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The Midbrain Dopaminergic System: Anatomy and Genetic Variation in Dopamine Neuron Number of Inbred Mouse Strains

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The mesotelencephalic dopamine system is genetically variable and affects motor behavior, motivation, and learning. Here we examine the genetic variation of mesencephalic DA neuron number in a quasi-congenic RQI mouse strain and its background partner and in a recombinant inbred strain with different levels of mesencephalic tyrosine hydroxylase activity (TH/MES). We used B6.Cb4i5-α6/Vad, C57BL/6By, and CXBI, which are known to express high, intermediate, and low levels of TH/MES, respectively. Unbiased stereological sampling with optical dissector counting methods were employed to estimate the number of TH-positive neurons in the A8–A9–A10 cell groups. Morphometric studies on the mesencephalic dopamine cell groups indicated that male mice of the B6.Cb4i5-α6/Vad strain were endowed with a significantly lower number of TH-positive cells than CXBI mice. In all strains studied, the right retrorubral field (A8 area) had a higher number of dopamine neurons compared to the left A8 area. The results suggest an inverse relationship between TH/MES and number of dopamine neurons in the A9–A10 cell groups and significant lateral asymmetry in the A8 cell group. A detailed anatomical atlas of the mesencephalic A8–A9–A10 dopaminergic cell groups in the mouse is also presented to facilitate the assignment of TH-positive neurons to specific cell groups.

KEY WORDS: Complex trait; QTL introgression; tyrosine hydroxylase; dopamine neuron; stereology; mesencephalon.

INTRODUCTION

In the past several decades, it was hoped that genetic strain differences in neural phenotypes (e.g., in brain morphology or in neurotransmitter level) would explain associated behavioral differences and that such a discovery would lead to the development of animal models for human pathological behavior. The mesotelencephalic dopamine system has been implicated in several neuropsychiatric disorders, such as schizophrenia (Meltzer and Stahl, 1976), Parkinson's disease (Hornykiewicz, 1979), attention-deficit hyperactivity disorder (Faraone and Biederman, 1998), substance abuse (Koob, 1999), and in the control of motor activity and learning (Beninger, 1983), attention (Yamaguchi and Kobayashi, 1998), and other behaviors. Although it is well established that genetic factors contribute to variations at both the neural and the behavioral levels, it has been difficult to clarify the mechanisms by which these behaviors are modulated by a specific genetic change in the mesotelencephalic dopamine system. The integrated use of more refined genomic, neurobiological, and behavioral strategies is needed for the better understanding of these mechanisms. Toward this end, we conceptualized and experimentally tested quasi-congenic recombinant QTL introgression (RQI) animal models for continuously distributed quantitative traits by the development of B6.C and B6.I replicate lines (Vadasz, 1990) and sets

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of RQI strains (Vadasz *et al.*, 1994a, b; 1996, 1998). These lines were constructed by repeated backcrosses to a background strain, C57BL/6By [B6; characterized by intermediate mesencephalic tyrosine hydroxylase (TH/MES)], with concomitant selection for high (B6.C) and low (B6.I) expression of TH/MES activity. Subsequently, using B6.C-derived inbred RQI strains we demonstrated that from the donor BALB/cJ strain (characterized by high TH/MES), virtually all major QTLs could be introgressed into a new strain, B6.Cb4i5-α6/Vad, which was about 95% identical genomically to the B6 background strain (Vadasz *et al.*, 1998). Here we present data on genetic variation of the number of TH-expressing neurons in three mesencephalic cell groups. To facilitate the proper cell-group assignment of TH-immunostained neurons, we provide an anatomical atlas[1] of the different TH-positive dopaminergic cell groups in the mesencephalon of the laboratory mouse.

METHODS

Animals

Animals were maintained in our Research Colony at The Nathan Kline Institute Animal Facility on a 12/12-h light/dark schedule (lights on at 0600, lights off at 1800) with free access to food (Purina No. 5008). After weaning at the age of 5–6 weeks (depending on the development of the litter), littermate males were housed together. For immunocytochemistry, 16 adult male mice weighing 22.5–30.4 g at the time of perfusion were used. Cell counting was carried out on four male mice per strain. Subsequently, data from one CXBI subject were excluded because of uneven staining in some of the sections.

To develop QTL introgression (QI) lines, we used BALB/cJ (C) and CXBI/By (I) as donor strains because previous research demonstrated that among several highly inbred strains, C and I had the highest and lowest TH/MES (Vadasz *et al.*, 1982). I is a recombinant inbred strain carrying C57BL/6ByJ (B6) and C genes (Bailey, 1981). B6 served as the background strain, because its TH/MES was intermediate between those of the donor strains, and it had already been used as a background strain for numerous congenic lines. QTLs responsible for the continuous variation of TH/MES were introgressed onto the B6 strain background from C and I donor strains with high and low TH/MES, respectively. F_2 generations were derived from mating B6 females to C or to I males. α and β closed replicate lines were created by equal division of each $(B6 \times C)F_2$ and $(B6 \times I)F_2$ litter, resulting in four QI lines: B6.C-α, B6.C-β, B6.I-α, and B6.I-β. The QTL transfer was carried out in two directions by four or five backcross–intercross cycles, with concomitant selection for the extreme high and low expression of TH/MES in replicates, resulting in four QTL introgression lines. In the B6.C and B6.I introgression lines the top and bottom one-third of the population were selected, respectively. A comparison of the population means for TH/MES after five backcross–intercross cycles (i.e., in the b_5i_7 generation) indicated that, within introgression type (B6.C or B6.I), the replicate lines were not significantly different; however, B6.C and B6.I replicate lines expressed significantly higher and lower TH/MES than that of the B6 background (one-way ANOVA followed by Tukey's post hoc multiple-comparison tests: B6.C-α, B6.C-β > B6 > B6.I-α, B6.I-β; HSD $\alpha = .05$, $df = 272$).

To drive the heterozygous genes into a homozygous condition (fixation), the QTL introgression phase was followed by initiation of b × s mating for at least 30 generations. As for the nomenclature, the full name of an RQI strain, for instance, B6.Cb_4i_5-α6/Vad, can be abbreviated C4A6. The first letter, I (or C), stands for the donor strain name; 4 designates the backcross–intercross series (b_4i_5); A (or B) indicates the replicate line α (or β); and the last character, 6, is the identification number of the strain. Here the RQI strain is referred to as "quasi-congenic" in the same sense that "congenic" is used for the recombinant congenic (RC) series (Demant and Hart, 1986).

Immunocytochemistry

Under deep anesthesia with a mixture of diazepam (220 μl) and innovar (85 μl), the animals were perfused through the heart with 30 ml of saline followed by 100 ml of an ice-cold solution containing 4% paraformaldehyde, 15% picric acid, and 0.05% glutaraldehyde in 0.1 *M* phosphate buffer (PB; pH 7.4). This was followed by 100 ml of the same fixative without glutaraldehyde. Brains were removed immediately and postfixed in the second fixative containing 30% sucrose for 1–2 days, before being cut into three series of 50-μm coronal sections using a sliding microtome. The first se-

[1]Figures 1 and 2 can be viewed in color on-line at website http://www.wkap.n1/journalhome.htm/0001-8244.

ries of sections was stained for TH, the second series was processed with cresyl violet, and the third series was stored in an antifreeze solution. Sections were rinsed in 0.1 *M* PB and incubated for 36 h at 4°C in a 1:100 dilution of a mouse monoclonal TH antiserum (Incstar) containing 0.5% Triton X-100 and 0.1% sodium azide. The sections were rinsed three times in PB before being incubated with biotinylated antimouse IgG (Vector; 1:100) for 2 h. Sections were subsequently treated with the coupled oxidation reaction of Itoh *et al.* (1979), with 3,3′-diaminobenzidine as chromogen intensified with ammonium nickel sulfate. The sections were then rinsed in PB, dehydrated, and coverslipped with DPX. Caudal diencephalic sections, starting where the first TH neurons (Fig. 1) the A9 cells appeared, were selected for subsequent morphometric analysis.

Stereological Analysis

All cell counts were carried out with the help of an interactive computer system (Neurolucida®, Stereo Investigator; MicroBrightField, Inc.) connected to a Zeiss Axioplan microscope. The outlines and landmarks of every third section from the midbrain, including the borders of the A8, A9, and A10 cell groups were drawn with a 5 × lens. For cytoarchitectonical landmarks the adjacent cresyl violet-stained sections were consulted. Additionally, several series of TH-stained sections, subsequent to the counting procedure, were counterstained with cresyl violet (Figs. 1, 2A–H). The outlined regions containing TH-positive neurons were scanned under a 100× objective lens (Zeiss Plan-NEOFLUAR; NA = 1.3) and neurons were recorded using systematic random sampling and optical dissector methods (West *et al.,* 1991, 1996). In all

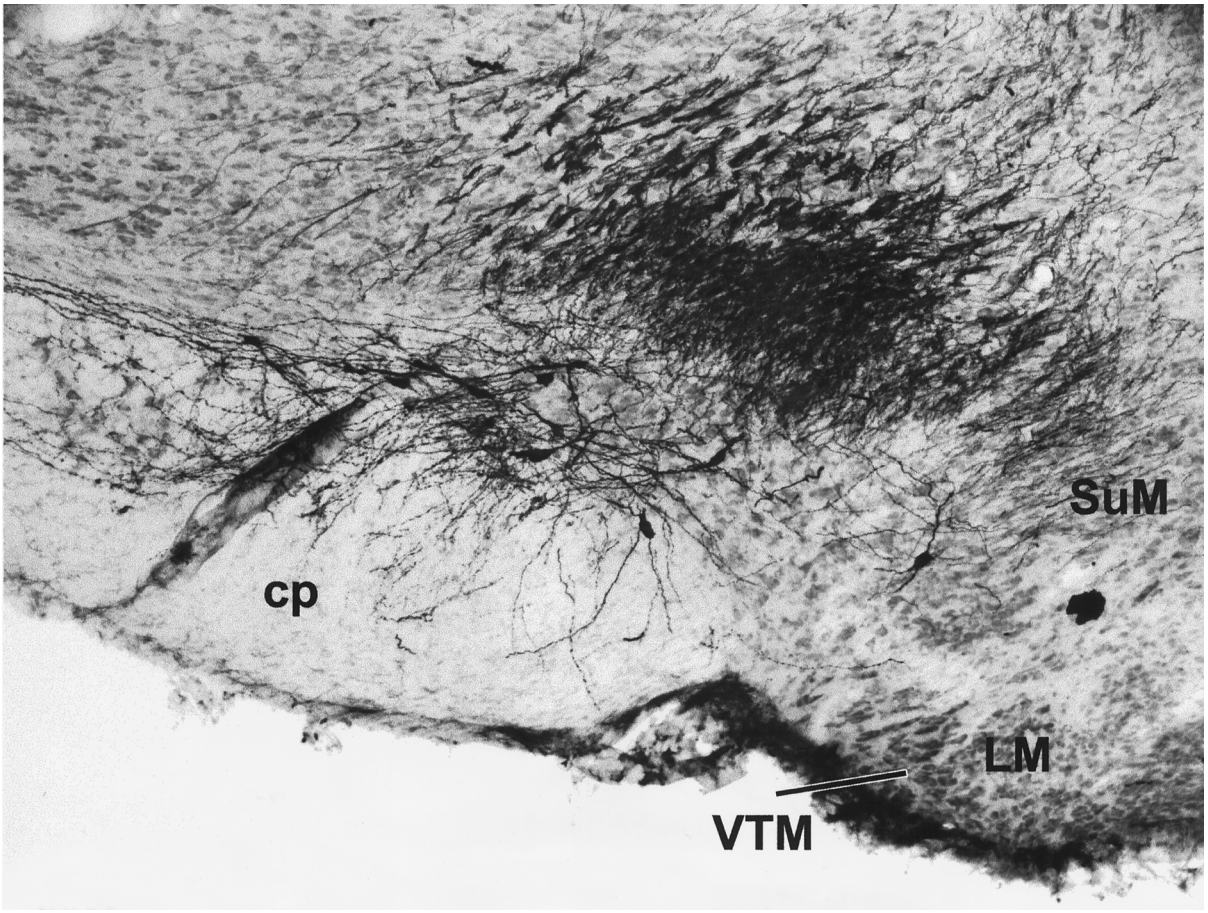


Fig. 1. High-resolution image showing the rostral part of the substantia nigra, pars compacta. The dense fiber bundle is the nigrostriatal tract. The original image was scanned with a Zeiss AxioCam digital camera with 2600 × 2060-pixel resolution using a Zeiss Axioscope microscope with a 10× Achroplan objective lens. The image was resized to 2492 × 1868 pixels using Adobe Photoshop 5.02. cp, cerebral peduncle; LM, lateral mammillary nucleus; SuM, supramammillary nucleus; VTM, ventral tuberomammillary nucleus.

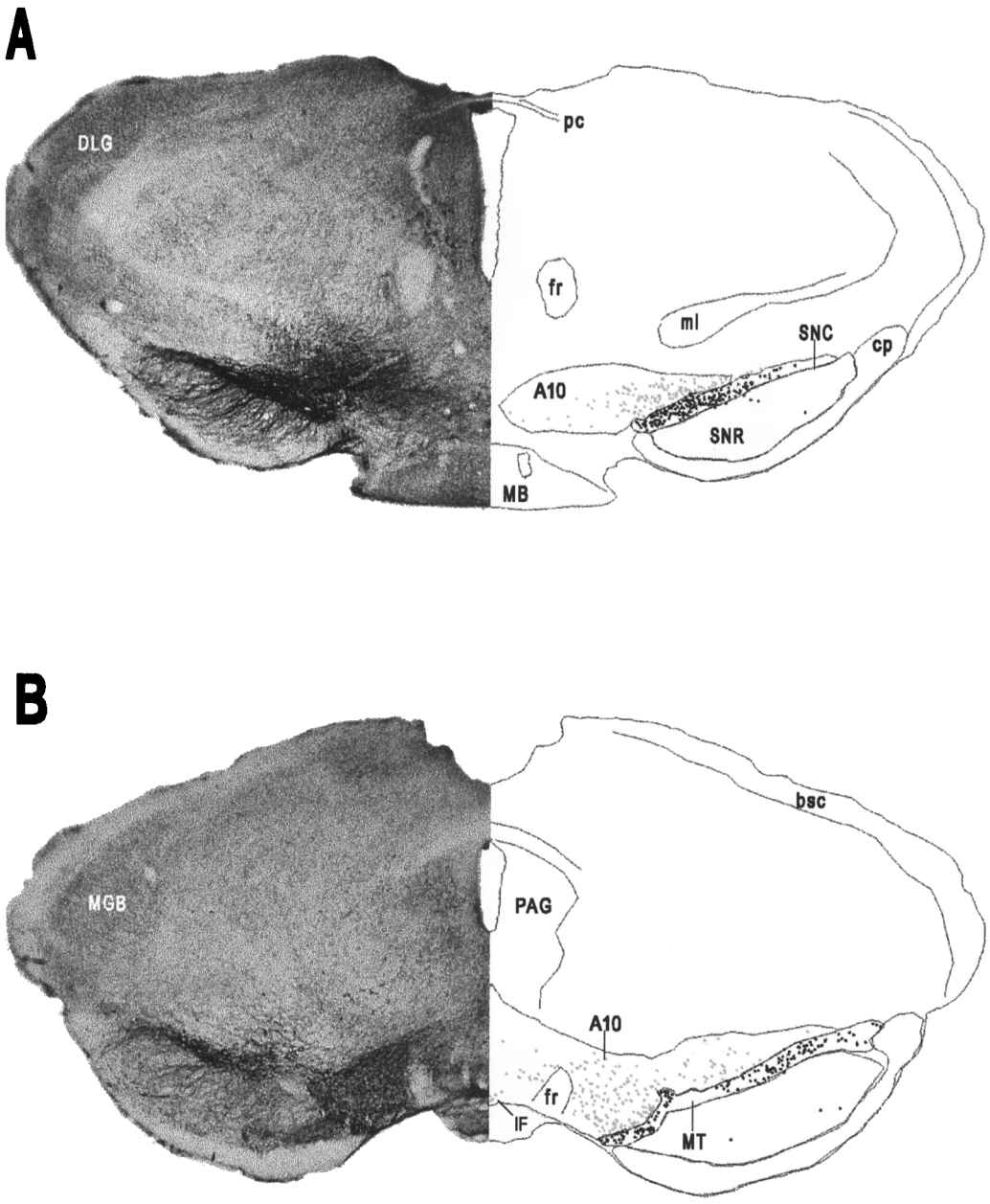


Fig. 2. Series of sections showing TH-positive neurons in the A10 (red), A9 (black), and A8 (green) cell groups. The left sides of the figures depict the original TH-stained sections counterstained with cresyl violet from a CXBI case (1483-1); the right sides show the distribution of TH-positive neurons as plotted from the same sections using a 20× objective lens and the Neurolucida® mapping software. Sections are separated by 100 μm, except between A and B and C and D, where the distance is 250 μm. A8–A10—catecholaminergic cell groups according to Dahlstrom and Fuxe (1964); 3—oculomotor nucleus; bp—brachium pontis; df—medial longitudinal fascicle; DLG—dorsal lateral geniculate nucleus; cp—cerebral peduncle; fr—fasciculus retroflexus; IP—interpeduncular nucleus; lfp—longitudinal fasciculus of the pons; ll—lateral lemniscus; m5—motor root of the trigeminal nerve; MB—mammillary body; MGB—medial geniculate body; ml—medial lemniscus; Mn—median raphe nucleus; MT—medial terminal nucleus; PAG—periaqueductal gray; PaR—pararubral nucleus; pc—posterior commissure; PMR—paramedian raphe nucleus; Pn—pontine nuclei; PPT—pedunculopontine tegmental nucleus; R—red nucleus; rs—rubrospinal tract; RtTg—reticulotegmental nucleus of the pons; SNC—substantia nigra, compact part; SNR—substantia nigra, reticular part; ts—tectospinal tract; VL—ventral nucleus of the lateral lemniscus; xscp—decussation superior of the cerebellar peduncle.

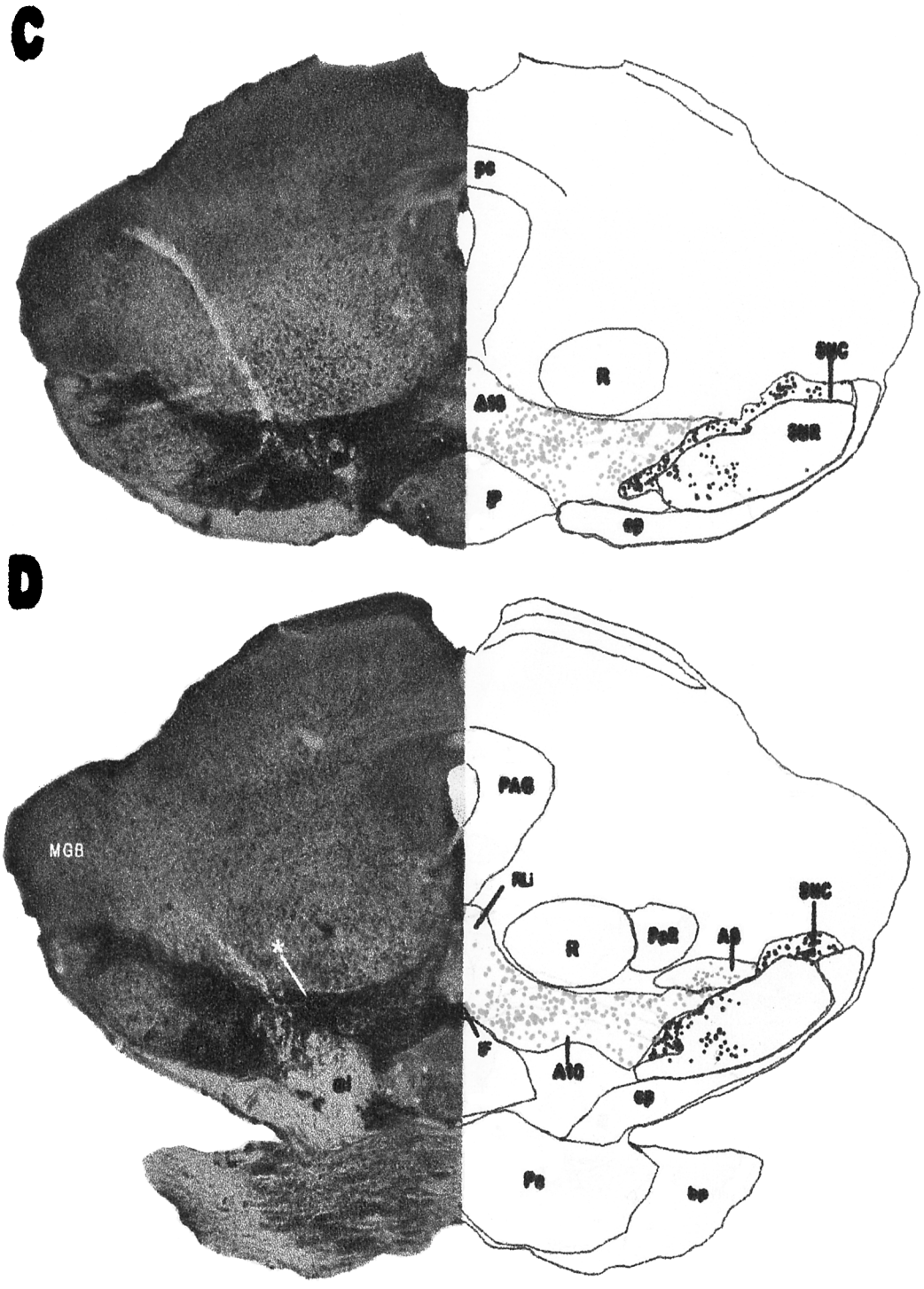
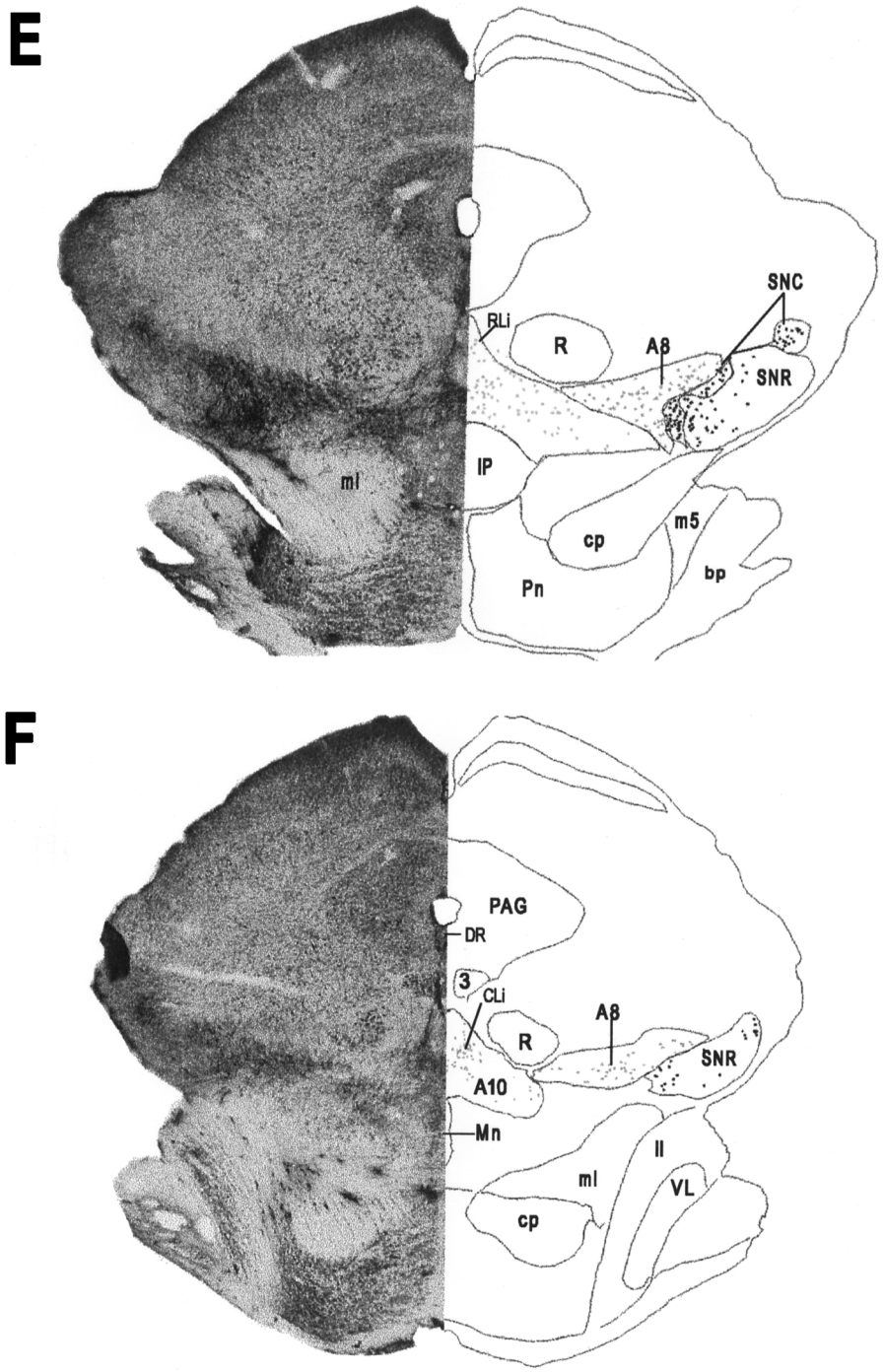


Fig. 2. Continued.

cases the following stereological parameters were used: sampling grid area, 10,000 μm^2; counting frame, 2500 μm^2; and height of optical disector, 14–15 μm, depending on the measured section thickness (20–21 μm). In each subdivision of the ventral mesencephalon the average number of cell bodies sampled in the disector (per animal and per side) was as follows: A8, 118 (52–195); A9, 264 (180–486); and A10, 386 (248–687).



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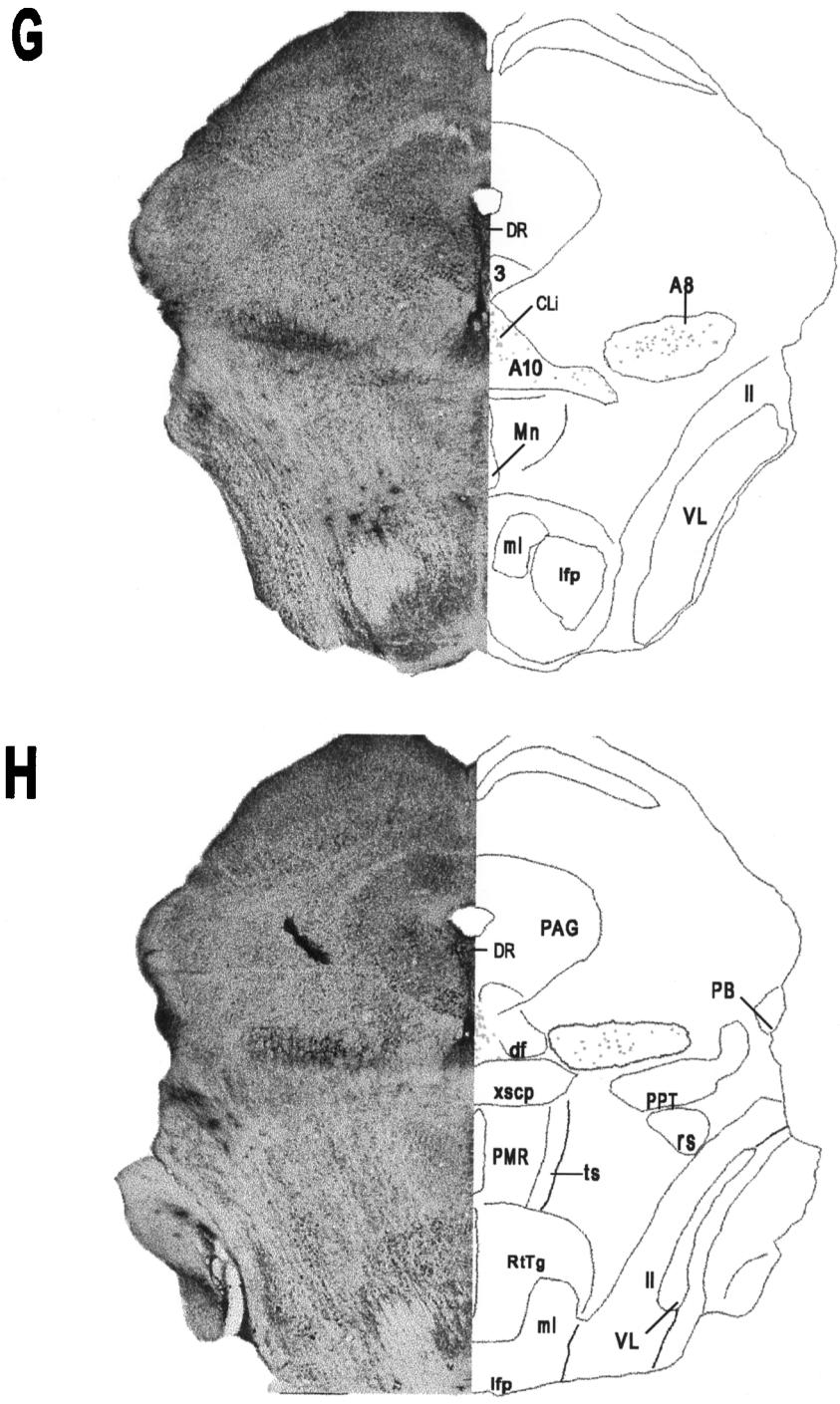
RESULTS

Anatomical Delineation of the Midbrain A8–A9–A10 Dopaminergic Cell Groups

For practical purposes we adopted a simple nomenclature following the original description of the catecholaminergic cell groups in the rat by Dahlstrom and Fuxe (1964).

A9 Cell Group. These TH-positive neurons are confined mainly to the pars compacta of the substantia nigra (SNC; Figs. 1, 2A and B). Although on cytoarchitectonic grounds, often the lateral part of the sub-



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stantia nigra is separated from the pars compacta (Franklin and Paxinos, 1997), in our study the laterally located TH-positive cells were included in the SNC. Additionally, scattered TH-positive cells can be observed, mostly confined to the medial part of the zona reticulata. The compact zone consists of three to six cell layers, to which dorsally and medially the lateral wing of the A10 cells joins. However, no sharp border exists rostrally between the A9 and the A10 cell groups. More caudally (Fig. 2C), the dorsomedial border of the A9 group is de-

fined by the ventral fibers of the medial lemniscus (ml). The TH-positive cells in the pars compacta often form pockets containing high-density TH-positive groups separated by TH-free zones. One conspicuous unstained area is represented by the small-celled medial terminal nucleus of the optic tract (MT; Fig. 2B). The ventrally located pars compacta cells give rise to dendrites extending ventrolaterally deep into the pars reticulata. Dendrites of dorsally placed neurons course mediolaterally. Reticulata TH-positive cells often show a random orientation.

A10 Cell Group. Dahlstrom and Fuxe (1964) described this group as the largest dopaminergic cell group in rat, situated mainly in the area dorsal to the interpeduncular nucleus, corresponding largely to the ventral tegmental area (VTA), and extending caudally into the rostral linear nucleus (RLi). On the basis of cytoarchitecture, in rat (Gonzalez-Hernandez and Rodriguez, 2000; Paxinos and Watson, 1998; Phillipson, 1979), mice (Franklin and Paxinos, 1997), cat (Taber, 1961), monkey, and human (Halliday and Tork, 1986; McRitchie *et al.,* 1998; Pearson *et al.,* 1990), several subgroups of the VTA have been distinguished: the paranigral nucleus, a compact cluster of bipolar or triangular cells medial to the pars compacta and dorsolateral to the interpeduncular nucleus; the parabrachial pigmented nucleus, large cells on the dorsal aspect of the VTA toward the medial lemniscus; and the interfascicular nucleus (IF), a dense small cell group located on either side of the dorsal aspect of the interpeduncular nucleus. Dorsal to the interfascicular nucleus are the rostral (RLi) and caudal linear (CLi) nuclei. TH-positive cells in these aggregates extend dorsally to the ventral aspect of the oculomotor nuclei (3; Fig. 2F) and the dorsal raphe nucleus (DR; Figs. 2D–H), respectively. There is considerable controversy as to the delineation of the different subdivisions of the A10 group. Some studies, for example, that by Swanson (1982), did not divide the VTA and use it as a synonym for the A10 group; others adopted different subdivisions (Deutch *et al.,* 1988; German and Manaye, 1993; Giolli *et al.,* 1985; Halliday and Tork, 1986). For cell-counting purposes we did not subdivide the A10 group.

In our study, the dorsal border of the A10 group was defined as where dense TH neuropil staining with a relatively sharp border blends into an area that contains only a few TH-positive fibers. Except for the most rostral part of this group, the midline was used as the medial border of the A10 (Fig. 2A). Cells of the A10 group show a characteristic position to the medial lemniscus as these fibers pierce through the ventral portion of the midbrain: rostrally, the medial lemniscus is dorsal to the A10 group (Fig. 2A); farther caudally (Fig. 2B), the A10 cells are increasingly admixed with the lemniscal fibers. Finally, at the level of Fig. 2E, most of the TH-positive cells occupy a position dorsal to the medial lemniscus. The interfascicular nucleus is characterized by very dense terminal staining that can be easily defined at the middorsal aspect of the interpeduncular nucleus (Figs. 2B–D). More caudally, with the appearance of the pontine nuclei (Pn; Fig. 2D), the ventromedial part of the A10 group is still occupied by the dense cells of the interfascicular nucleus, but the rest of the VTA can be subdivided into a ventrolateral zone, which contains a few small cells but is occupied by a dense neuropil, and a dorsal area, which contains larger cells with less dense neuropil staining. These two areas may correspond to the paranigral and parabrachial pigmented nuclei of rodents and humans, respectively. Between these two subdivisions the neuropil displays a reticular structure with few or no TH-positive cells. At the level of Fig. 2D, in the dorsolateral portion of the A10 groups, a densely stained triangular group of large cells lies between the substantia nigra and the A8 cell group (asterisk; Fig. 2D). Above the interfascicular nucleus, there is an oval-shaped area devoid of cells that is flanked by the cell-dense rostral linear nuclei. Further caudally, the A10 group is split by the crossing fibers of the pedunculus cerebellaris superior (xscp; Fig. 2H) into a cell-dense dorsal area, which constitutes the caudal linear nucleus, and a ventral area, which contains a few elongated cells that are displaced ventrolaterally by this fiber system. Neither TH-positive cells in the supramammillary region nor those in the dorsal raphe nucleus are included in the A10 group as defined here.

A8 Cell Group. In our delineation of the A8 cell group we followed the description of Deutch *et al.* (1988). This cell group appears rostrally as a small group of neurons between the dorsolateral group of the A10 cell group and the A9 neurons of the substantia nigra (Figs. 2D and E). More caudally (Fig. 2G) the bilateral A8 groups become very prominent and a dorsally situated dense and a ventrally located, more diffuse cell group can be distinguished. At the larger expanse of the crossing of the pedunculus cerebellaris superior (xscp), only the compact dorsal group remains (Fig. 2H). At the level of the parabigeminal nucleus (PB), the most caudal portion of the A8 cell group is located dorsomedially to the pedunculopontine tegmental nucleus (PPT).

Genetic Variation in the Number of TH-Positive Neurons in the Mesencephalon

We applied unbiased stereological methods using optical dissector counting to estimate the number of TH-positive neurons in the A8–A9–A10 dopaminergic cell groups in three mouse stains with different TH/MES levels. Based on previous results, the B6, C4A6, and CXBI strains were chosen, with intermediate, high, and low TH/MES, respectively (Vadasz *et al.*, 1982, 1998). The number of TH-positive neurons was counted separately in the left and right sides of the A8, A9, and A10 cell groups. Preplanned comparison of the strains with the highest and lowest TH/MES showed that CXBI mice are endowed with a significantly higher number of TH-positive cells in the A9–A10 cell groups than C4A6 mice [A9, 14,492 $\pm$ 597 vs 7787 $\pm$ 1162 (mean $\pm$ SE), $p < .005$; A10, 20,720 $\pm$ 1359 vs 12,102 $\pm$ 1395, $p < .01$; Fig. 3].

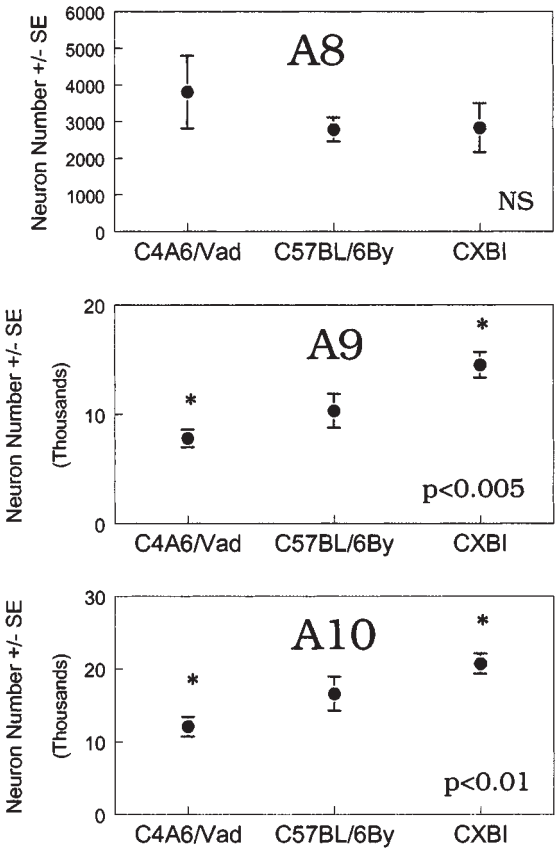

Fig. 3. Strain-dependent variation in mean dopamine neuron number. Significant differences were found between C4A6 and CXBI in cell groups A9 ($p < .05$) and A10 ($p < .01$; preplanned comparisons, independent-sample *t* test). Error bars show standard errors of the means (SE).

However, no significant strain differences were found in the A8 cell group.

No significant lateral differences were detected in the number of TH-positive neurons in the A9–A10 cell groups. In contrast, in all three mouse strains studied, the right A8 area had a higher number of TH neurons compared to the left A8 area, indicating lateral asymmetry across strains. Combining individuals from the three strains, the average $\pm$ SE TH neuron number in the left A8 area was 1461 $\pm$ 221; while in the right A8 region we counted 1704 $\pm$ 194 neurons (paired-sample *t* test, $N = 11$, $p = .01$).

DISCUSSION

Using a simple and reproducible delineation scheme the main results of this work are the following: (1) the number of TH-positive cells is significantly higher in the CXBI strain compared to the C4A6 strain in the A9–A10 cell groups, and (2) there is a significant lateral asymmetry in the number of TH-positive cells in the A8 area in all strains investigated. Because previous studies established the midbrain TH activities in these strains (Vadasz *et al.*, 1998), these results suggest an inverse relationship between cell number and TH activity in the midbrain.

Although the exact relationships between regional brain neuron number and behavior is not known, it has been suggested that quantitative variation in neuron number is one of the significant variables underlying individual differences in behavior or predisposing to neurobehavioral disorders (Reis *et al.*, 1981). Control of complex traits, such as neuron number, involves both genetic and environmental factors and their interactions in the course of development. Examples of neuron number variations include mouse strain differences in granule cell number in the area dentata (Wimer *et al.*, 1978), in 5-HT neurons in the dorsal raphe nucleus (Daszuta and Portalier, 1985), and in retinal ganglion cells (Williams *et al.*, 1998). Genetic variation in dopamine neuron number was associated with strain-dependent differences in midbrain TH activity (Baker *et al.*, 1980; Harris and Nestler, 1996; Ross *et al.*, 1976) and behavior (Reis *et al.*, 1981).

Strain-dependent variation in brain dopamine levels and TH activity have been reported by several laboratories (Ciaranello *et al.*, 1972; Kessler *et al.*, 1971; Ross *et al.*, 1976; Vadasz *et al.*, 1982, 1985, 1986, 1987; Waller *et al.*, 1983). It was suggested that a 20–50% difference in midbrain TH activity between the mouse strains, BALB/c and CBA, was consequent

to a 20% difference in the number of DA neurons in the midbrain (Baker *et al.*, 1980; Ross *et al.*, 1976). Also, the size of the caudate putamen in BALB/c was significantly greater than that in CBA (Baker *et al.*, 1980). Examination of midbrain dopamine cell density in two mouse lines selected for high (NR) and low (NNR) cataleptic response to haloperidol suggested that the increase in midbrain D2 autoreceptor density within the substantia nigra zona compacta (SNzc), but not the ventral tegmental area (VTA), was associated with a 41% increase in DA neuron number in the NNR line, while no difference between lines was found in DA neuron number in the VTA (Hitzemann *et al.*, 1993). These results were confirmed, demonstrating that the TH-positive neuron number was increased in NNR/Np mice compared to NR/Np mice, and the highest difference in DA neuron number between BALB/crl and C57BL/crl was 43% in both rostral SNzc and caudal VTA (Hitzemann *et al.*, 1994). Using MPTP treatment for modeling Parkinson's disease, Muthane *et al.* (1994) noted that MPTP treatment dramatically reduced striatal levels of DA in C57BL/6 mice, while the effect in CD-1 mice was minimal. In MPTP-treated animals the reduction in the number of SNzc TH-positive neurons was significantly greater in C57BL/6 mice than in CD-1 mice. On the other hand, there was no difference between the two strains in a subpopulation of dopaminergic neurons that coexpress TH with calbindin. Studies on the striatal terminal field indicated that mesostriatal neurons give rise to comparable axonal branching within the striatum in BALB/c and CBA strains (Mattiace *et al.*, 1989), even though the BALB/c strain has significantly more midbrain DA neurons than the CBA (Baker *et al.*, 1980; German *et al.*, 1983; Ross *et al.*, 1976). Mice of the BALB/cJ strain were more sensitive to the action of amphetamine than those of the CBA strain (Reis *et al.*, 1983), suggesting that strain-dependent differences in TH-positive cell number are associated with differential DA-mediated behaviors in BALB/c and CBA.

According to our study, midbrain TH activity was significantly higher in the B6.Cb4i5-α6/Vad strain compared to CXBI mice (Vadasz *et al.*, 1998), thus suggesting an inverse relationship between A9–A10 TH activity and dopamine cell number. A similar inverse relationship was observed between TH activity and cocaine-induced running (Vadasz, 1994a). Our findings are in line with those of Harris and Nestler (1996), who observed that 50% fewer TH-positive neurons in the VTA of the Lewis rats were associated with a 45% higher level of TH compared to Fischer 344 rats (Harris and Nestler, 1996). Similar studies on BALB/c and CBA/J mice, however, suggested positive association between TH activity and cell number (Baker *et al.*, 1980; Ross *et al.*, 1976). The discrepancies among the reported studies in regional TH activity vs TH-positive neuron number may derive from numerous factors, including the use of different species, strains, measurements of TH (activity vs amount of protein), maintenance conditions affecting gene expression, genetic susceptibility to drug-induced TH-mRNA expression (Marcel *et al.*, 1998), anatomical delineation of dopaminergic cell groups, and methods of counting neurons (West *et al.*, 1991).

The underlying mechanism for this inverse relationship is unknown. It is possible that genetically based variation in DA neuron number can lead to compensatory processes, such as altering TH activity per DA neuron. Such genetically initiated plasticity can lead to different behavioral processes depending on the affected target areas of the mesotelencephalic dopaminergic system, including the nucleus accumbens, the prefrontal cortex, and the dorsal striatum.

The functional significance of A8 dopamine neurons is not well known, and this is the first report to demonstrate lateral asymmetry in this cell group. In the rat, A8 neurons were shown to contribute to the dopaminergic innervation of the hypothalamic median eminence, an area concerned with neuroendocrine regulation (Kizer *et al.*, 1976), the striatum, nucleus accumbens, olfactory tubercle, amygdala, and bed nucleus of the stria terminalis, as well as innervating the pyriform and entorhinal cortices (Deutch *et al.*, 1988). In addition, *via* direct connections, A8 dopamine cells may modulate the functional activity of the A9 and A10 cell groups (Deutch *et al.*, 1988). Supporting this notion, feline studies have shown that a lesion in the retrorubral area, which includes the A8 cell group, produces motor programming deficits inherent to a hypofunction of the A9 system (Arts *et al.*, 1998). In nonhuman primates, the A8 cell group innervates the frontal cortex, which is commonly implicated in psychiatric and neurological disorders (Williams and Goldman-Rakic, 1998). Also, it was suggested that the A8–A10 projection to the hippocampus may have a role in metamphetamine-produced hypermotility and modulation of memory processes (Gasbarri *et al.*, 1996, 1997).

Animal and human experiments have shown that neurochemical and anatomical asymmetries exist within the basal ganglia. These asymmetries correlate with preferred direction of rotation and limb preference (Glick,

1985; Kooistra and Heilman, 1988; Richter *et al.*, 1999). Recent works focusing on attention-deficit/hyperactivity disorder (ADHD) suggest a possible abnormal hemispheric asymmetry of attention functions in boys with ADHD: they react more slowly to uncued targets in the left visual field (Nigg *et al.*, 1997). Electroencephalographic studies also support lateralization and a gender-specific atypical frontal brain activation in ADHD (Baving *et al.*, 1999). Because dopamine neurotransmission has been implicated in ADHD and in attention (Swanson, 2000; Swanson *et al.*, 2000; Yamaguchi and Kobayashi, 1998), it is possible that the A8 dopamine cell group asymmetry found in our study plays a role in the lateralized attention deficits in ADHD.

The progress in understanding the genetic control of quantitative traits has been slow due to the involvement of multiple genes, the presence of multiple alleles, and their complex interactions. Using the RQI method it is possible to transfer a QTL(s) onto the same homogeneous genetic background (Vadasz, 1994) and distribute the QTLs in recombinant strains (Vadasz *et al.*, 1998). After successful intogression of QTLs responsible for a complex trait, an RQI animal model can be characterized using the armamentarium of modern neuroscience to elucidate how genetic predisposition could lead to quantitative differences in neural circuitry and behavior. Phenotyping and genotyping of more than 100 RQI strains will also allow the mapping of relevant QTLs. The joint use of advanced animal models and molecular genetic manipulation of dopamine system-specific genes can shed light on questions about genetic differences in DA neuron number and information processing in circuitries. For example, if a genetically determined higher number of neurons is detected in one of the nodes of a neural circuitry, would this gain be expressed in the target area and in each node of a pathway? What is responsible for cell-number variation in the dopaminergic system: genetic differences in neurogenesis or in postnatal survival (Baker *et al.*, 1982)? Answers to these and similar questions, identification of the involved genes, and further work on specific genetic manipulation of the mesotelencephalic dopamine system can provide new insights into mechanisms related to Parkinson's disease (de Silva *et al.*, 2000; Morino *et al.*, 2000; Polymeropoulos *et al.*, 1997; Shimura *et al.*, 2000), ADHD (Swanson *et al.*, 2000), substance abuse (Brodie and Appel, 2000; Duaux *et al.*, 2000; Noble, 2000), and other complex diseases (Ciaranello and Boehme, 1981; Dikeos *et al.*, 1999; Ebstein *et al.*, 1997; Meloni *et al.*, 1995; Serretti *et al.*, 1999; Thibaut *et al.*, 1997).

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