention (Jobling et al., 2004).

Experiments performed by the late Prof. Dmitry K. Belyaev at the Institute of Cytology and Genetics in Novosibirsk, Russia, are one brilliant exception. He and his collaborators selectively bred a number of wild mammalian species over several decades for the absence of aggressive and fearful behavior towards humans. This resulted in a population of foxes (*Vulpes vulpes*) that reached such a level of tameness that their behavior is, by many counts, akin to that of dogs (Belyaev, 1969, 1979; Hare et al., 2005; Trut et al., 2004). In another series of experiments, Belyaev subjected a population of 233 wild-caught gray rats (*Rattus norvegicus*) to selection for tameness as well as increased defensive aggression towards humans. In each generation, the rats were tested for their reaction to a human hand's approach in their cage, and the 30% showing the least and most aggressive behaviors, respectively, were allowed to breed. Neither line was ever smaller than approximately 60 animals in size. After 8–10 generations under constant selection, the tame line exhibited a lack of aggressive and defensive reactions towards humans, whereas the aggressive line exhibited fiercely aggressive behavior directed at humans (Belyaev and Borodin, 1982; Blanchard et al., 1994; Naumenko et al., 1989; Plyusnina and Oskina, 1997). Levels of offensive aggression directed at conspecifics remained unchanged (Naumenko et al., 1989). Until today, both lines have been subjected to constant selection based solely on their reaction to an approaching human hand. No other parameter, in appearance, in behavior or in physiology, has been used as a selective criterion. Both strains are maintained under identical conditions, with minimal handling. Thus, the tame rats are not trained or otherwise more accustomed to human presence than the aggressive rats, and aggressive rats are not subjected to stressful treatment. The behavior and physiology of both lines have been extensively studied (Belyaev, 1969; Belyaev and Borodin, 1982; Blanchard et al., 1994; Naumenko et al., 1989; Nikulina et al., 1992; Oskina et al., 2003; Plyusnina, 2004; Plyusnina and Oskina, 1997; Popova et al., 2000, 2005; Prasolova et al., 2004; Shishkina et al., 1993; Trut et al., 2004). However, research into the genetic basis for the animals' tameness is only just beginning (Kukekova et al., 2007).

We have recently established daughter colonies of both the tame and the aggressive rats in Leipzig, Germany, from 15 unrelated tame and 15 unrelated aggressive rats (5 males, respectively) from the 64th generation of selection. In order to obtain a more complete view of phenotypic differences between the tame and the aggressive rats, we have performed a broad experimental screen, of their behavior, physiology and neurochemistry. Here, we present results of behavioral experiments measuring the rats' level of tameness, fear and anxiety, physiological measures including organ weights, hormone levels and other serological parameters and concentrations of several neurotransmitters in the brain.

Materials and methods

A more detailed methods description can be found in the supporting online material.

Animals

Rats (*R. norvegicus*) were obtained from the Institute of Cytology and Genetics in Novosibirsk, Russia. They stem from two divergent lines derived from the same wild population, which have been selected over 32 years for either the absence of or enhanced defensive aggression towards humans. Selection has been imposed on every generation since the initiation of the two lines and is continued in the present populations. Animals were housed under standard laboratory conditions with an artificial 12:12-h light/dark cycle (lights on at 01:00 am) and treated and handled identically. The study was approved by the regional government of Saxony (TVV 29/05).

Behavioral tests

All behavioral tests were performed in the same order, starting with animals of 45–60 days of age. The "glove test" measures an animal's level of tameness/aggressiveness when confronted with a gloved human hand (see supplementary movies). Following a 5-min adaptation period during which animals were left undisturbed in the test cage, experimenters followed a standardized procedure, and experiments were video-recorded. Individual behaviors (see Supplementary Table 1) were scored by an observer blind to the animals' identity and to further data analysis. Principal component analysis was used to integrate behaviors into quantifiable measures. Other behavioral tests (hole board test, light–dark test, open field test, startle response test) were performed as in previous studies (Plyusnina, 2004; Plyusnina and Oskina, 1997; Popova et al., 2000) using automated measuring technology (TSE Systems, Bad Homburg, Germany).

Cross-fostering

On postnatal day 2, mothers were separated from their pups and pups were exchanged by placing them into the cage of a mother from the other strain. Mothers were then returned to their cages and to the newly exchanged pups. Only pups born on the same day were exchanged. Pups from the same litter were neither mixed with those from other litters nor separated from each other.

Clinical laboratory tests and tissue collection

Animals were dissected within 2 weeks after behavioral testing, between 2:00 pm and 6:00 pm (during the dark phase of the light cycle). Animals were weighed, anesthetized with CO_2 and killed by cervical dislocation. Blood was collected immediately after death by heart puncture and separated into serum and blood cells by centrifugation after complete coagulation. Tissues were weighed and frozen at −80 °C; serum was frozen at −20 °C. Electrolytes, metabolites, immunological parameters, enzymes and hormones were analyzed in serum or, where appropriate, whole blood, according to the guidelines of the German Society of Clinical Chemistry and Laboratory Medicine, using a Hitachi PPE-Modular analyzer, an Accu-Check® blood glucose measurement device (both Roche Diagnostics, Mannheim, Germany) and an electrospray ionization tandem mass spectrometer (ESI-MS/MS) (API 2000, Applied Biosystems, Darmstadt, Germany) (Ceglarek et al., 2002; Mueller et al., 2003). Corticosterone was measured using an enzyme-linked immunosorbent assay (ELISA) (IDS, Boldon, England). Testosterone levels were measured using the Elecsys assay system (Roche-Diagnostic, Mannheim, Germany). Fecal corticosterone metabolites were measured with a 5α-pregnane-3β,11β,21-triol-20-one EIA (Lepschy et al., 2007; Touma et al., 2003) from feces collected after the animals had been undisturbed for 2 days and then transferred to a sampling cage at 08:00 am. Feces were collected at 12:00 pm (morning collection) and the procedure repeated in the afternoon (afternoon collection, transfer to sampling cage at 1:00 pm, collection at 5:00 pm). Because the time lag between circulating corticosterone and corticosterone metabolites in feces is 14 h (Lepschy et al., 2007), these measurements represent corticosterone levels prevailing while the animals were still undisturbed.

Neurochemistry

Neurotransmitters (glutamate, serotonin, GABA, dopamine and taurine) were measured using high performance liquid chromatography as previously

described (Felice et al., 1978; Piepponen and Skujins, 2001; Sperk, 1982; Sperk et al., 1981), in nine brain regions: cingulate cortex area 1, prelimbic cortex, infralimbic cortex, caudate–putamen, nucleus accumbens, lateral globus pallidus, central amygdaloid nucleus, hippocampus and ventral tegmental area.

Statistical analyses

For each measure, plots of the residuals against the group means calculated by the respective statistical model were inspected. Where the variance appeared to be dependent on the mean, the raw data were transformed using the natural logarithm prior to statistical analyses. Measures collected in both sexes were analyzed by two-way analysis of variance (ANOVA) with line (tame vs. aggressive) and sex as factors. For measurements taken in males only, two-sided *T*-tests were used. In the glove test, the fractions of animals showing a given behavior were compared using Fisher's exact test. The open field test was analyzed using an ANOVA with line and sex as between-subject factors and trial as within-subject covariate. Startle responses were analyzed with an ANOVA with line and sex as between-subject factors, body weight as a between-subject covariate and trial as a within-subject covariate. Individual startle response trials were compared using two-sided *T*-tests, separately for each sex. Organ weights were analyzed using an ANOVA with line and sex (not for testis) as factors and body weight as covariate. Fecal measurements were analyzed with an ANOVA with line as between-subjects factor and time (morning vs. afternoon collection) as a within-subject factor. Neurotransmitter concentrations were analyzed using two-way ANOVA with line and brain region as factors. For each brain region and neurotransmitter, two-sided *T*-tests were performed. Principal component analysis of the glove test data was performed using the software package SPSS (Version 13). All other analyses were conducted in R (R Development Core Team, 2007).

Results

Behavioral tests

In the glove test designed to test a rat's reaction towards an approaching human hand and eventual attempts of handling, we found that all but two (freezing upon the approach of a gloved hand and stretch attended postures) of 13 behaviors showed significant differences between the two lines of rats, either in the fraction of animals exhibiting them and/or in the number and duration with which they occurred (Fig. 1, Table 1). Tame rats tolerated being touched and handled and showed almost no antagonistic behaviors. Some animals approached and investigated the glove. The aggressive rats attacked the glove, showed defensive upright or "boxing" postures (Barnett, 1963; Blanchard et al., 2003) and screamed loudly. When the experimenter attempted to handle or pick them up, they fled or escaped immediately from the experimenter's hand (for detailed results,

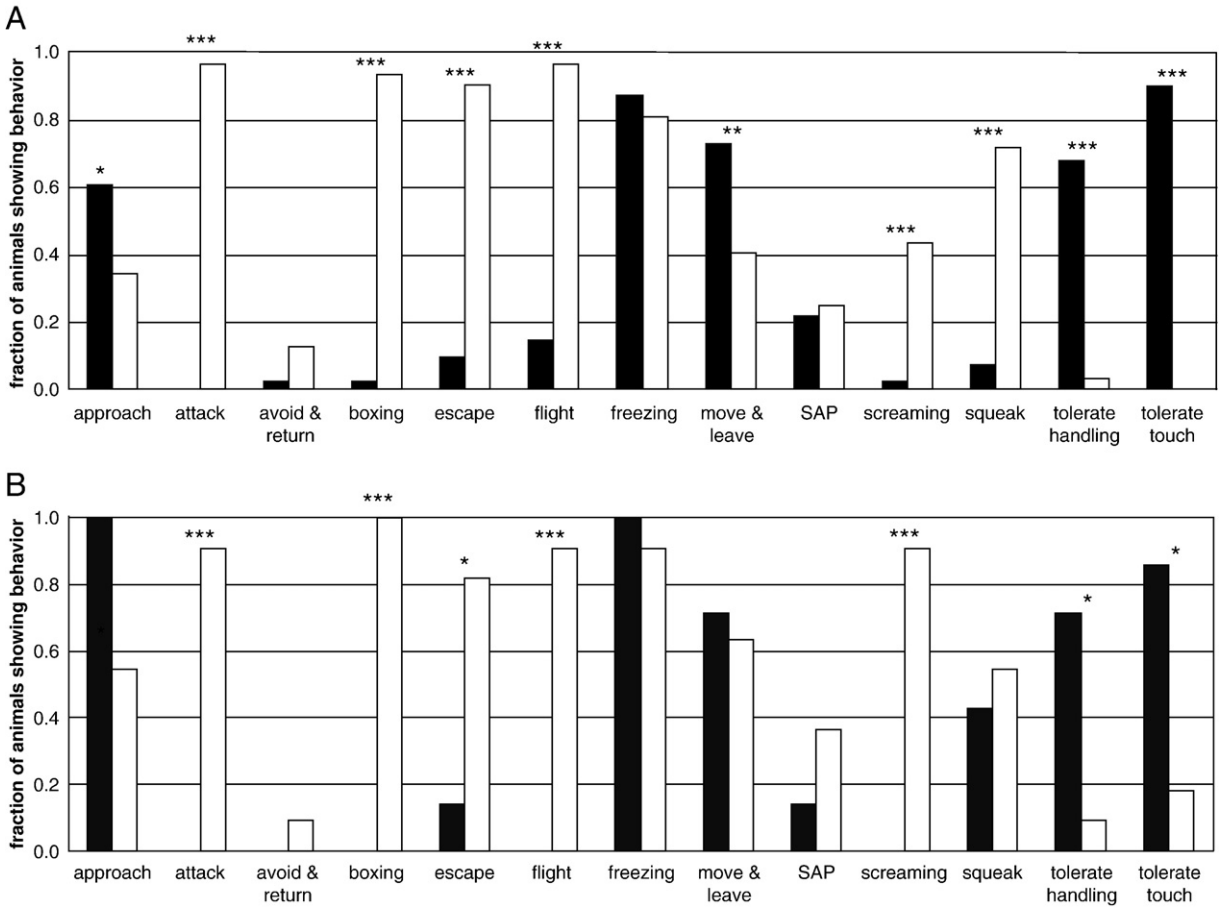

Fig. 1. Results of 13 behaviors measured in the glove test. Fraction of animals showing respective behavior. (A) Results from normally raised individuals of the two rat lines (41 tame rats (23 males) and 32 aggressive rats (16 males)); (B) results from cross-fostered animals (7 tame rats (1 male) and 11 aggressive rats (7 males)), respectively. Solid bars: tame rats. Open bars: aggressive rats. Significance levels (Fisher's exact test): ***$p<0.001$; **$p<0.01$; *$p<0.05$. SAP: stretch attended posture.

Table 1
Behavior in response to a human hand

	Significance	Males		Females	
		TR (n=23)	AR (n=16)	TR (n=18)	AR (n=16)
No. of occurrences/animal					
Attack	***	0±0	6.9±0.6	0±0	5.9±1.2
Approach	*, &	0.6±0.1	0.6±0.2	1.1±0.2	0.3±0.2
Boxing	***	0.1±0.1	2.4±0.4	0±0	3.2±0.4
Escape	***	0.2±0.1	2.3±0.3	0.1±0.1	2.9±0.5
Flight	***	0.1±0.1	3.1±0.5	0.2±0.1	4.1±0.6
Move and leave	**	1.2±0.2	0.6±0.2	1.2±0.2	0.6±0.2
SAP		0.3±0.1	0.3±0.1	0.2±0.1	0.3±0.2
Squeak	***	0.1±0.1	1.6±0.4	0.6±0.5	2.1±0.5
Scream	***, #, &&	0±0	1.3±0.4	0.1±0.1	0.3±0.2
Duration (s)					
Boxing	***	0.1±0.1	4.2±0.8	0±0	6.2±1.0
Freezing		23.1±3.2	18.5±3.9	22.1±4.0	21.7±3.1
Scream	***, #, &&	0±0	1.7±0.5	0.1±0.1	0.3±0.1
Tolerate handling	***	5.3±1.1	0.7±0.7	5.7±1.2	0±0
Tolerate touch	***	15.3±1.3	0±0	13.4±1.9	0±0

Mean values±SEMs are presented. TR: tame rats; AR: aggressive rats. Significance levels: *, **, ***: line differences. #, ##, ###: sex differences. &, &&, &&&: interaction line and sex ($p<0.05$; $p<0.01$; $p<0.001$, respectively, calculated by two-way ANOVA with line and sex as factors).

see Fig. 1, Table 1; for statistical analyses, see Supplementary Table 2; videos of typical behaviors are available in the supporting online material). The results of a principal component analysis of the glove test data are shown in Fig. 2 and Table 2. The first principal component (PC1), which explained 42% of the overall variance, corresponded to behaviors intuitively associated with tameness and aggression (e.g. tolerating touching and handling vs. attacking, boxing and flight). An ANOVA of PC1 scores using the two factors "sex" and "line" was highly significant for "line" ($F_{1,\ 69}=240$, $p<0.001$), whereas neither "sex" nor the interaction term "sex-line" showed a significant effect ($F_{1,\ 69}<2.5$, $p>0.12$). The second principal component (PC2) was associated primarily with screaming (Table 2), which was almost absent in the tame animals, but more prevalent in aggressive males than in aggressive females (Figs. 1 and 2, Table 1). PC2 scores differed significantly between the lines ($F_{1,\ 69}=7.1$, $p=0.009$) and the sexes ($F_{1,\ 69}=5.7$, $p=0.02$; interaction line and sex: $F_{1,\ 69}=9.5$, $p=0.003$).

In locomotion-based tests, the tame line spent more time in anxiogenic surroundings and was more active in them (Table 3; for statistical analyses, see Supplementary Table 3), whereas there was no consistent difference in overall activity across tests. For instance, tame rats spent more time and moved more slowly in the light area of the light–dark test and spent more time in the center and less time in the corners of the open field than aggressive rats (for reviews of the measures described here, see Bourin and Hascoët, 2003; Prut and Belzung, 2002). Tame rats also showed more rearing behaviors. In a startle–response test, where the animals were exposed to a series of sounds and their reaction was measured as the pressure exerted on the floor when they startled, both lines became habituated to the sounds, but the tame rats' responses were on average only half as strong as those of the aggressive rats (Fig. 3).

Cross-fostering

The patterns of individual behaviors in the cross-fostered animals were almost identical to those of the lines from which they were derived (Figs. 1 and 2) and were radically different from those of their foster mothers (data not shown). Consequently, cross-fostered animals' PC1 scores did not differ significantly from those of animals raised by their own mothers (nursing effect: $F_{1,\ 83}=2.63$, $p=0.11$ in an ANOVA with line, sex and nursing as factors, $F_{1,\ 83}<2.39$, $p>0.13$ for all interaction terms).

Body and organ weights

There was no overall difference in body weight between the lines (Supplementary Table 4a). The weights of spleens, kidneys, hearts, lungs and brains were significantly affected by overall body weight (spleen: $F_{1,56}=155$, $p<0.001$; kidney, heart and lung: $F_{1,\ 57}>79$, $p<0.001$; brain: $F_{1,\ 42}=15$, $p<0.001$); whereas for testes ($F_{1,\ 31}=3.94$, $p=0.055$) and adrenal glands ($F_{1,\ 55}=4.18$, $p=0.046$), body weight had smaller effects. Although the weights of the hearts, lungs and testes did not differ between the tame and the aggressive rats when overall body weight was taken into account, kidneys, spleens and brains were larger than expected from body weight in the tame rats. This is particularly the case for spleens, which were about 25% larger in tame rats. By contrast, adrenal glands were larger in aggressive rats. The complete statistical analyses can be found in Supplementary Table 4b.

Blood and serum chemistry

The complete results are given in Supplementary Table 5a. Out of 12 proteinaceous amino acids analyzed, 10 were higher in tame

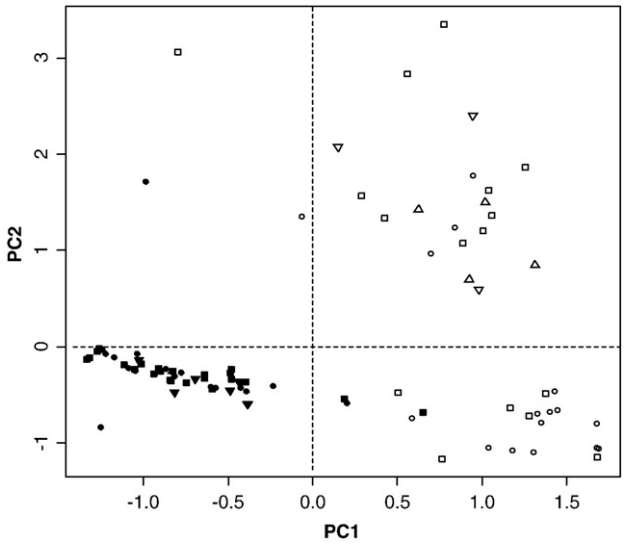

Fig. 2. Principal components in the glove test. Plotted are individual rats' scores in the first two principal components. Solid symbols: tame rats; open symbols: aggressive rats; circles: normally raised females; squares: normally raised males; triangles pointing downwards: cross-fostered females; triangles pointing upwards: cross-fostered males. PC1: first principal component; PC2: second principal component.

Table 2
Principal component analysis of the glove test

Measure	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Attack[a]	0.86						
Boxing[a]	0.91						
Escape[a]	0.90						
Flight[a]	0.77						
Tolerate handling[a]	−0.75						
Tolerate touch[a]	−0.79						
Duration boxing	0.80						
Duration tolerate handling	−0.65						
Duration tolerate touch	−0.79						
Latency attack	−0.86						
Latency boxing	−0.91						
Latency escape	−0.90						
Latency flight	−0.77						
Number of attacks	0.78						
Number of boxing	0.87						
Number of escapes	0.79						
Number of flights	0.73						
Screaming[a]		0.90					
Duration screaming		0.88					
Latency screaming		−0.91					
Number of screams		0.91					
Move and leave[a]			−0.90				
Latency move and leave			0.91				
Number of move and leave			−0.89				
Approach[a]				0.94			
Latency approach				−0.95			
Number of approaches				0.91			
Squeak[a]					0.75		
Latency squeak					−0.75		
Number of squeaks					0.83		
SAP						0.92	
Number of SAP						0.93	
Avoid and return[a]							−0.60
Freezing[a]							0.73
Duration freezing							0.72
% Variance	42	13	9	7	4	4	4

Principal component (PC) loadings after varimax rotation are shown. The loading indicates the degree to which a measure contributes to the respective PC. Only PC with eigenvalues >1 are shown because PC with smaller eigenvalues represent only small portions of the overall variation. Only loadings >0.6 are shown. [a]Variable is '1' if rat showed behavior, '0' if behavior did not occur. SAP: stretch attended posture.

than in aggressive rats and 5 of these were statistically significantly so (*T*-test, all $p<0.05$). In addition, C18 and C18:1 acylcarnitines (long-chain saturated and mono-unsaturated fatty acids bound to the carrier carnitine; Scaglia and Longo, 1999) were significantly higher in tame rats (*T*-test, both $p<0.01$), whereas glucose levels were lower (*T*-test, $p=0.03$). Tame rats also had higher albumin levels and lower β- and γ-globulin serum protein fractions than aggressive rats (for statistical analyses, see Supplementary Table 5b).

Hormonal status

Among the hormones analyzed, the androgen testosterone did not differ between the two rat lines when both sexes were considered together ($F_{1,\ 57}<1$, $p=0.43$, ANOVA of log-transformed data with line and sex as factors), although female tame rats did have significantly higher testosterone levels than female aggressive rats (*T*-test of log-transformed data: $p=0.04$). In contrast, serum corticosterone levels in males and females were 16% and 34% higher, respectively, in aggressive than in tame rats (Supplementary Table 5a; for statistical analyses, see Supplementary Table 5b). Fecal corticosterone metabolites were about half as abundant in the tame as in the aggressive rats ($F_{1,\ 17}=9.49$, $p=0.007$, ANOVA of log-transformed data with line as between-subject and collection time as within-subject factor; effect of collection time: $F_{1,\ 17}=37.24$, $p<0.001$, interaction: $F_{1,\ 17}<1$, $p=0.68$). For the number of fecal boli, there was a strong interaction of line and collection time ($F_{1,\ 18}=124$, $p<0.001$, ANOVA as for fecal corticosterone metabolites, but with untransformed data) in addition to main effects for line and collection time ($F_{1,\ 18}>39$, $p<0.001$).

Brain neurotransmitters

We measured the concentrations of four neurotransmitters (glutamate, γ-aminobutyric acid (GABA), dopamine and serotonin) as well as of taurine, an amino acid implicated in several brain processes (Albrecht and Schousboe, 2005), in nine brain

Table 3
Behavior in locomotion-based tests

	Significance	Males		Females	
		TR ($n=19$)	AR ($n=17$)	TR ($n=18$)	AR ($n=11$)
Activity (%)					
HBT	**	33±0.8	39±1.6	36±1.1	40±2.3
LDT[a]	#	49±1.5	50±3.1	55±1.7	56±2.7
OFT	**, ###	40±2.1	33±2.7	48±2.0	40±2.9
Rearing (number)					
HBT		18±1.0	20±2.2	18±1.5	20±1.9
LDT[a]	***, ##	38±1.8	26±2.2	44±2.7	34±3.3
OFT	***, ###	33±2.4	19±2.8	46±2.5	26±3.3
Spatial patterns (%)					
HBT—center	#	15±2.0	12±1.8	18±2.9	20±4.1
HBT—corners		37±3.7	36±3.4	36±3.7	40±4.8
LDT—light part	**, #	29±3.5	22±3.0	39±3.2	28±3.6
OFT—center	*	4±1.0	3±0.9	4±0.8	3±0.6
OFT—corners	**	49±3.4	62±4.5	53±3.5	64±5.1
Locomotion speed (cm/s)					
HBT	###, &	25±0.8	28±1.6	34±1.5	30±1.9
LDT—light area	**, ##	28±1.2	33±1.4	33±1.1	37±1.9
LDT—dark area	#	25±1.4	26±1.6	29±1.6	28±1.4
OFT	***, ###, &&	29±1.0	26±1.4	35±1.0	28±1.3

Mean values±SEMs are shown. TR: tame rats; AR: aggressive rats; HBT: hole board test; LDT: light–dark test; OFT: open field test. [a]Sum of activity in light and dark compartments. Activity is measured as the percentage of time an animal spent moving. Rearing indicates the number of times an animal reared on its hind legs, interrupting the upper photo beams. Spatial patterns are expressed as the amount of time an animal spent in the given area. Animals behaved similarly in the two consecutive trials of the open field test ($F_{1,\ 63}<3.94$, $p>0.052$ for the effect of trial for all measures). We present data from the first trial. Significance levels: *, **, ***: line differences; #, ##, ###: sex differences; &, &&, &&&: interaction line and sex ($p<0.05$; $p<0.01$; $p<0.001$, respectively, calculated by ANOVA with line and sex (and trial for OFT) as factors). There was no line difference in duration or latency of head dips in HBT (not shown).

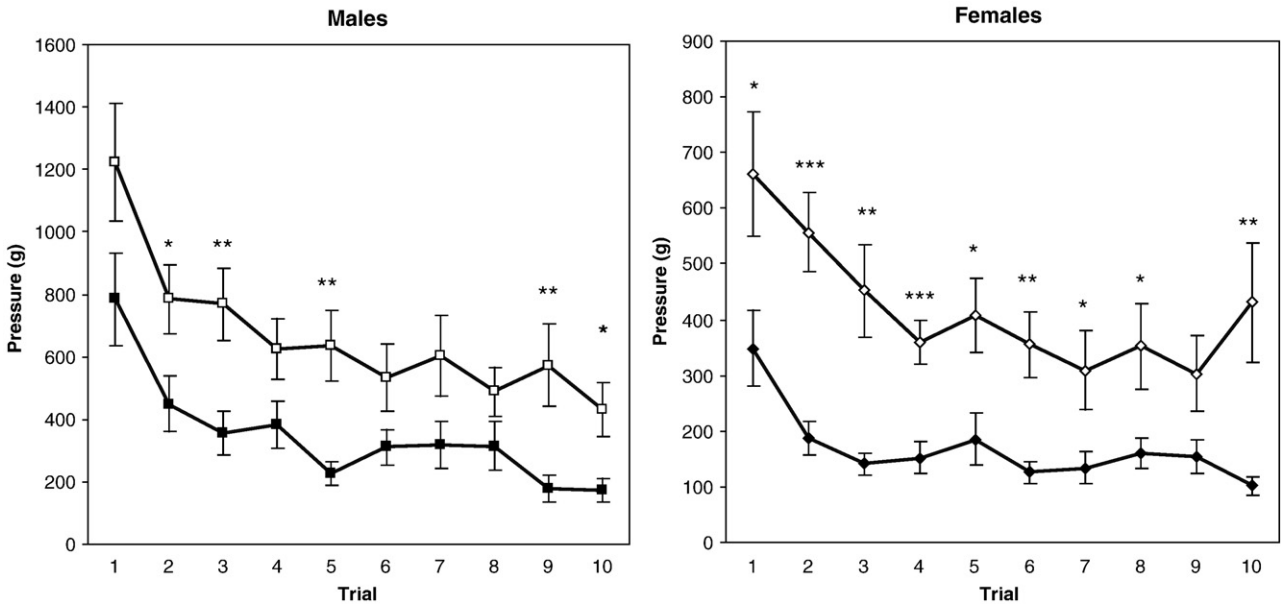

Fig. 3. Startle response to auditory stimuli. The maximum pressure applied to the sensor is shown. Solid symbols: tame rats ($n=37$, 17 female). Open symbols: aggressive rats ($n=30$, 15 female). Significance levels (T-test for individual trials): ***$p<0.001$; **$p<0.01$. Error bars: SEM. ANOVA showed main effects of line and body weight ($F_{1,60}>22$, $p<0.001$) as well as of trial ($F_{1,600}=119$, $p<0.001$) but no effect of sex ($F_{1,60}<1$, $p=0.63$). The interaction terms for line and weight ($F_{1,60}=4.4$, $p=0.04$) as well as for trial/sex and trial/line ($F_{1,600}>3.9$, $p<0.048$) were also significant.

regions. The results are summarized in Supplementary Table 6a. Overall, no significant differences between the rat lines were observed for dopamine and glutamate (for statistical analyses, see Supplementary Table 6b). By contrast, GABA, the major inhibitory neurotransmitter in the vertebrate central nervous system, was lower in tame rats than in aggressive rats. Similarly, tame rats had lower serotonin in cingulate cortex, nucleus accumbens and putamen. In tame rats, taurine was higher in four brain regions, including all cortical regions measured.

Discussion

Behavioral response to humans

To analyze the behavior of the two rat lines with respect to human contact and handling, we designed a test that closely models the paradigm used during selection of the rat lines (Naumenko et al., 1989; Plyusnina and Oskina, 1997). It exposes the rats to increasingly intense human contact in a standardized way, with a gloved human hand first approaching the rat, then touching it and eventually attempting to pick it up. This test differs from earlier studies of defensive aggression towards an experimenter (Albert et al., 1984, 1986b) in that it exposes the rats exclusively to a human rather than to a series of mostly inanimate objects. A set of 13 behaviors, which were scored from video recordings of the tests by an independent observer, included evasive behaviors such as escape and flight, vocalization such as squeaking and screaming, aggressive behaviors such as attacking as well as non-aggressive approaches and tolerance for touching and handling (Supplementary Table 1). Tame and aggressive rats differed dramatically in their response to a human hand. Tame rats were tolerant of handling, whereas aggressive rats attacked and fled.

In a principal component analysis of the behaviors scored in this test, PC1 corresponded to behaviors intuitively associated with tameness and aggression (Table 2) and hence is likely to reflect differences in behaviors that were primary targets of selection. Consequently, the lines differed strongly in their PC1 scores, whereas there was no detectable influence of sex (Fig. 2). The absence of a sex difference in the level of tameness/aggression is consistent with earlier reports on defensive aggression elicited by lesions of brain regions (Albert et al., 1986b), suggesting that the neural substrate of defensive aggression is not sexually dimorphic. Interestingly, screaming during the glove test corresponded to PC2 (Table 2), which is by definition uncorrelated with PC1. It thus appears that screaming, while occurring almost only in aggressive animals (Fig. 1), is uncorrelated with other expressions of defensiveness within the aggressive line, suggesting that it might reflect a separate physiological or psychological mechanism.

Our experiments confirm previous reports (Plyusnina, 2004; Plyusnina and Oskina, 1997; Popova et al., 2000) showing that the tame and aggressive rats behave drastically differently in response to the approach of a human hand. In rodents, two main forms of aggression can be distinguished. Offensive aggression is usually targeted at conspecifics in response to perceived challenges to access to important resources, whereas defensive aggression is displayed in response to perceived threats to an animal's bodily integrity (Blanchard et al., 2003). In this regard, it is noteworthy that the tameness and aggression displayed by the two rat lines in response to humans is reflected in the behavior of the rats toward one another. Tame rats show fewer displays of defensive aggression directed toward other rats in several paradigms (Naumenko et al., 1989; Nikulina et al., 1992). Further, when males are brought together after copulation, there is a tendency for more severe fighting among the

aggressive rats than among the tame rats (I. Z. Plyusnina, unpublished observation). On the other hand, during normal maintenance of the lines, aggressive rats do not exhibit more offensive aggression among themselves than tame rats. This is in agreement with earlier findings that offensive intermale aggression in both lines remained unchanged over the course of selection (Naumenko et al., 1989). Hence, the behavior of the tame rats seems to reflect the absence of defensive rather than of offensive aggression.

Cross-fostering

To investigate whether the behavioral differences between the tame and the aggressive rats are influenced by factors during maternal care and rearing of the offspring, we exchanged pups between the tame and the aggressive lines shortly after birth so that they were reared by mothers of the other line. The behavior in the glove test of both tame and aggressive cross-fostered rats was virtually indistinguishable from that of rats raised by their own mothers (Figs. 1 and 2). Thus, cross-fostering had no measurable effect on the behavioral differences observed between the tame and aggressive lines. Although these experiments cannot dismiss the effects of prenatal environment (e.g. the mother's hormonal levels), the results strongly suggest that the behavioral differences observed between the lines have a genetic basis.

Anxiety and fear

There was no overall difference in locomotor activity between the tame and aggressive rats. However, when activity in the more aversive areas such as the open field test or the light area of the light–dark test is considered, the tame rats were consistently more active than the aggressive rats. They also spent less time in the protected corners of the open field. Similarly, the tame rats responded less to acoustic stimulation in the startle experiment. This indicates that tame rats are less anxious and fearful than their aggressive counterparts and is in agreement with earlier reports (Plyusnina, 2004; Plyusnina and Oskina, 1997; Popova et al., 2000). Low levels of anxiety can be correlated with either high (Veenema et al., 2007) or low (Guillot and Chapouthier, 1996) offensive aggression depending on the rodent model used. However, they are thought to be associated with decreased defensive aggression (Blanchard et al., 2003), in agreement with our observations of the tame rats.

Hormonal status

In agreement with earlier studies (Shishkina et al., 1993), we found no difference between the two rat lines in testosterone levels. Although testosterone has been associated with offensive, intermale aggression in rats (Albert et al., 1986a; but see also Archer, 2006; Wingfield, 2005), defensive aggression seems to be independent of testosterone and to involve neural mechanisms different from those involved in offensive aggression (Albert et al., 1986b). In conjunction with the fact that the sexes did not differ in their response to humans, although they obviously differ drastically in testosterone levels, it seems clear that differences in testosterone are not involved in the behavioral differences between the two lines.

In contrast, although serum corticosterone levels were relatively high for both rat lines, they were significantly lower in the tame than in the aggressive rats. This is in agreement with previous findings of lower plasma corticosterone in tame rats compared to aggressive rats, both after stress and in a restive state, and is well in line with previous work showing a lower reactivity of the tame rats' hypothalamic–pituitary–adrenocortical (HPA) axis in response to restraint stress, pain and injections of noradrenalin and serotonin (Naumenko et al., 1989; Oskina et al., 2003; Plyusnina and Oskina, 1997).

Fecal corticosterone metabolites

Although it is clear that tame rats have lower levels of serum corticosterone than their aggressive counterparts, it is unclear whether corticosterone levels differ in the complete absence of humans, who presumably constitute a stressful stimulus at least for the aggressive rats. Further, the serum corticosterone levels we obtained were high for both rat lines (compared e.g. to Vahl et al., 2005). Hence, they do not reflect baseline or "unstressed" values. To test whether baseline levels of corticosterone differ between the lines in the absence of humans, we collected feces after the animals were left undisturbed for 2 days and measured fecal corticosterone metabolites. These reflect serum corticosterone levels with a time lag of 14 h and allow adrenocortical activity to be integrated over a longer time period (Lepschy et al., 2007). We found that fecal corticosterone metabolites were about half as abundant in the tame as in the aggressive rats. Because daily exposure to the mild stressors of normal animal maintenance were absent in this experiment and therefore cannot explain the difference in corticosterone metabolites, the tame and the aggressive rats' HPA axes may have different set points for basally secreted corticosterone, consistent with previous data from rat strains with genetically determined differences in their response to stress (Dhabhar et al., 1997). This might also help to explain the differences in adrenal gland size. Still, it is possible that the level of perceived stress in the tame rats might be lower, even in the absence of stressors other than those constantly present in the colony room environment (e.g. the sound of air conditioning). Differences in basal corticosterone and adrenal gland or spleen size might then be secondary effects of this difference in perception.

Conclusions

The tame and aggressive rat lines have been selected over more than 65 generations based on one simple criterion—their behavioral response to a human hand. This selection regime has resulted in drastic differences in the rats' behavior (Fig. 1 and Table 1, see also the supporting online video material). Indeed, out of 13 components that we scored in the behavior of rats towards a human hand, the two lines differed significantly in 11.

Given that the selection was based on a narrowly defined trait, it is striking that this regime has resulted in animals that differ at almost every phenotypic level we have studied. In

principle, there are three possible reasons for why phenotypic differences might co-occur in the two lines. First, both traits may be influenced by genetic variants that were selected for. They may either be directly caused by the same genetic variants ("primary" effects) or by one primary effect causing another effect ("secondary" effects). Second, traits may co-occur with selected traits because genes influencing them are genetically linked to, i.e. situated close to, those causing the selected trait. Third, the traits may occur together in the two lines by chance, e.g. the genetic variants causing them may have become frequent or fixed due to random genetic drift during the selection process. In crosses between the tame and the aggressive rats and subsequent intercrosses, unlinked genetic variants will be separated by recombination from selected genetic variants, allowing to distinguish the effects of drift from those of selection. Such experiments are currently underway in our laboratory.

It will be particularly interesting to what extend tameness/defensive aggression correlates with differences in anxiety and in the stress response. A number of differences between the lines, in particular the smaller adrenal glands and lower corticosterone levels, are compatible with a reduced stress response in tame rats, which in turn could influence many aspects of physiology (Belyaev, 1979). A number of other differences, such as those in spleen size or serum albumin, could potentially be secondary to such an effect. However, other differences, such as in brain size, serum levels of amino acids or brain taurine (see also Supplementary discussion), cannot easily be explained as secondary effects of a lower stress response and await further exploration.

It is noteworthy that one motivation for the initiation of the long-term selection regime in these rats was Belyaev's (1969, 1979) hypothesis that selection for tameness (i.e. the absence of hostile behavior towards humans) might bring about many of the phenotypic changes observed in a range of domestic animals, and that this would be caused by reduced emotional reactivity and altered activity of the hormonal system, including the hypothalamic-pituitary-adrenocortical axis (Belyaev, 1979). Because many of the differences between the two rat lines, both reported here and previously (Blanchard et al., 1994; Naumenko et al., 1989; Nikulina et al., 1992; Oskina et al., 2003; Plyusnina, 2004; Plyusnina and Oskina, 1997; Popova et al., 2000, 2005; Prasolova et al., 2004; Shishkina et al., 1993), are consistent with a lower stress response in tame rats, these findings support Belyaev's original hypotheses. Nevertheless, the primary cause of the differences between tameness and defensive aggression towards humans remains to be uncovered.

Acknowledgments

We thank Josep Call for invaluable advice on the glove test; Roger Mundry for help with statistical analyses; Norbert Sachser and Volker Stefanski for advice on behavioral testing; Sandra Peter, Susann Lautenschläger, Claudia Schmidt, Daniela Hedwig, Christof Neumann, Christel Schneider and Jenny Bielig for experimental assistance; and the Max Planck Society for financial support. We are grateful to two anonymous reviewers for their constructive comments on an earlier version of the manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.yhbeh.2007.11.010.

References

Albert, D.J., et al., 1984. Handling from weaning to adulthood does not prevent hyperdefensiveness induced by septal medial accumbens or medial hypothalamic lesions. Behav. Neural Biol. 41, 127–134.
Albert, D.J., et al., 1986a. Testosterone removal in rats results in a decrease in social aggression and a loss of social dominance. Physiol. Behav. 36, 401–407.
Albert, D.J., et al., 1986b. Defensive aggression toward an experimenter no differences between males and females following septal medial accumbens or medial hypothalamic lesions in rats. Physiol. Behav. 38, 11–14.
Albrecht, J., Schousboe, A., 2005. Taurine interaction with neurotransmitter receptors in the CNS: an update. Neurochem. Res. 30, 1615–1621.
Andersson, L., Georges, M., 2004. Domestic-animal genomics: deciphering the genetics of complex traits. Nat. Rev., Genet. 5, 202–212.
Archer, J., 2006. Testosterone and human aggression: an evaluation of the challenge hypothesis. Neurosci. Biobehav. Rev. 30, 319–345.
Barnett, S.A., 1963. A study in Behavior—Principles of Ethology and Behavioural Physiology, Displayed Mainly in the Rat. Methuen & Co, London.
Belyaev, D.K., 1969. Domestication of animals. Sci. J. 1, 47–52.
Belyaev, D.K., 1979. Destabilizing selection as a factor in domestication. J. Heredity 70, 301–308.
Belyaev, D.K., Borodin, P.M., 1982. The influence of stress on variation and its role in evolution. Biol. Zent.bl. 100, 705–714.
Blanchard, D.C., et al., 1994. Defensive reactions of "wild type" and "domesticated" wild rats to approach and contact by a threat stimulus. Aggress. Behav. 20, 387–397.
Blanchard, R.J., et al., 2003. Problems in the study of rodent aggression. Horm. Behav. 44, 161–170.
Bourin, M., Hascoët, M., 2003. The mouse light/dark box test. Eur. J. Pharmacol. 463, 55–65.
Ceglarek, U., et al., 2002. Validation of the phenylalanine/tyrosine ratio determined by tandem mass spectrometry: sensitive newborn screening for phenylketonuria. Clin. Chem. Lab. Med. 40, 693–697.
Dhabhar, F.S., et al., 1997. Adaptation to prolonged or repeated stress—comparison between rat strains showing intrinsic differences in reactivity to acute stress. Neuroendocrinology 65, 360–368.
Felice, L.J., et al., 1978. Determination of catecholamines in rat brain parts by reverse phase ion pair liquid chromatography. J. Neurochem. 31, 1461–1466.
Guillot, P.-V., Chapouthier, G., 1996. Intermale aggression and dark/light preference in ten inbred mouse strains. Behav. Brain Res. 77, 211–213.
Hare, B., et al., 2005. Social cognitive evolution in captive foxes is a correlated by-product of experimental domestication. Curr. Biol. 15, 226–230.
Jobling, M.A., et al., 2004. Human Evolutionary Genetics. Garland Publishing, New York.
Kukekova, A.V., et al., 2007. A meiotic linkage map of the silver fox, aligned and compared to the canine genome. Genome Res. 17, 387–399.
Larson, G., et al., 2005. Worldwide phylogeography of the wild boar reveals multiple centers of pig domestication. Science 307, 1618–1621.
Leonard, J.A., et al., 2002. Ancient DNA evidence for Old World origin of New World dogs. Science 298, 1613–1616.
Lepschy, M., Touma, C., Hruby, R., Palme, R., 2007. Non-invasive measurement of adrenocortical activity in male and female rats. Lab. Anim. 41, 372–387.
Mueller, P., et al., 2003. Validation of an ESI–MS/MS screening method for acylcarnitine profiling in urine specimens of neonates, children, adolescents and adults. Clin. Chim. Acta 327 (1–2), 47–57.
Naumenko, E.V., et al., 1989. Behavior, adrenocortical activity, and brain monoamines in Norway rats selected for reduced aggressiveness towards man. Pharmacol. Biochem. Behav. 33, 85–91.

Nikulina, E.M., et al., 1992. Selection for reduced aggressiveness towards man and dopaminergic activity in Norway rats. Aggress. Behav. 18, 65–72.

Oskina, I.N., et al., 2003. Relationship between behavioral selection and primary and secondary immune response in wild gray rats. Bull. Exp. Biol. Med. 136, 404–407.

Piepponen, T.P., Skujins, A., 2001. Rapid and sensitive step gradient assays of glutamate, glycine, taurine and gamma-aminobutyric acid by high-performance liquid chromatography-fluorescence detection with *o*-phthalaldehyde-mercaptoethanol derivatization with an emphasis on microdialysis samples. J. Chromatogr., B 757, 277–283.

Plyusnina, I.Z., 2004. Locomotor activity—exploration or "panic"? Rossisky Fiziologichesky Zhurnal, vol. 90, p. 84. In Russian.

Plyusnina, I.Z., Oskina, I., 1997. Behavioral and adrenocortical responses to open-field test in rats selected for reduced aggressiveness toward humans. Physiol. Behav. 61, 381–385.

Popova, N.K., et al., 2000. Expression of the startle response reaction in rats genetically predisposed towards different types of defensive behavior. Neurosci. Behav. Physiol. 30, 321–325.

Popova, N.K., et al., 2005. Reduction in 5-HT1A receptor density, 5-HT1A mRNA expression and functional correlates for 5-HT1A receptors in genetically defined aggressive rats. J. Neurosci. Res. 80, 286–292.

Prasolova, L.A., et al., 2004. Morpho-functional characteristics of the spleen in rats with different behavior after exposure to restriction stress. Russ. J. Morphol. 25, 59–63.

Price, E.O., 2002. Animal Domestication and Behavior. CABI Publishing, Oxon/New York.

Prut, L., Belzung, C., 2002. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur. J. Pharmacol. 463, 3–33.

R Development Core Team, 2007. R: A Language and Environment for Statistical Computing. R foundation for statistical computing, Vienna, Austria.

Savolainen, P., et al., 2002. Genetic evidence for an East Asian origin of domestic dogs. Science 298, 1610–1613.

Scaglia, F., Longo, N., 1999. Primary and secondary alterations of neonatal carnitine metabolism. Semin. Perinatol. 23, 152–161.

Shishkina, G.T., et al., 1993. Sexual maturation and seasonal changes in plasma levels of sex steroids and fecundity of wild Norway rats selected for aggressiveness toward humans. Physiol. Behav. 53, 389–393.

Sperk, G., 1982. Simultaneous determination of serotonin 5 hydroxy IAA 3 4 di hydroxyphenyl acetic-acid and homo vanillic-acid by high performance liquid chromatography with electrochemical detection. J. Neurochem. 38, 840–843.

Sperk, G., et al., 1981. Kainic-acid induced changes of serotonin and dopamine metabolism in the striatum and substantia nigra of the rat. Eur. J. Pharmacol. 74, 279–286.

Sutter, N.B., et al., 2007. A single IGF1 allele is a major determinant of small size in dogs. Science 316, 112–115.

Touma, C., et al., 2003. Effect of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen. Comp. Endocrinol. 130, 267–278.

Troy, C.S., et al., 2001. Genetic evidence for Near-Eastern origins of European cattle. Nature 410, 1088–1091.

Trut, L.N., et al., 2004. An experiment on fox domestication and debatable issues of evolution of the dog. Russ. J. Genet. 40, 644–655.

Vahl, T.P., et al., 2005. Comparative analysis of ACTH and corticosterone sampling methods in rats. Am. J. Physiol: Endocrinol. Metab. 289, E823–E828.

Van Laere, A.-S., et al., 2003. A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. Nature 425, 832–836.

Veenema, A.H., et al., 2007. Low inborn anxiety correlates with high intermale aggression: link to ACTH response and neuronal activation of the hypothalamic paraventricular nucleus. Horm. Behav. 51, 11–19.

Vila, C., et al., 2001. Widespread origins of domestic horse lineages. Science 291, 474–477.

Wingfield, J.C., 2005. A continuing saga: the role of testosterone in aggression. Horm. Behav. 48, 253–255.