Genes, Brain and Behavior
Official publication of the International Behavioural and Neural Genetics Society

Genes, Brain and Behavior (2016) ***15:*** *733–740* doi: 10.1111/gbb.12313

Interplay between aggression, brain monoamines and fur color mutation in the American mink

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Domestication of wild animals alters the aggression towards humans, brain monoamines and coat pigmentation. Our aim is the interplay between aggression, brain monoamines and depigmentation. The Hedlund white mutation in the American mink is an extreme case of depigmentation observed in domesticated animals. The aggressive (-2.06 ± 0.03) and tame ($+3.5 \pm 0.1$) populations of wild-type dark brown color (standard) minks were bred during 17 successive generations for aggressive or tame reaction towards humans, respectively. The Hedlund mutation was transferred to the aggressive and tame backgrounds to generate aggressive (-1.2 ± 0.1) and tame ($+3.0 \pm 0.2$) Hedlund minks. Four groups of 10 males with equal expression of aggressive (−2) or tame (+5) behavior, standard or with the Hedlund mutation, were selected to study biogenic amines in the brain. Decreased levels of noradrenaline in the hypothalamus, but increased concentrations of the serotonin metabolite, 5-hydroxyindoleacetic acid and dopamine metabolite, homovanillic acid, in the striatum were measured in the tame compared with the aggressive standard minks. The Hedlund mutation increased noradrenaline level in the hypothalamus and *substantia nigra*, serotonin level in the *substantia nigra* and striatum and decreased dopamine concentration in the hypothalamus and striatum. Significant interaction effects were found between the Hedlund mutation and aggressive behavior on serotonin metabolism in the substantia nigra ($P < 0.001$), dopamine level in the midbrain ($P < 0.01$) and its metabolism in the striatum ($P < 0.05$). These results provide the first experimental evidence of the interplay between aggression, brain monoamines and the Hedlund mutation in the American minks.

Keywords: Aggressive behavior, brain, dopamine, fur color, genetics, Hedlund mutation, mink, neurochemistry, noradrenaline, serotonin, tameness

Received 15 March 2016, revised 27 May 2016 and 15 June 2016, accepted for publication 28 July 2016

Aggression is a major public health problem worldwide (Krug *et al.* 2002). Defensive aggression, which is a kind of aggressive behavior (Maxson 1999; Moyer 1968), seems to be a major type of human aggression (Albert *et al.* 1993). Wild animals who do not consider humans as potential preys show active avoidance or defensive aggression towards humans. Genetic and neural mechanisms of defensive behavior were studied in silver foxes and Norway rats selectively bred for aggressive or tame behavior towards humans (Albert *et al.* 2008, 2009, 2012; Heyne *et al.* 2014; Plyusnina *et al.* 2012; Popova 2006; Trut 1999).

It was shown that brain monoamines, serotonin (5-HT), dopamine (DA) and noradrenaline (NA) are involved in the regulation of aggression towards humans (Popova 2006). Selective breeding of silver foxes and Norway rats for tame or aggressive behavior towards humans was accompanied by similar changes of brain monoamines. Elevated levels of 5-HT and its main metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the hypothalamus and midbrain (Naumenko *et al.* 1989; Popova *et al.* 1991a), an increased activity of the key enzyme of 5-HT synthesis, tryptophan hydroxylase 2, in the hypothalamus, as well as an increased density of 5-HT_{2A} receptors in the frontal cortex (Popova *et al.* 1991a) and 5-HT_{1A} receptors in the hypothalamus and frontal cortex (Popova *et al.* 1998, 2007) were shown in tame Norway rats. Selective breeding of silver foxes for tame behavior also increased 5-HT, 5-HIAA levels and tryptophan hydroxylase 2 activity in the hypothalamus and midbrain (Popova *et al.* 1991b).

Selective breeding for tame behavior (domestication) is often accompanied by color mutations. Many tame (domestic) animals are piebald with large and irregular white patches in specific body areas due to the disturbance of melanoblast distribution and development (Reissmann & Ludwig 2013; Trut 1999). A relationship between the distribution and development of melanoblasts, brain monoamine levels and aggressive behavior was expected (Reissmann & Ludwig 2013). The Hedlund white (h/h) mutation appeared in 1944 in the population of wild-type dark brown standard (+/+) American minks of a commercial mink farm (owner E. Hedlund, St. Paul, Minnesota) and was described as a recessive mutation with incomplete recessive effect (Shackelford & Moore 1954). Homozygous h/h minks have a white coat color, while heterozygous animals (h/+) are piebald (Markakis *et al.* 2014).

The aim of the present study was to investigation the possible interplay between the h/h mutation, aggressive behavior and brain monoamines. It was intended (1) to evaluate

the effect of the Hedlund mutation on aggressive behavior towards humans; (2) to generate standard (+/+) tame, +/+ aggressive, h/h tame and h/h aggressive minks; (3) to compare the levels of monoamines and their metabolites in the brain of minks from these four stocks.

Materials and methods

Animals

The experiment was carried out on farm-bred American minks (*Neovison vison*) of Hedlund white (h/h), and dark brown wild-type standard (+/+) genotypes maintained in the Experimental Fur Farm of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences (RFMEFI61914X0005 and RFMEFI61914X0010). The minks were treated in compliance with standard requirements established for farm-bred fur animals. After weaning at the age of 45 days all animals were individually housed in standard rearing wire mesh cages ($90 \times 45 \times 45\,cm^3$) with a $45 \times 45 \times 45\,cm^3$ wood nest box under a natural light–dark cycle and fed basic ready-mix farm feed and fresh water available *ad libitum*. The contacts with the staff were strictly standard for caged mink breeding (feeding, veterinary treatment, general care). The animal genotype (h/h, h/+ or +/+) was clearly detected by the animal color (white, piebald or brown, respectively). All animals were tested by the same rater for their reaction to human at the age of 5 months in September. Long time observations show that the behavior phenotype (aggressive or tame) determined at this age persists for 2–3 years (Trapezov *et al.* 2008, 2012). The experiments were carried out at day time. The procedures were in compliance with the European Communities Council directive 2010/63 and were approved by the Institute's ethics committee. All efforts were made to minimize the number of animals used and their suffering.

The lines of aggressive or tame minks were selectively bred during 17 successive generations for aggressive or tame behavior from the initial farm-bred population of +/+ minks (Fig. S1, Supporting Information).

The population of aggressive h/h minks was bred according to the following protocol: 30 males of farm-bred h/h minks (0 score) were hybridized with 30 females of aggressive +/+ minks from the 15th generation of selection (–3 score), then 35 h/+ males were crossed with 46 h/+ females to produce F_2 intercrosses. Twenty F_2 h/h males were crossed with 20 F_2 h/h females. A population of 97 male and female aggressive h/h minks was obtained (Fig. 1).

In order to generate the population of tame h/h minks, 30 males of farm-bred h/h minks (0 score) were hybridized with 30 females of tame +/+ minks from the 15th generation of selection (+5 score), then 40 h/+ males were crossed with 50 h/+ females to produce a F_2 intercross. Thirty h/h males from the F_2 generation were crossed with 30 h/h females from the F_2 generation. A population of 126 male and female tame h/h minks was obtained (Fig. 1).

Four experimental groups of 10 adult males from different parents were selected for brain monoamines assay. They included +/+ minks of the 17th generation of selection for aggressive or tame behavior, as well as aggressive or tame h/h minks bred as above. All animals were 11-month old and weighed 1843 ± 38 g. They expressed the maximal score of aggressive or tame phenotype, respectively: all aggressive +/+ and h/h minks had the –2 score (Fig. S2), while all tame +/+ and h/h minks had the +5 score at the age of 5 months (Fig. S3).

Test for aggressive or tame behavior towards human

Minks were tested for behavior through the hand-catch test. The rater faces the caged mink, opens the cage and attempts to catch the mink with his gloved hand. The scores of reaction to the rater are: –4 (attack at the sight of the hand), –3 (attack outside the cage), –2 (attack from the cage), –1 (threatening behavior), 0 (escape behavior), +1 (exploratory response), +2 (calm response to contact), +3 (active contact with the hand), +4 (the mink allows to touch any part of its body) and +5 (the mink allows to be handled) (Trapezov *et al.* 2008, 2012) (Table S1).

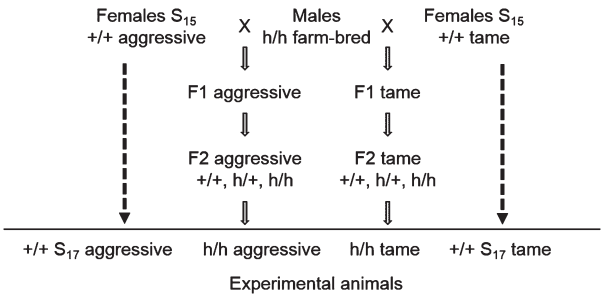

Figure 1: Protocol of the generation of experimental animals. The population of aggressive and tame Hedlund (h/h) minks were obtained from the F_2 intercrosses between the 15th generation (S_{15}) of standard brown (+/+) minks selectively bred for aggressive or tame behavior towards human and farm-bred h/h minks. They were compared to animals from the 17th generation of selection (S_{17}).

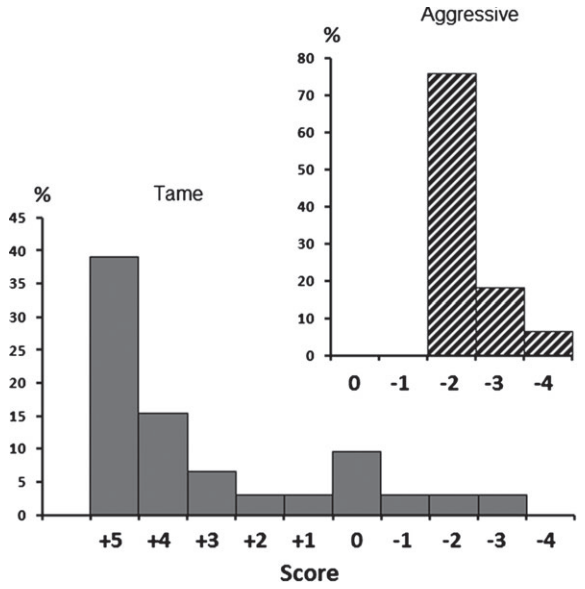

Figure 2: Distribution of behavioral phenotypes in the aggressive and tame lines of standard mink after 17 generations of selective breeding for aggressive and tame behavior towards man.

High-pressure liquid chromatography

Minks were euthanized by carbon dioxide asphyxiation followed by decapitation according to the published protocol (Fitzhugh *et al.* 2008) and the institutional guidelines on animal welfare. The midbrain, *substantia nigra* (*s. nigra*), hypothalamus, striatum and hippocampus were rapidly dissected and frozen in liquid nitrogen, and then stored at –70°C until extraction of monoamines. The tissue samples were homogenized in $HClO_4$ (0.8 M, 300 µl) and aliquots (180 µl) of homogenate were centrifuged (15 min, 14 000 ***g***, 4°C). The supernatants were diluted twice with sterile water, and the precipitate was stored at –20°C until protein quantitation by the Bradford method. The levels of monoamines and their metabolites were then analyzed by high-pressure liquid chromatography on a Luna C18(2) column (5 µm, 75 mm × 4.6 mm, Penomenex, Torrance, CA, USA), with electrochemical detection (500 mV, Coulochem III; ESA Chelmsford, MA,

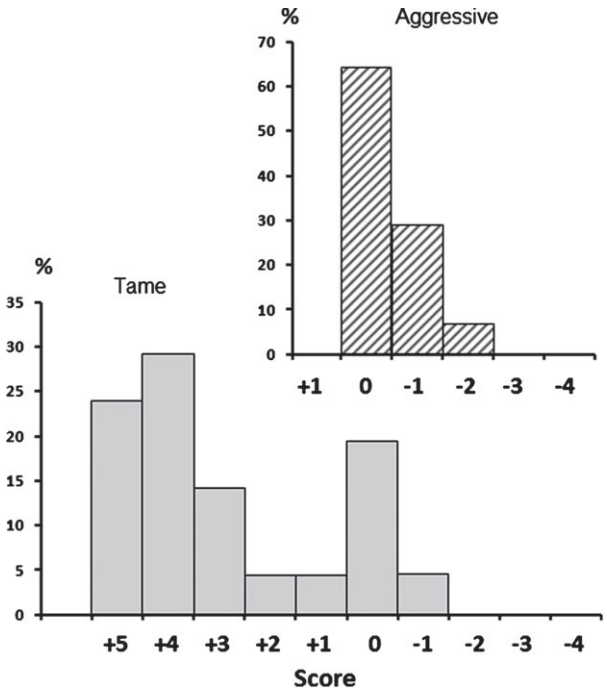

Figure 3: Distribution of behavioral phenotypes in the aggressive and tame lines of Hedlund white mink created from the F_2 intercrosses between farm-bred Hedlund white minks with standard minks of the 15th generation of selective breeding for aggressive and tame behavior.

USA; flowcell BASi, West Lafayette, IN, USA) using the solvent delivery module LC-20 AD (Shimadzu Corporation, Columbia, MD, USA) and the autosampler Optimas type 820 (Spark Holland B.V., Emmen, The Netherlands). The mobile phase contained KH_2PO_4 (100 mM, pH = 3.45), EDTA (0.1 mM), 1-octanesulfonic acid sodium salt (1.4 mM; Sigma-Aldrich, St. Louis, MO, USA) and methanol (15 v%; Vekton Ltd., Dzerzhinsk, Russia) with a flow rate of 0.8 ml/min.

The standard mix containing 5 ng each of NA, 5-HT, 5-HIAA, DA, DOPAC and HVA was repeatedly assayed throughout the entire procedure. The areas and heights of peaks were estimated using MultiChrom v.1.5 software (Ampersand Ltd., Moscow, Russia) and calibrated against the corresponding standards. The contents of NA, 5-HT, 5-HIAA, DA, 3,4-difydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were expressed in ng/mg protein. The index of 5-HT metabolism was calculated as the ratio of 5-HIAA/5-HT and the index of DA metabolism was calculated as (HVA + DOPAC)/DA.

Statistics

All data are presented as the means ± SEM. The levels of biogenic amines and their metabolites were compared by two-way ANOVA followed by the Fisher's post-hoc test if the null hypothesis of equality of the values in all four groups was rejected by one-way ANOVA. The behavioral scores were compared by Student *t*-test (aggressive vs tame) or one-way ANOVA (h/h, h/+ and +/+ in F_2). The numbers of h/h, h/+ and +/+ animals in the F_2 were compared by χ^2 test.

Results

The mean scores in the aggressive and tame populations of +/+ minks were -2.6 ± 0.03 and $+3.5 \pm 0.1$ ($t_{125} = 35.0$, $P < 0.0001$), respectively. The segregation of the color genotypes in the F_2 population obtained from aggressive females was 41 h/h, 79 h/+ and 36 +/+ and did not differ from the Mendelian segregation ($\chi^2 = 0.34$, $P > 0.05$). No difference in the aggression score between h/h (-1.8 ± 0.1 score), h/+ (-1.9 ± 0.09 score) and +/+ (-2.0 ± 0.1 score)

Table 1: ANOVA results on the effects of h/h mutation, behavioral selection (aggressive/tame) and their interaction on 5-HT and 5-HIAA levels and 5-HIAA/5-HT ratio in brain structures of the American mink

	Effect of mutation	Effect of selection	Effect of interaction
Midbrain			
5-HT	$F_{1,34} = 2.99$, $P > 0.05$	$F_{1,34} = 4.83$, $P < 0.05$	$F_{1,34} = 2.63$, $P > 0.05$
5-HIAA	$F_{1,34} < 1$	$F_{1,34} < 1$	$F_{1,34} < 1$
5-HIAA/5-HT	$F_{1,34} = 1.15$, $P > 0.05$	$F_{1,34} = 3.79$, *P= 0.06*	$F_{1,34} < 1$
Substantia nigra			
5-HT	$F_{1,36} = 245.60$, $P < 0.001$	$F_{1,36} = 10.75$, $P < 0.01$	$F_{1,36} = 12.49$, $P < 0.001$
5-HIAA	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} = 2.04$, $P > 0.05$
5-HIAA/5-HT	$F_{1,36} = 80.51$, $P < 0.001$	$F_{1,36} < 1$	$F_{1,36} = 6.30$, $P < 0.05$
Hypothalamus			
5-HT	$F_{1,36} = 19.24$, $P < 0.001$	$F_{1,36} < 1$	$F_{1,36} = 2.02$, $P > 0.05$
5-HIAA	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} = 3.48$, $P > 0.05$
5-HIAA/5-HT	$F_{1,36} = 9.71$, $P < 0.01$	$F_{1,36} = 3.69$, *P= 0.06*	$F_{1,36} < 1$
Striatum			
5-HT	$F_{1,33} = 31.43$, $P < 0.001$	$F_{1,33} = 5.17$, $P < 0.05$	$F_{1,33} < 1$
5-HIAA	$F_{1,33} < 1$	$F_{1,33} = 8.64$, $P < 0.01$	$F_{1,33} = 1.05$, $P > 0.05$
5-HIAA/5-HT	$F_{1,33} = 13.18$, $P < 0.001$	$F_{1,33} = 1.20$, $P > 0.05$	$F_{1,36} < 1$
Hippocampus			
5-HT	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} < 1$
5-HIAA	$F_{1,36} < 1$	$F_{1,36} = 1.99$, $P > 0.05$	$F_{1,36} < 1$
5-HIAA/5-HT	$F_{1,36} < 1$	$F_{1,36} = 5.87$, $P < 0.05$	$F_{1,36} < 1$

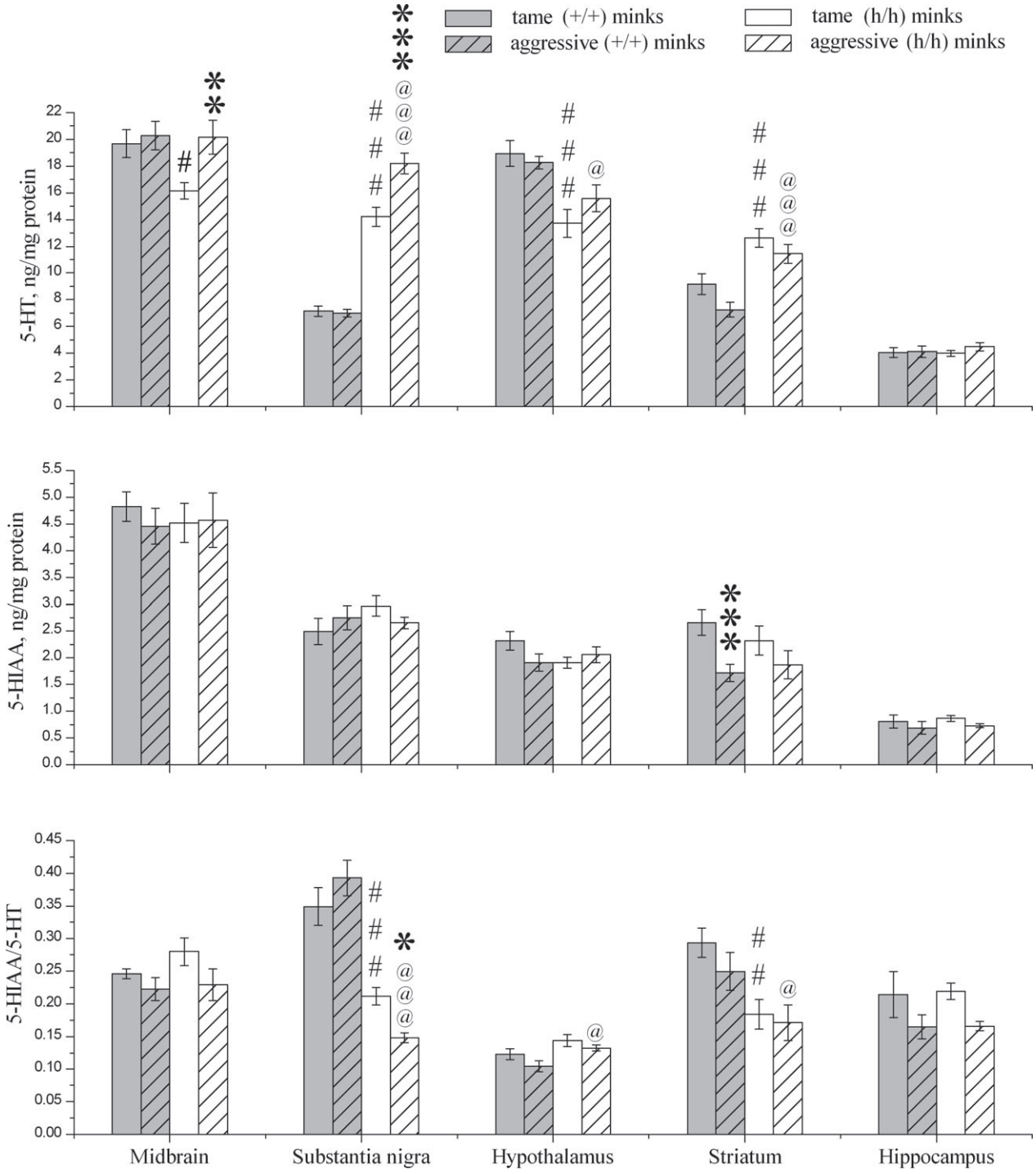

Figure 4: Levels of 5-HT and 5-HIAA as well as 5-HIAA/5-HT ratio in brain regions of standard (+/+) and Hedlund white (h/h) minks with tame or aggressive behavior towards man. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. corresponding tame minks; $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. tame +/+ minks; $@P < 0.05$, $@@@P < 0.001$ vs. aggressive +/+ minks.

Table 2: ANOVA results on the effects of h/h mutation, behavioral selection (aggressive/tame) and their interaction on NA, DA, DOPAC and HVA levels and (HVA + DOPAC)/DA ratio in brain structures of the American mink

	Effect of mutation	Effect of selection	Effect of interaction
Midbrain			
NA	$F_{1,34} < 1$	$F_{1,34} < 1$	$F_{1,34} < 1$
DA	$F_{1,34} < 1$	$F_{1,34} < 1$	$F_{1,34} = 7.45$, $P < 0.01$
DOPAC	$F_{1,34} = 6.95$, $P < 0.01$	$F_{1,34} < 1$	$F_{1,34} = 3.23$, $P > 0.05$
HVA	$F_{1,34} < 1$	$F_{1,34} < 1$	$F_{1,34} = 3.64$, *P = 0.06*
(HVA + DOPAC)/DA	$F_{1,34} < 1$	$F_{1,34} = 1.36$, $P > 0.05$	$F_{1,34} < 1$
Substantia nigra			
NA	$F_{1,36} = 25.12$, $P < 0.001$	$F_{1,36} = 3.86$, *P = 0.057*	$F_{1,36} < 1$
DA	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} = 3.72$, $P > 0.05$
DOPAC	$F_{1,36} = 2.42$, $P > 0.05$	$F_{1,36} < 1$	$F_{1,36} < 1$
HVA	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} = 3,73$, *P = 0.06*
(HVA + DOPAC)/DA	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} = 3.25$, $P > 0.05$
Hypothalamus			
NA	$F_{1,36} = 7.72$, $P < 0.01$	$F_{1,36} = 2.41$, $P > 0.05$	$F_{1,36} = 3.35$, $P > 0.05$
DA	$F_{1,36} = 4.96$, $P < 0.05$	$F_{1,36} < 1$	$F_{1,36} < 1$
DOPAC	$F_{1,36} < 1$	$F_{1,36} < 1$	$F_{1,36} < 1$
HVA	$F_{1,36} = 1.37$, $P > 0.05$	$F_{1,36} = 1.27$, $P > 0.05$	$F_{1,36} < 1$
(HVA + DOPAC)/DA	$F_{1,36} = 10.97$, $P < 0.01$	$F_{1,36} < 1$	$F_{1,36} < 1$
Striatum			
NA	$F_{1,33} = 2.03$, $P > 0.05$	$F_{1,33} < 1$	$F_{1,33} < 1$
DA	$F_{1,33} = 5.68$, $P < 0.05$	$F_{1,33} < 1$	$F_{1,33} < 1$
DOPAC	$F_{1,33} = 21.80$, $P < 0.001$	$F_{1,33} < 1$	$F_{1,33} = 3.27$, $P > 0.05$
HVA	$F_{1,33} = 4.58$, $P < 0.05$	$F_{1,33} = 2.53$, $P > 0.05$	$F_{1,33} = 3.47$, $P > 0.05$
(HVA + DOPAC)/DA	$F_{1,33} = 1.10$, $P > 0.05$	$F_{1,33} = 2.75$, $P > 0.05$	$F_{1,33} = 4,45$, $P < 0.05$
Hippocampus			
NA	$F_{1,36} = 9.29$, $P < 0.01$	$F_{1,36} < 1$	$F_{1,36} < 1$

animals was observed ($F_{2,153} < 1$) (Fig. 2). At the same time, the behavior score of +/+ F_2 animals was significantly lower compared with that of the aggressive line of standard minks ($t_{180} = 11.1$, $P < 0.0001$). The segregation of the color genotype in the F_2 population obtained from tame females was 63 h/h, 112 h/+ and 59 +/+ and did not differ from the Mendelian segregation ($\chi^2 = 0.56$, $P > 0.05$). No difference in the tame score between h/h (+3.9 ± 0.2 score), h/+ (+3.6 ± 0.2 score) and +/+ (+3.5 ± 0.2 score) animals was observed ($F_{2,231} < 1$). Moreover, the behavior score of +/+ F_2 animals did not differ from that of the tame line of standard minks ($t_{184} = 0$, $P > 0.05$). The scores of the final experimental populations of aggressive and tame minks with the Hedlund mutation (h/h) were −1.2 ± 0.1 and +3.0 ± 0.2, respectively (Fig. 3).

The mutation influenced 5-HT level and 5-HIAA/5-HT ratio in the *s. nigra*, hypothalamus and striatum. The behavioral selection influenced 5-HT level in the midbrain, *s. nigra*, striatum, 5-HIAA level in the striatum and 5-HIAA/5-HT ratio in the hippocampus. The mutation × selection interaction on 5-HT level and 5-HIAA/5-HT ratio in the *s. nigra* was significant (Table 1). Serotonin levels were higher in the *s. nigra* ($P < 0.0001$) and striatum ($P < 0.0001$), but lower in the hypothalamus ($P < 0.05$) of h/h minks compared with +/+ animals. In h/h minks the level of 5-HT was higher in the midbrain ($P < 0.01$) and *s. nigra* ($P < 0.001$) of aggressive animals. In +/+ minks, the level of 5-HIAA was lower in the striatum of aggressive animals ($P < 0.01$). The 5-HIAA/5-HT ratio was lower in the *s.nigra* ($P < 0.001$) and striatum ($P < 0.05$) in h/h minks compared with +/+ animals. In h/h minks, this trait was lower in aggressive animals ($P < 0.05$) (Fig. 4).

The mutation influenced NA levels in the *s.nigra*, hypothalamus and hippocampus (Table 2). The NA level in h/h minks was increased in the *s. nigra* ($P < 0.0001$) and hypothalamus ($P < 0.01$), but decreased in the hippocampus ($P < 0.01$) compared with +/+ animals (Fig. 5). NA concentration was increased in the hypothalamus of aggressive +/+ minks compared to tame +/+ minks (post hoc analysis, $P < 0.05$) (Fig. 5).

No DA, 3,4-dihydroxyphenylacetiv acid (DOPAC) and homovanillic acid (HVA) were detected in the hippocampus. The mutation influenced DA levels in the hypothalamus and striatum, DOPAC levels in the midbrain and striatum, HVA level in the striatum and (DOPAC + HVA)/DA ratio in the hypothalamus and striatum (Table 2). At the same time, the mutation × selection interaction was significant on the DA level in the midbrain and the (DOPAC + HVA)/DA ratio in the striatum (Table 2). Dopamine concentrations in the hypothalamus ($P < 0.05$) and striatum ($P < 0.05$) in +/+ minks were higher than those in h/h minks. They tended to decrease ($P = 0.06$) in the aggressive +/+ minks, but to increase ($P = 0.06$) in the aggressive h/h minks compared with their corresponding tame counterparts (Fig. 6). DOPAC level significantly increased in the midbrain ($P < 0.01$), but decreased in the striatum ($P < 0.0001$) in h/h compared with +/+ minks (Fig. 6). HVA concentration in the striatum was higher in the tame +/+ minks compared with the tame

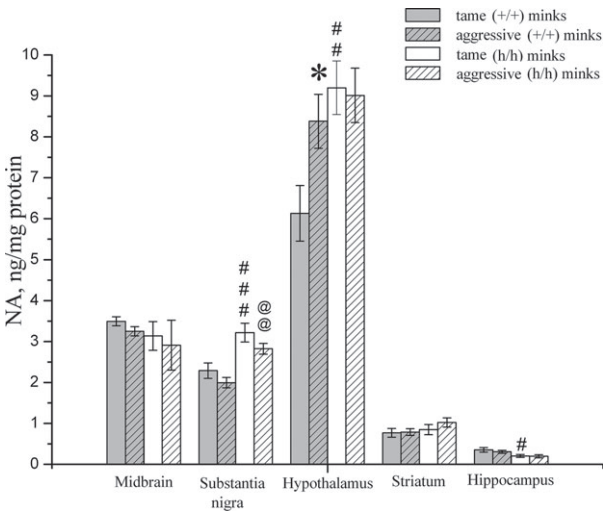

Figure 5: Level of NA in brain regions of standard (+/+) and Hedlund white (h/h) minks with tame and aggressive defensive behavior towards man. *$P < 0.05$ vs. corresponding tame minks; ##$P < 0.01$, ###$P < 0.001$ vs. tame +/+ minks; @@$P < 0.01$ vs. aggressive +/+ minks.

h/h minks ($P < 0.01$). The selective breeding for aggressive behavior decreased the HVA level in the striatum ($P < 0.05$) in +/+ minks without any effect in h/h minks (Fig. 6). The (HVA + DOPAC)/DA ratio increased in the hypothalamus of h/h minks compared with +/+ minks ($P < 0.01$). The selective breeding for aggressive behavior significantly decreased this index in the striatum of +/+ minks ($P < 0.01$), but not in h/h minks (Fig. 6).

Discussion

Selective breeding of wild-type colored standard minks for tame behavior was followed by the elevation of 5-HIAA and HVA levels in the striatum indicating the increase of 5-HT and DA metabolisms, respectively, in this structure. In contrast to tame silver foxes (Popova *et al.* 1991b) or Norway rats (Naumenko *et al.* 1989; Popova *et al.* 1991a), the tame minks did not show any significant alteration in the concentration or metabolism of 5-HT in the midbrain or hypothalamus. In general, the alterations in the brain 5-HT system in minks during the selective breeding for tame/aggressive behavior were moderate compared with those observed during the selection of silver foxes (Popova *et al.* 1991b) and Norway rats (Popova *et al.* 1991a). Furthermore, in contrast to silver foxes (Trut *et al.* 2000), a significant elevation of the NA

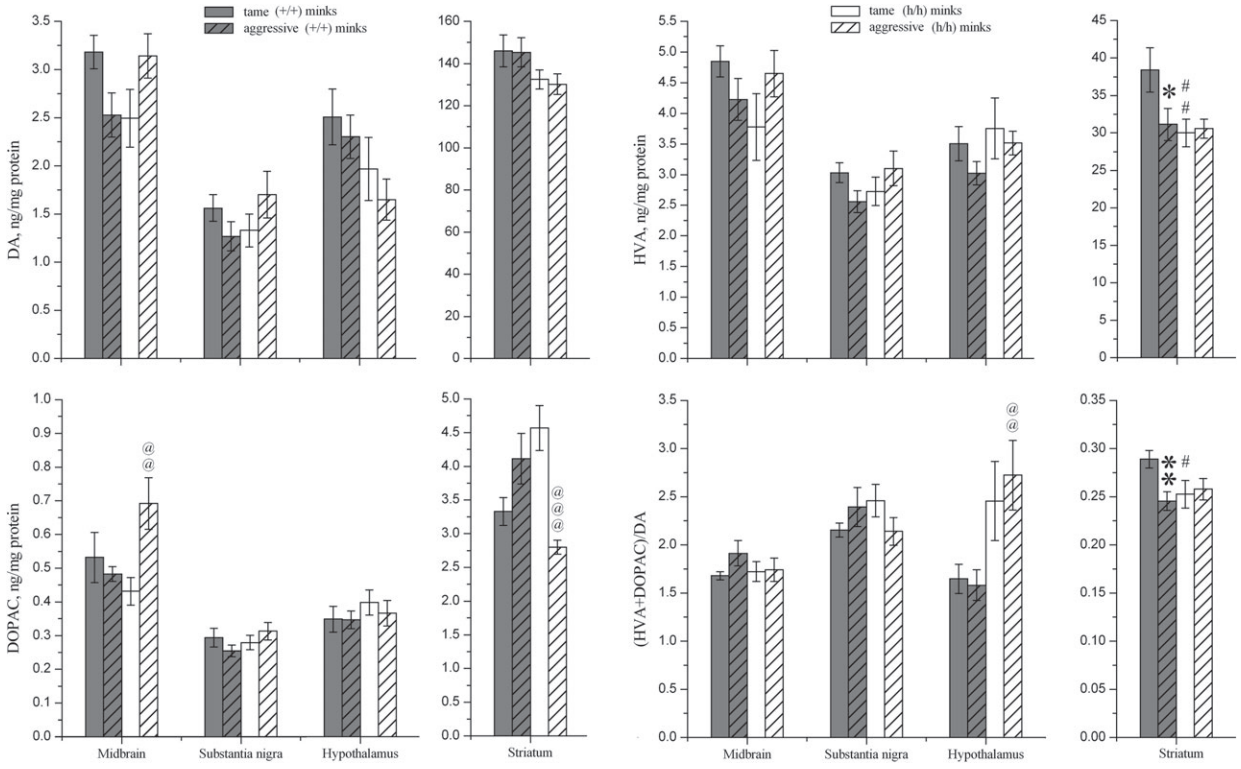

Figure 6: Levels of DA, DOPAC and HVA as well as (HVA + DOPAC)/DA ratio in brain regions of standard (+/+) and Hedlund white (h/h) minks with tame or aggressive behavior towards man. *$P < 0.05$, **$P < 0.01$ vs. corresponding tame minks; #$P < 0.05$, ##$P < 0.01$ vs. tame +/+ minks; @$P < 0.05$, @@@$P < 0.001$ vs. aggressive +/+ minks.

level in the hypothalamus of aggressive minks compared with their tame counterparts was observed. This elevation is consistent with the key role of NA in the mechanism of fear (Itoi & Sugimoto 2010).

The h/h color mutation transferred to the aggressive or tame backgrounds did not modify the reaction towards humans in the American mink. The comparison of h/h, h/+ and +/+ minks in the F_2 intercrosses did not reveal any effect of this mutation on their aggressive or tame behavior. However, the crossing of +/+ minks bred for aggressive or tame with farm-bred h/h minks significantly decreased aggressive score, but not tame score of the +/+ F_2 intercrosses. It can be supposed that farm-bred h/h minks carry other alleles modifying aggressive reaction towards humans. In order to avoid a possible influence of the genetic variability in reaction towards humans we compared biogenic amine concentrations in the brain of +/+ and h/h minks showing the same score of aggressiveness (−2) or tameness (+5).

Domestication leads to piebaldism in different species (Reissmann & Ludwig 2013; Trut 1999). A cause of piebaldism, accompanied by partial or total deafness is a disturbance of the migration, distribution and survival of the neural crest-derived melanoblasts across the entire body surface. The h/h phenotype may be considered as an extreme case of piebaldism when no pigment cells survival, while h/+ minks are piebald. In cats, dogs (Stritzel *et al.* 2009), cattle (Philipp *et al.* 2005), horses (Hauswirth *et al.* 2012) and humans (Tietz and Waardenburg syndromes) piebaldism, total or partial deafness is associated with mutations in the gene encoding microphthalmia-associated transcription factor (MITF), which is necessary for the migration and differentiation of melanoblasts (Tachibana 2000). Moreover, MITF regulates the transcriptional network of many developmental pathways (Hallsson *et al.* 2007). At the same time, the sequence analysis did not reveal any mutation in the MITF gene in the Hedlund white compared with standard brown minks (Markakis *et al.* 2014). So the question about a possible interaction between brain monoamines, behavior and the MITF gene is still open.

Domestication is an unconscious selection for reduction of aggression towards humans (Belyaev 1979). The latter is *per se* a selection for the activity of the brain neurotransmitter system involved in the regulation of defensive aggression (Popova 2006). During natural selection the behavior of wild animals was integrated with other physiological (seasonality) and morphological traits (coat color) to maximize adaptation to survive in the wild. Domestication disrupts the links between the behavior, physiology and morphology formed during the natural selection of the species (Belyaev 1979; Trut, 1999). In the present study the effect of the interaction between the h/h color mutation and the type of behavior on brain monoamines in minks was shown. Although this mutation does not alter the behavioral reaction towards human, it modifies the neurochemical mechanisms underlying aggressive and tame behavior. Taking into consideration this interaction we can hypothesize two mechanisms of modification of the brain neurotransmitter systems involved in the regulation of defensive behavior: (1) mutations of genes encoding the enzymes of metabolism, transporters and receptors of brain neurotransmitters (Popova 2006) or (2) modifications of the activity or expression of these enzymes, transporters and receptors via alterations in genes with pleiotropic effect such as the genes of pigmentation.

Conclusion

There are numerous color mutations in the American minks (Anistoroaei *et al.* 2012) and, therefore, this species is a suitable experimental model for the investigation of interplay between the coat color aberrations and behavior. The present study provides the first experimental evidence of the interplay between color mutation, reaction towards human and brain monoamines. The Hedlund white color mutation modifies the genetically defined association between brain monoamines and reaction towards human inverting the relationship between aggressive behavior and 5-HT and DA metabolism in the *s. nigra* and striatum.

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Acknowledgments

The maintenance of mink populations was supported by the basic project No 0324-2015-0004. The study of behavior and biogenic amines in the brain was supported by the Russian Science Foundation (grant No 14-25-00038). The authors declare that they have no conflict of interest.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Figure S1: Reorganization of the responses towards human in Standard mink in the course of the breeding program designed to the study the effects of selection on aggression and tameness: (A) dynamics of the tame and aggredssive scores and (B) structure of the tame and aggressive populations. The selection has been carried for 36 years (Trapezov *et al.* 2008, 2012).

Figure S2: Aggressive Standard (left) and Hedlund white (right) minks. These animals have aggressive score = −2.

Figure S3: Tame Standard (left) and Hedlund white (right) minks. These animals have tame score = +5.

Table S1: Scores of mink's defensive reaction to rater (from Trapezov *et al.* 2008).