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Regulation of Genetic Aggression by Central Dopamine System
−Plurality of Dopamine Receptor−

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Abstract □ Two types of aggressive behavior were produced by selective breeding in ICR mice. NC900 line mice exhibited high level of species-typical, isolation-induced aggression, conversely, NC100 line mice exhibited little aggression. The present study tested the hypothesis that these differences involved brain monoamine systems. Comparisons of microdissected samples from various brain regions showed that NC100 line mice had significantly lower concentrations of dopamine, DOPAC and HVA in the nucleus accumbens (NAB) and caudate nucleus (NCU) than NC900 line. Homogenate binding studies demonstrated that NC100 mice had significantly increased density of D_1 dopamine receptor, but not D_2 dopamine receptor in the caudate nucleus. These results support the hypothesis that central dopamine pathways play an important role in modulating the genetically selected differences in aggressive behavior, and of which intensity differs from D_1 and D_2 dopamine receptors.

Keywords □ Selective breeding, aggression, HPLC, dopamine, receptor binding

Cairns *et al.*[1], selectively bred ICR mice from the same foundational stock for high or low levels of aggression. Selection effects appeared rapidly, line differences in attack obtained by the fourth generation. The breeding outcomes were unidirectional; NC100 line mice that failed to exhibit the expected, species-typical aggression following isolation housing[2]. The low-aggressive line mice was more likely freezing and immobile in response to contact with test partner than the high aggressive line mice.

The neurobiology of aggression in animals has been the focus of a great deal of research[3,4]. Much of this work has examined the role of monoamines in mediating aggressive behavior induced by isolation[5,6]. However, few attention has been given to the neurobiology of mice which fail to exhibit agonistic behavior following isolation[7].

The present study was undertaken to examine the neurobiological mediation of genetic differences in mice of the same strain, selectively bred for high or low levels of aggression. Furthermore, regional functions of dopamine receptors were also investigated by examining the effect of receptor bindings in the same nuclei.

EXPERIMENTAL METHODS

Behavioral test

ICR mice were used, and attack behavior was the sole criterion for selection for breeding. The same criterion was employed in successive generations. In brief, in each generation, male mice were tested at 45 days of age for aggressive behavior in dyadic test following postweaning rearing (21 days of age) in individual cages. The test cage was constructed of plexiglass (20×21×31 cm), with a removable sheet-metal panel which divided the compartment in two separated chambers. In the dyadic test, the subject was placed on one side of the test cage and a same-age, group-reared male of the unselected line (NC600) was placed on the other side. After five minutes, the panel was removed and interactions between the subject and the partner were scored for 10 min. After testing, both animal were weighed and returned to their home cages. The social interactions observed in the dyadic test were scored such that both the initiator actions and the others responses were identified. Thirty-three categories of social behavior were used in the coding

of dyadic interaction.

Microdissection of selected brain nuclei

After the dyadic test, mice were sacrificed by decapitation, and brains were rapidly removed and frozen on dry ice with care taken to avoid compression. Brain slices (250 μm thick) were then cut on a microtome maintained at −20℃.

The micropunch techenique described by Palkovits and co-workers was used to dissect specific mouse brain nuclei from unfixed, frozen, colonal brain sections[8-10]. Individual slices were arranged on dissecting surfaces maintained at −15℃, and selected nuclei were micropunched using stainless steel cannulae with an internal diameter of either 300 μm or 500 μm. Micropunched samples were blown into polypropylene microcentrifuge tubes, placed on dry ice and stored at −70℃ until assayed. To validate the location of each punch, melted frozen sections were examined under magnification.

HPLC analyses of monoamines and metabolites

The concentrations of monoamines from various micropunched brain regions were quantified by HPLC procedure using electrochemical detection[11,12]. Differences between mouse lines under specified experimental conditions were analyzed by compound for each brain region. Quantitative determinations of monoamine and metabolite concentrations were made using amperometric detection of the column effluent with a potential of +0.75 V *vs* a Ag/AgCl reference electrode. Chromatographic separations were performed using a stainless steel column (150 mm×4.6 mm i.d.) packed with 3 micron C18 bonded micropaticulate silica (Phenomenox, Rancho Palos Verdes, CA). The mobile phase was 0.05 M Na_2HPO_4 containing 0.03 M citric acid, 0.1 mM disodium ethylenediaminetetraacetate (EDTA), 0.042% sodium octyl sulfate (SOS), and 25% methanol, with a final pH of 3.4 and a flow rate of 0.75 m*l*/min. Blank-corrected standard curve for the quantification of all compounds [dopamine (DA), serotonin (5-HT), norepinephrine (NE) and their biologically important metabolites, including homovanillic acid (HVA), dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindolacetic acid (5-HIAA)] were prepared by analyzing a series of standard solutions containing a fixed amount of the internal standard and varying amounts of each compound.

The standard solutions, in appropriate volumes of mobile phase, were subjected to the identical preparative steps used to assay unknown brain samples. Data were collected and digitized *via* Nelson Analytic data modules, and sample concentrations were calculated using the peak height for each analyte relative to the appropriate internal standard. The correlation coefficient of the standard curve obtained by linear regression analyses was routinely greater than 0.999.

Homogenate radioligand binding

^{3}H-SCH23390 binding: After dissection, mouse striata (n=4 or 5) were pooled and homogenized in 10 m*l* of ice cold 50 mM HEPES buffer pH 7.4 (25°C), using teflon-glass homogenizers. Tissue was centrifuged at 27,000 g for 10 min, the supernatant was discarded, and the pellet was resuspended in 10 m*l* ice cold buffer and centrifuged again. The final pellet was suspended at a concentration of approximately 1.0 mg wet weight/m*l*. Assay tubes (1 m*l* final volume) were incubated at 37℃ for 15 min. Nonspecific binding of ^{3}H-SCH23390 was defined by adding unlabeled SCH23390 at a concentration of 1 μM. Binding was terminated by filtering with 15 m*l* ice cold buffer on a Scatron cell harvester (Skatron INC, Sterling, VA) using glass fiber filter mats (Skatron #7034, Sterling, VA). Filters were allowed to dry and 3.0 m*l* of Scintiverse E (Fischer Scientific Co., Fair Lawn, NJ) was added. After shaking for 30 min, radioactivity was determined on a LKB Rack Beta liquid scientillation counter. K_d and B_{max} values were experimentally determined from Scatchard analyses.

Binding of ^{3}H-spiperone: The general binding protocol was identical to that described above with the following exception. Nonspecific binding of ^{3}H-spiperone was defined by adding unlabeled chlorpromazine (1 μM), ketanserin-tartarate (50 nM) was used to mask binding of spiperone to $5\text{-}HT_2$ receptors.

Measurement of protein concentrations: Quantification of protein concentrations for the neurochemical and receptor assays was performed using the method of Lowry *et al.*,[13] adapted for use with a Technicon Autoanalyzer I (Technician Instruments

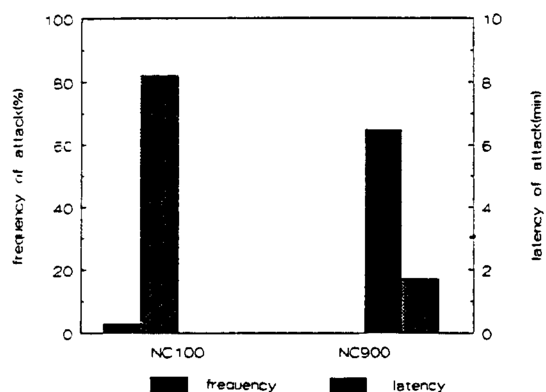

Fig. 1. Frequency (%) and latency (min) of attacks for high and low aggressive mice.

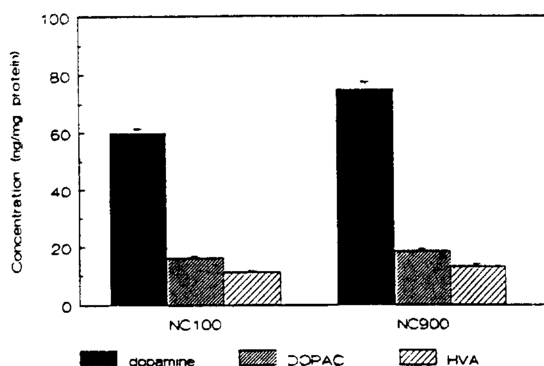

Fig. 3. Concentrations of DA, DOPAC and HVA in the nucleus accumbens of high and low aggressive mice.

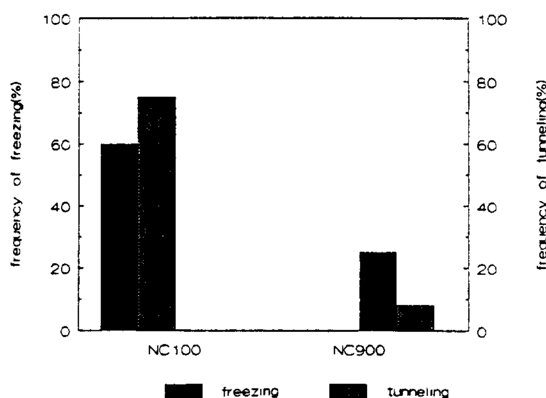

Fig. 2. Frequency of freezing (%) and tunneling (%) for high and low aggressive mice.

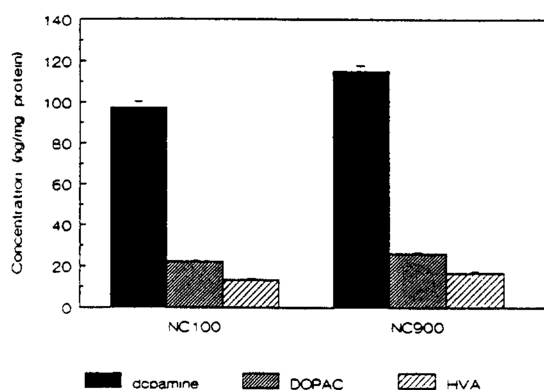

Fig. 4. Concentrations of DA, DOPAC and HVA in the nucleus caudate of high and low aggressive mice.

Corp., Chauncey, NJ) using bovine serum albumin as a standard.

RESULTS

Effects on behavior

Significant differences in aggression were seen between the NC900 and NC100 lines, using either attack frequency, or latency to attack (Fig. 1). NC100 mice also exhibited remarkably higher levels of freezing and tunneling (Fig. 2).

Line differences of dopamine activity

In the nucleus accumbens of NC100, the concentration of dopamine was significantly decreased as well as its acidic metabolites DOPAC and HVA (Fig. 3). Similar effects were found in the caudate nucleus, although the magnitude of the line differences was smaller than that observed in the nucleus accumbens.

As depicted in Fig. 4, in this brain region NC100 mice had decrease in the concentrations of dopamine, DOPAC and HVA. Also, we have not found evidence for line differences in other monoamines (5-HT or NE) in these terminal areas.

Line differences of dopamine receptor function

As shown in Table I, a significant difference in the density of binding sites for the D_1 ligand ^{3}H-SCH23390 was found with NC100 mice having the higher receptor density, in caudate nucleus. While an increase in the density of the selective D_2 radioligand ^{3}H-spiperone was not to be statistically reliable.

Table I. Dopmine receptor binding in the nucleus caudate of high and low aggressive mice

	[^{3}H]-SCH23390		[^{3}H]-Spiperone	
	B_{max} (fmol/mg protein)	K_d (nM)	B_{max} (fmol/mg protein)	K_d (nM)
NC900	2594± 103	0.26± 0.03	895± 45	0.07± 0.01
NC100	2863± 73*	0.30± 0.02	979± 64	0.08± 0.01

All values expressed as mean± SEM.
*Significantly greater than NC900, $p<0.05$

DISCUSSION

The main purpose of this study was to examine the neulobiological characteristics of behavior differences in mice, selectivily bred for high or low levels of aggression. The study of central neurotransmitter in aggression has been the focus of a great deal of research[14]. A number of these studies has compared the role of monoamines; NE, DA and 5HT, in mediating such behavior[15,16]. Other research has manipulated monoamine levels by drug administration or chemical lesions to evaluate how such changes affect aggressive behavior[17,18]. However, few attention has been given to the neurobiology of mice which fail to exhibit agonistic behavior following isolated-housing.

Considerable data support a facillitatory role for DA in defensive aggression[19,20]. Because the NAB and the NCU are typical terminal areas of DA-containing neurones, it was resonable to expect that DA functions within the NAB and NCU might be responsible for at least aggressive behavior.

This hypothesis was tested by quantifying monoamine and monoamine metabolite concentrations in microdissected brain nuclei from NC100 and NC 900 line mice. In NC100 line, mice concentrations of DA, DOPAC and HVA were significantly decresed, but not other monoamines or metabolite in NAB and NCU. This result supported the hypothesis that alteration in dopamine activity play an important role on low aggressive behavior in NC100 line mice. It is generally accepted that there are two major subclasses of dopamine receptors, D_1 and D_2[21]. Until recently, the D_2 class was believed responsible for dopamine-mediated psychopharmacological and neurochemical effects. However, with the availability of selective D_1 antagonist, it became clear that dopamine D_1 receptors also had profound functional effects which were mediated, in part, by the interaction of D_1 and D_2 receptors[22]. Therefore, although the receptor function leading to aggressive behavior following selective breeding is unkown, it may also be very interesting to examine the receptor function on this genetic differences. The homogenate binding data was seen a significant increase in D_1 receptor binding but not D_2 in NCU. This additional finding of increased dopamine receptor densities supported further the role played by dopamine in mediating genetic differences, and suggested that dopamine receptor in NC100 line mice is may be hypersensitivity. In this respect, D_1 receptor is assumed to participate to a greater extent in this genetic differences. The present data suggest that decrease in dopamine function may be an important role in aggressive behavior induced by selective-breeding. Some pharmacological investigations along this line are now in progress.

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