# GATA-3 Is Involved in the Development of Serotonergic Neurons in the Caudal Raphe Nuclei

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The GATA-3 transcription factor shows a specific and restricted expression pattern in the developing and adult mouse brain. In the present study we investigated the role of GATA-3 in the caudal raphe system, which is known to operate as a modulator of motor activity. We demonstrate that virtually all neurons in the caudal raphe nuclei that express GATA-3 also produce serotonin. Absence of GATA-3, as analyzed in chimeric −/− mice, affects the cytoarchitecture of serotonergic neurons in the caudal raphe nuclei. As a result the chimeras show a serious defect in their locomotor performance on a rotating rod. In sum, we conclude that GATA-3 plays a major role in the development of the serotonergic neurons of the caudal raphe nuclei, and that it is crucial for their role in locomotion.

*Key words: locomotion; rotorod; transcription factors; (tau-)lacZ; nucleus raphes obscurus; nucleus raphes pallidus; rhombomere 4*

The development of the neural tube into the complex adult CNS is regulated by a large number of interacting transcription factors. Some of these transcription factors belong to the GATA family. They are characterized by two zinc finger domains of which the carboxyl finger binds the sequence WGATAR (Ko and Engel, 1993; Whyatt et al., 1993; Merika and Orkin, 1995). The six GATA factors present in mammals have overlapping expression regions in embryonic and adult tissues (for review, see Simon, 1995). Each of the GATA members might confer specificity to the binding sites in their target genes through the amino zinc finger (Whyatt et al., 1993), or they might interact with each other (Crossley et al., 1995) or with other zinc finger proteins (Merika and Orkin, 1995; Tsang et al., 1997; Wadman et al., 1997) through the N-terminal significantly divergent regions outside of the DNA binding domain. The GATA factors have very diverse patterns of expression, and all but GATA-5 have been shown to be indispensable for embryonic development. Absence of GATA-1 and -2 results in lethal hematopoietic defects (Pevny et al., 1991; Tsai et al., 1994). GATA-4 absence leads to an arrest in cardiac development (Molkentin et al., 1997), whereas GATA-6 absence leads to an extraembryonic defect early in development (Koutsourakis et al., 1999). The absence of GATA-3 also leads to embryonic death with a number of defects apparent (Pandolfi et al., 1995).

GATA-3 shows a dynamic mRNA expression pattern during mouse embryogenesis. It is expressed at an early stage in the ectoplacental cone (day 8), and over the course of the next days, GATA-3 expression increases and/or declines at numerous sites in the developing fetus. The most abundant expression is seen in the developing central and peripheral nervous systems, otic and optic vesicles, kidney, liver, adrenal gland, endothelial cells, and the primitive thymus (Oosterwegel et al., 1992; George et al., 1994). Mice carrying two GATA-3 knock-out alleles die between embryonic day 9.5 (E9.5) and E11.5 and have an abnormal morphology of the brain, defective liver hematopoiesis, abdominal hemorrhaging, and retardation of the development of the lower jaw. The brains of mice that survive until E11.5 show smaller and collapsed ventricles and a thinned and highly disorganized neuroepithelial layer. Half of the knock-out mice have a distorted spinal cord presumably because of an abnormal formation of their spines (Pandolfi et al., 1995). The brains of GATA-3−/− embryos show a much more distorted phenotype than might have been expected on the basis of the restricted expression pattern: *in situ* data of GATA-3 expression in the embryonic brain show restricted staining at E10.5 in the diencephalon and in the hindbrain, which by E11.5 is localized in the mesencephalon and parts of the pontine area (Oosterwegel et al., 1992; George et al., 1994).

The malformed brain phenotype in knock-out mice prompted us to investigate the localization and function of GATA-3 in the mature brain. A detailed expression study of GATA-3 expression in the brain was carried out using heterozygous mice in which a lacZ reporter gene is under the control of GATA-3 regulatory

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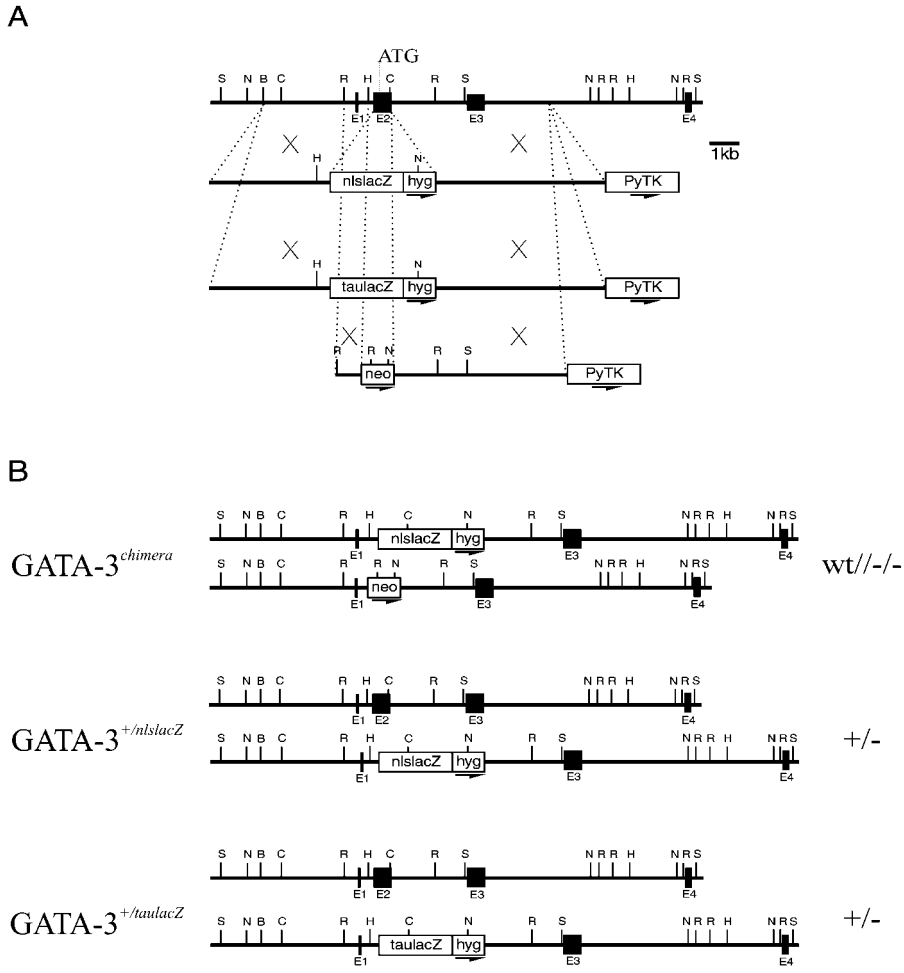


*Figure 1.* GATA-3 targeting constructs and generation of GATA-3 mutant mice. *A*, Schematic representation of the genomic wild-type GATA-3 allele (*top*) and the three GATA-3 targeting vectors used to generate the mice that were investigated in this study. *Black boxes* depict the exons (*E1–E4*) present in this region. The transcription orientations of the inserted cassettes are indicated by *arrows*. *X*, Regions of homology between the GATA-3 gene and the targeting constructs; *hyg*, hygromycin-selectable marker; *neo*, neomycin-selectable marker; *PyTK*, polyoma thymidin kinase. Restriction enzyme recognition sites: *B*, *Bam*HI; *C*, *Cla*I; *H*, *Hin*dIII; *N*, *Nco*I; *R*, *Eco*RI; *S*, *Sac*I. *B*, Schematic representation of the targeted alleles of *GATA-3*$^{nlslacZ/neo}$ chimeric mice, heterozygous *GATA-3*$^{+/nlslacZ}$ mice, and heterozygous *GATA-3*$^{+/taulacZ}$ mice.

regions. One of the major systems in the brain that was found to prominently express GATA-3 was the raphe system. The raphe nuclei are widely overlapping the groups of serotonergic neurons in the brainstem, and serotonin is considered the most important neurotransmitter in this system (for review, see Nieuwenhuys, 1985). Thus, we carried out experiments in chimeric GATA-3−/− mice to find out whether the development of serotonergic neurons in the raphe nuclei is affected by the absence of GATA-3. The same chimeras were subjected to locomotor tests to investigate the functional consequences of a partial GATA-3 deficiency.

## MATERIALS AND METHODS

*Creation of mutants.* To follow the expression of GATA-3, mice were created in which a *lacZ* gene was introduced at the ATG of the *GATA-3* gene (Fig. 1*A*). The *lacZ* gene was either fused to a nuclear localization signal sequence (*nlslacZ*) or to the *tau* gene (*taulacZ*) to follow the expression in the projecting axons. To generate the *GATA-3-nlslacZ* targeting vector, a 3.5 kb *Bam*HI–*Cla*I fragment containing exons 1 and 2 of the *GATA-3* locus was isolated and inserted into the multiple cloning site of pBluescript KS+. The ATG start codon in exon 2 was PCR-modified into an *Nco*I site, and a *Bsp*HI–*Xho*I *nlslacZ* cassette (kindly provided by Dave Michalovich, SmithKline Beecham) was placed in-frame into exon 2 in between the *Nco*I and *Sal*I sites. This subclone was flanked on the 3′ end with the *PGK*-hygromycin cassette (kindly provided by Hein te Riele, NKI), thereby deleting the coding sequence until the *Cla*I site in exon 2 (Fig. 1*A*). An additional 3 kb *GATA-3* genomic *Cla*I–*Sal*I fragment containing the Polyoma *thymidine kinase* gene obtained from a previously described vector (Pandolfi et al., 1995) was added 3′ of the construct. The *GATA-3-taulacZ* targeting vector was based on this *GATA-3-nlslacZ* targeting construct by exchanging a fragment encompassing the *nls* with a fragment of the ETL plasmid containing the bovine *tau* gene (kindly provided by Peter Mombaerts, Rockefeller University). Embryonic stem (ES) cells (either AB1 cells derived from 129/Sv mouse line or E14 cells derived from 129/Ola mouse line) were transfected with these constructs, and homologous recombinants were screened by Southern analysis using 5′ external probe, which corresponds to the *Nco*I–*Bam*HI fragment. An *Nco* digest reveals the 11, 8.5, and 9.5 kb fragments for the wild-type allele and the *nlsLacZ*- and *tauLacZ*-targeted alleles, respectively (data not shown). ES cells with a homologous recombination were injected into C57Bl/6J blastocysts to generate either heterozygous *GATA-3*$^{+/nlslacZ}$ or *GATA-3*$^{+/taulacZ}$ mice. The mice were genotyped and separated into wild-type or heterozygous mice by incubating unfixed tail tips in 5-bromo-4-chloro-3-indolyl-β-D-galactopyranosidase (X-gal) staining solution at 30°C and by Southern blot analysis. *GATA-3*$^{nlslacZ/neo}$ chimeric mice ($n = 15$) were generated by electroporation of the *GATA-3+/neo* cell line with the *GATA-3+/nlslacZ* targeting construct (also see Pandolfi et al., 1995), and double-targeted ES cells were used for C57Bl/6J blastocyst injection.

*Morphological analysis.* Mutant mice and control animals were anesthetized with an overdose of Hypnodil or Nembutal (0.5 ml) and transcardially perfused with saline and 4% paraformaldehyde in 0.1 M phosphate buffer. The brain was cryoprotected in 30% sucrose and embedded in gelatin. For serotonin immunocytochemistry, 40 μm sections were cut on a cryotome, rinsed in Tris-buffered saline (TBS), blocked in 10% normal goat serum, incubated overnight in antiserum against serotonin (1:20,000; Incstar, Stillwater, MN) in a solution of TBS with 2% normal goat serum and 0.4% triton, rinsed, processed with biotinylated goat anti-rabbit antibody (1:200) in the same solution, and incubated for visualization of the reaction product in ABC and DAB solutions (Elite ABC kit, Vectastain; Vector Laboratories, Burlingame, CA). The lacZ staining of the neurons in the sections was obtained by incubation in X-gal staining solution at 30°C. Subsequently, the sections were mounted with Permount and covered. LacZ staining of neurons in embryonic and adult whole-mount preparations were obtained likewise.

Cell counts of the percentages of lacZ-labeled and immunocytochemically labeled neurons were obtained by screening every other section containing one or more of the brainstem nuclei of interest. For this relative cell count analysis only profiles with a nucleus were used. Neurons were considered lacZ-labeled and/or immunostained when their entire nucleus and/or their entire cytoplasm was stained with blue and/or brown reaction products, respectively. For the absolute cell counts volume and density measurements were made and corrected for section thickness according to standard procedures in our laboratory (for details, see Uylings et al., 1986).

*Behavioral analysis: motor coordination tests.* The rotorod tests were performed according to the protocol described by De Zeeuw et al. (1998). The rotorod consists of a smooth plastic roller (8 cm diameter, 14 cm long) flanked by two large round plates (30 cm diameter) to prevent animals from escaping. In short, a mouse was placed on the roller, and the time it remained on the stationary or rotating (2 rpm) roller was measured. A maximum of 60 sec was allowed for each animal for all motor skill tests. The thin rod consists of a smooth plastic rod (1.5 cm diameter, 50 cm long) held horizontally on both ends. A mouse was placed on the midpoint of the rod, and the time it remained on the rod was measured.

## RESULTS

### Spatial distribution of GATA-3

The expression of GATA-3-directed nlslacZ in heterozygous *GATA-3*$^{+/nlslacZ}$ mice was observed in neurons of a restricted number of brainstem areas including nuclei of the auditory system, visual system, and raphe system (for whole-mount preparation, see Fig. 2*A,B*); no staining was found in the cerebellum or cerebral cortex, and in the spinal cord some minute labeling was observed in areas controlling the proximal musculature. Regions of the auditory and visual system that express GATA-3 include, for example, the cochlea and the inferior colliculus and the pretectal nuclei and the superior colliculus, respectively. However, the most prominent labeling occurred in the raphe system, which is the focus of the present study. The labeling in the raphe system was remarkable in that all its nuclei were GATA-3-positive; thus, the caudal raphe nuclei including the nucleus raphes pallidus (RPa), raphes obscurus (ROb), nucleus raphes magnus (RMg), and B4 contained GATA-3-labeled neurons, and the rostral raphe nuclei including the pontine raphe nuclei (PnR), dorsal (DR), and ventrolateral (DRVL) raphe nuclei expressed GATA-3 (for identification of nuclei, see Franklin and Paxinos, 1997). The expression pattern of GATA-3-directed taulacZ in heterozygous mice confirmed that of the *GATA-3*$^{+/nlslacZ}$ mice (Fig. 2*C–E*); apart from the labeled auditory and visual pathways, it was found that the ascending projections from the rostral raphe nuclei via the medial forebrain bundle as well as the descending projections from the caudal raphe nuclei to the spinal cord (bulbospinal serotonergic pathway) were positively labeled (Fig. 2*C*).

During early development three populations of GATA-3-positive cells were observed in the brainstem of whole-mount preparations (Fig. 2*F,G*). At 9 d postcoitum (dpc) we observed that GATA-3 is prominently expressed in the ventral area of rhombomere 4 (r4) in the hindbrain, whereas at 10 dpc another bipartite domain of GATA-3-positive cells occurs dorsally to this r4 region. The latter population generally extends from the caudal end of the neural tube to the midbrain–hindbrain junction, but it can be divided in two groups, which occur at slightly different stages: the first one occurs at ~10 dpc and is centered rostrally of r4, whereas the other fully develops at ~11.5 dpc and is centered caudally of r4 (for stages at 10.5 and 11.5 dpc, see Fig. 2*F,G*). The location of these latter two groups corresponds to that of the serotonergic neurons of the rostral (PnR, DR, DRVL, and B9) and caudal (ROb, RPa, RMg, and B4) raphe nuclei during early development (Aitken and Törk, 1988; Hansson et al., 1998).

### Colocalization of GATA-3 and serotonin

Although generally not more than half of the raphe nuclei neurons use serotonin as their neurotransmitter (Wiklund and Bjorklund, 1980), serotonin is still considered the most important neurotransmitter of this system, because >90% of the serotonin supply of the entire brain is derived from the raphe system (Nieuwenhuys, 1985). To determine to what extent the GATA-3-positive neurons in the raphe nuclei coincide with populations of serotonin-producing cells, double-labeling experiments were performed on brainstem sections of *GATA-3*$^{+/nlslacZ}$ mice. After X-gal staining combined with serotonin immunohistochemistry, we found a prominent overlap of both labeling patterns (Fig. 3*A,B*). In all raphe nuclei the vast majority of the lacZ-labeled neurons contained serotonin (varying from 64 to 92%; Fig. 4). Vice versa, we also observed that many of the serotonergic neurons expressed GATA-3; in the caudal raphe nuclei virtually all serotonergic neurons were positively labeled with X-gal (Fig. 3*B*), whereas in the rostral raphe nuclei approximately half (46%) of the serotonergic neurons were shown to express GATA-3. In fact, the colocalization of GATA-3 and serotonin even extended beyond the complex of raphe nuclei, because it also prominently occurred in the lateral paragigantocellular nucleus (Fig. 3*A, LPGi*). Thus, in the caudal raphe nuclei and adjacent area the two populations of cells, i.e., the GATA-3 and serotonergic cells, substantially overlap, whereas in the rostral raphe nuclei, most of the GATA-3-expressing neurons contain serotonin, but only half of the serotonergic neurons express GATA-3.

### Morphological effects in GATA-3$^{nlslacZ/neo}$ chimeric mice

To examine whether GATA-3 is necessary for a normal development of the serotonergic neurons in the raphe nuclei, we investigated the effects of a lack of GATA-3 in adult *GATA-3*$^{nlslacZ/neo}$ chimeric mice in which both GATA-3 alleles were knocked out (Fig. 1*B*). Because such chimeric mice die *in utero* when they have a high contribution of −/− cells (Pandolfi et al., 1995), only mice with a relatively low chimerism (20–40%) were investigated. The morphological composition of the rostral raphe nuclei of *GATA-3*$^{nlslacZ/neo}$ chimeric mice appeared unchanged, whereas the cytoarchitecture of the caudal raphe nuclei, especially that of the ROb, changed. While the ROb of normal mice showed a characteristic pattern of rather thin, dorsoventrally oriented cells in two stripes along the midline (Fig. 3*A,B*), the ROb of chimeric *GATA-3*$^{nlslacZ/neo}$ mice was chaotically organized and contained cells of many different sizes oriented in many different directions (Fig. 3*C,D*); these included both GATA-3−/− lacZ-stained cells and wild-type serotonergic cells. In the chimeric *GATA-3*$^{lslacZ/neo}$ mice the percentage of lacZ-labeled cells in the caudally located raphe nuclei that contained serotonin was significantly smaller (ROb, $p < 0.0005$; RPa, $p < 0.005$; RMg, $p < 0.005$; and B4, $p < 0.001$, Mann–Whitney $U$ test) than in the *GATA-3*$^{+/nlslacZ}$ mice (Fig. 4), but the absolute number of cells remained unchanged. The reducing effect on the percentage of lacZ-labeled serotonergic neurons was most pronounced in the ROb, in which the number of double positives decreased from 92% in heterozygous *GATA-3*$^{+/nlslacZ}$ mice to 30% in chimeric *GATA-3*$^{nlslacZ/neo}$ mice. In the more rostral nuclei the absence of GATA-3 did not directly affect the serotonergic neurons (Figs. 3*E*, 4, see *PnR, DR, DRVL, B9*). Thus, GATA-3 is necessary for a normal development of

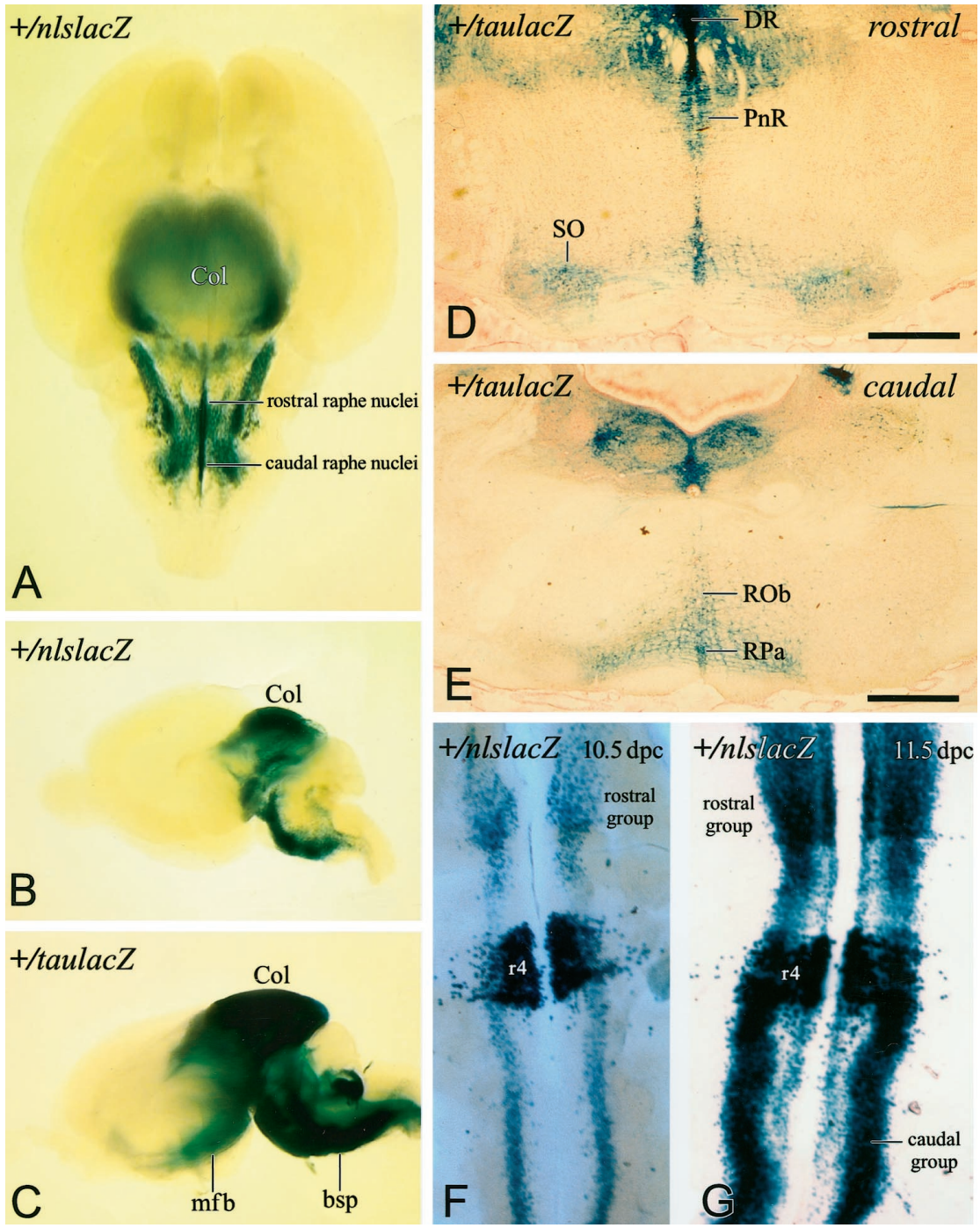


*Figure 2.* GATA-3-directed LacZ labeling in brain tissue from adult (*A, B*) and young (*F, G*), heterozygous *GATA-3*$^{+/nlslacZ}$ mice and adult *GATA-3*$^{+/taulacZ}$ mice (*C–E*). In the adult *GATA-3*$^{+/nlslacZ}$ mice the labeled cells occurred throughout the brainstem including both rhombencephalon and mesencephalon. Extensive labeling appeared, for example, in the colliculi (*Col*), superior olive (*SO*), and caudal and rostral raphe nuclei (for whole-mount preparation, see *A, B*; for coronal sections, see *D, E*). In the *GATA-3*$^{+/taulacZ}$ mice the same distribution pattern can be observed plus the major projection pathways; these include, for example, the medial forebrain bundle (*mfb*) and the descending projections from the raphe nuclei to the spinal cord [bulbospinal serotonergic pathway (*bsp*)]. The levels of the coronal sections with the rostral and caudal raphe nuclei depicted in *D* and *E* correspond to the levels indicated in the whole-mount preparation in *A*. During early development at embryonic days 10.5 (*F*) and 11.5 (*G*), three populations of GATA-3-positive cells can be observed in a frontal view of the brainstem. In the middle, one can see a dense population of neurons at the level of rhombomere 4 (r4), which presumably give rise to the efferents of the auditory system, whereas somewhat more rostrally and caudally of this region one can observe the other two groups of neurons. Considering the identical distribution of serotonergic neurons during early development (Aitken and Törk, 1988), it appears most likely that these latter rostral and caudal groups of positive GATA-3 neurons give rise to the serotonergic neurons of the rostral (PnR, DR, DRVL, and B9) and caudal (ROb, RPa, RMg, and B4) raphe nuclei, respectively. Scale bars (in *D, E*), 0.9 mm.

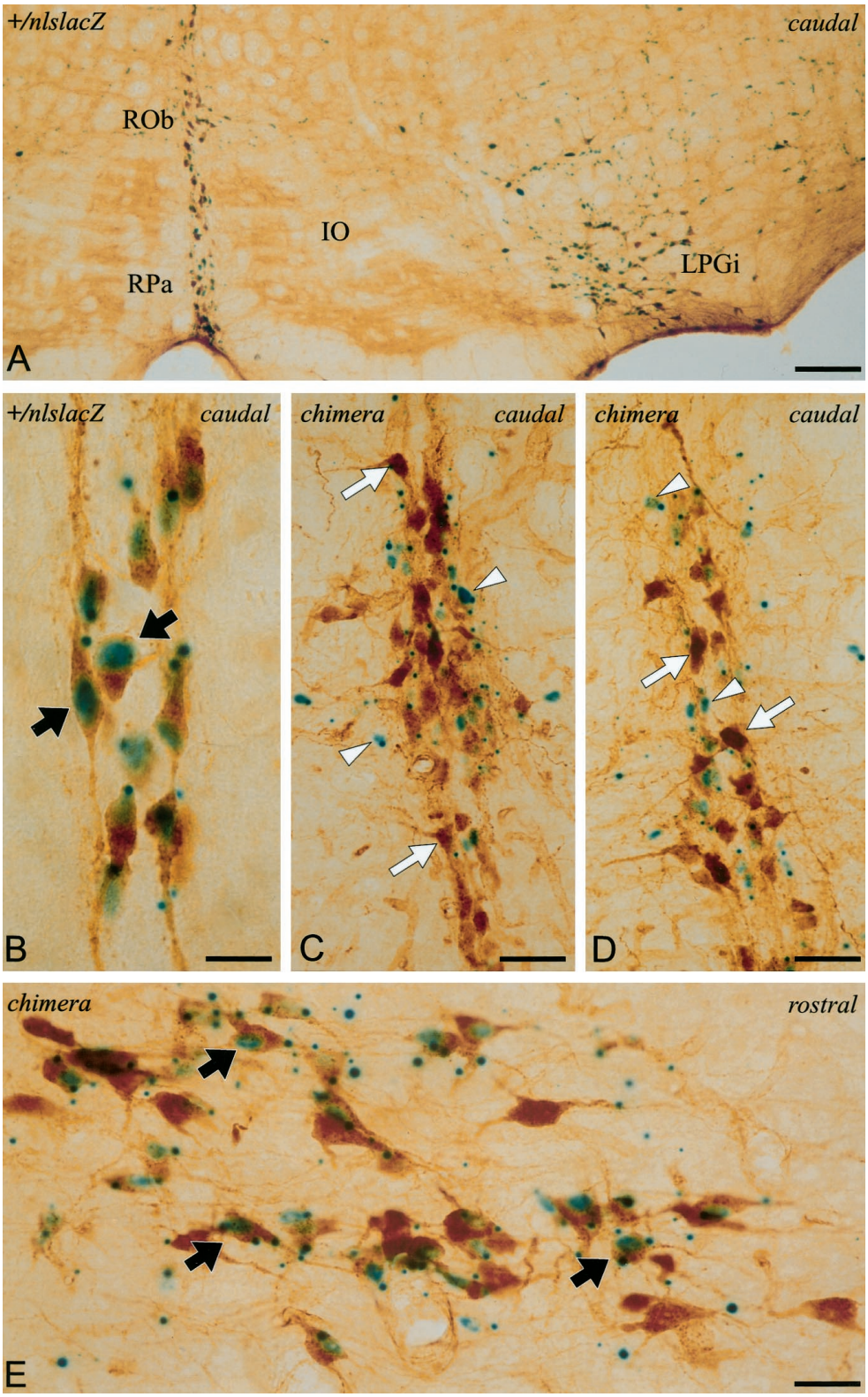


*Figure 3.* Colocalization of lacZ labeling and serotonin in heterozygous *GATA-3*$^{+/nlslacZ}$ mice and *GATA-3*$^{nlslacZ/neo}$ chimeras. In the caudal raphe nuclei of the heterozygous *GATA-3*$^{+/nlslacZ}$ mice the vast majority of the lacZ-labeled neurons were positively labeled with antisera against serotonin (*A, B*), whereas in those of the chimeras only a minority of the lacZ-labeled neurons contained serotonin (*C, D*). However, in the rostral raphe nuclei of both the *GATA-3*$^{+/nlslacZ}$ mice and GATA-3 chimeras most of the lacZ-labeled neurons produce serotonin (*E*). *IO*, Inferior olive. *Black arrows, white arrows*, and *white arrowheads* indicate double-labeled neurons, single serotonergic-labeled neurons, and single lacZ-labeled neurons, respectively. Scale bars: *A*, 100 μm; *B*, 15 μm; *C, D*, 40 μm; *E*, 25 μm.

serotonergic neurons in the caudal raphe nuclei, but not in the rostral raphe nuclei.

### Behavioral effects in GATA-3$^{nlslacZ/neo}$ chimeric mice

To find out whether an absence of GATA-3 can lead to physiological deficits, we subjected adult chimeric *GATA-3*$^{nlslacZ/neo}$ mice ($n$ = 9, obtained from two different lines) and age- and background-matched (129/C57Bl/6J) controls ($n$ = 10, including 3 heterozygous *GATA-3* chimeric mice, 3 *Sp1* chimeric mice, and 4 wild types) to three sets of motor assays (De Zeeuw et al., 1998). The animals were put on a stationary rotorod, a stationary horizontal thin rod, and a running rotorod, and the duration for which the animals remained on the apparatus (retention dura-

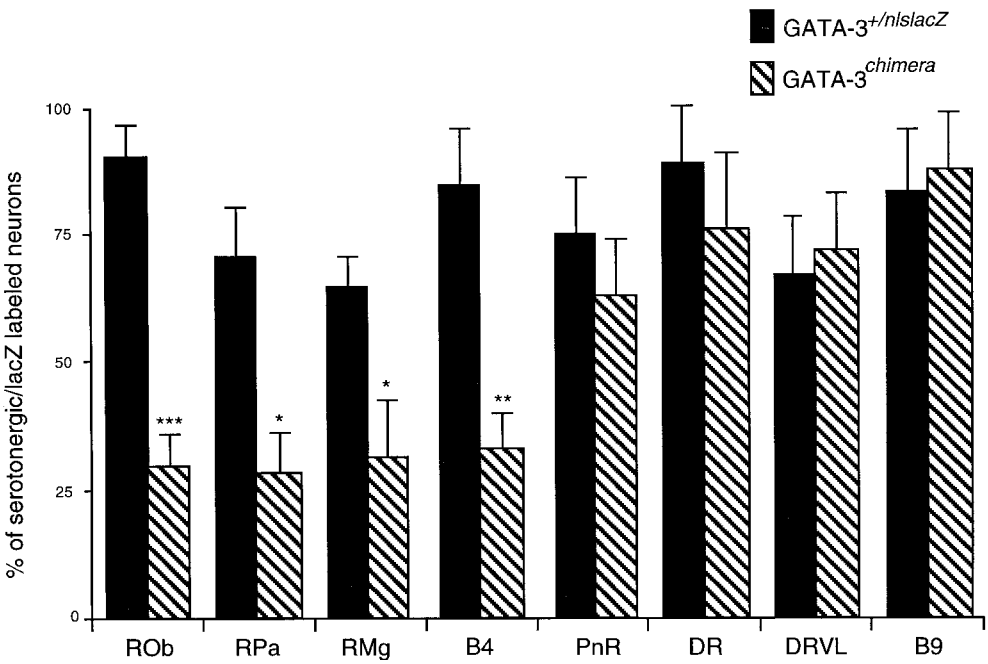


*Figure 4.* Histogram showing the percentage of lacZ-labeled neurons that were serotonergic in the raphe nuclei of the *GATA-3* $^{+/nlslacZ}$ mice and the *GATA-3*$^{nlslacZ/neo}$ chimeric mice. In the ROb, RPa, RMg, and B4 the percentage of double-labeled neurons in the chimeric mice was significantly smaller than in the *GATA-3* $^{+/nlslacZ}$ mice ($*p < 0.005$; $**p < 0.001$; $***p < 0.0005$), whereas in the more rostrally located raphe nuclei (PnR, DR, DRVL, and B9) there was no difference. Error bars indicate SD.

tion) was determined over six consecutive trials. In the two tests with the stationary rods the performances of the chimeric *GATA-3*$^{nlslacZ/neo}$ mice generally did not differ significantly from those of controls (Fig. 5); in both tests the retention duration of both types of mice increased over the trials to 60 sec. In the running rotorod test, however, the retention duration of the *GATA-3*$^{nlslacZ/neo}$ chimeras was significantly lower ($p < 0.01$, Mann–Whitney $U$ test) during the five last trials of the test; thus, whereas the retention duration of the controls increased from 5 to 36 sec over the six trials, most of the *GATA-3*$^{nlslacZ/neo}$ chimeric mice did not stay on the running rotorod for >10 sec. Interestingly, in some of the chimeric mice in which the −/− cells did not contribute to the caudal raphe nuclei, as observed by the lacZ labeling, the locomotion behavior was not impaired (data not included in Fig. 5). As an additional positive control we subjected adult lurcher mice (B6CBACa; $n = 6$), which lack Purkinje cells (Caddy and Biscoe, 1979), to the same rotorod tests. These mice completely lacked the ability to control their movements; in all three tests their average retention duration remained between 0 and 10 sec (see also De Zeeuw et al., 1998). We conclude from these experiments that, in contrast to lurchers, which have a general impairment of motor control, chimeric *GATA-3*$^{nlslacZ/neo}$ mice in which the caudal raphe nuclei are affected have a specific deficit in their locomotor activity.

## DISCUSSION

The present study demonstrates that many serotonergic neurons in the brain express the transcription factor GATA-3. In addition, we demonstrate that GATA-3 is essential for a proper development of the serotonergic neurons in the caudal raphe nuclei neurons and possibly also for their role in locomotion. To our knowledge, this is one of the first transcription factors known to be involved in these processes (see also Ye et al., 1998).

The serotonergic neurons in the different raphe nuclei subserve various functions. In general, the rostral raphe nuclei and their ascending projections are mainly involved in the control of cerebral blood flow (Edvinsson et al., 1983), sleep (Puizillout et al., 1981), and mood (van Praag and Korf, 1974; Stockmeier, 1997), whereas the caudal raphe nuclei and their descending projections are probably involved in the control of cardiovascular function (Loewy and Neil, 1981), nociception (Basbaum and Fields, 1984), and locomotion (Anderson and Proudfit, 1981). The present finding that absence of GATA-3 leads to an aberrant development of the serotonergic neurons in the caudal raphe nuclei and concomitantly to reduced locomotor activity supports the putative role of these neurons in locomotion. Especially the fact that the locomotion behavior was not affected in the chimeras in which the −/− cells did not contribute to the formation of the caudal raphe nuclei, although they did contribute to other parts of the brain, provides supportive evidence for this role. Probably the role of the caudal raphe neurons in locomotion is mediated by a direct projection from the serotonergic neurons in the ROb and RPa to motoneurons in the ventral horn of the spinal cord (Holstege and Kuypers, 1987). In fact, the caudal raphe neurons have been demonstrated to be maximally activated during repetitive locomotor movements (Veasey et al., 1995), and electrical stimulation of these nuclei as well as local application of serotonergic agonists increase the excitability of motoneurons (Roberts et al., 1988; Fung and Barnes, 1989; White et al., 1996). Thus, partial absence of GATA-3 may distort the cytoarchitectonic organization of the serotonergic neurons in the caudal raphe nuclei and thereby possibly their motor control, although the total number of serotonergic neurons may be upregulated to a normal level.

It would require considerable additional studies to determine why the serotonergic neurons in the caudal raphe nuclei were affected by the absence of GATA-3. One of the possibilities is that GATA-3 directly regulates the production of serotonin by controlling the production of one of its producing enzymes, tryptophane hydroxylase, which contains several GATA recognition sites in its promoter region (database accession numbers, X53503 and M23598). However, the fact that the rostral raphe nuclei were not affected by the lack of GATA-3 argues against this possibility. A more likely explanation appears to be that GATA-3 controls the formation of serotonergic raphe nuclei neurons early during

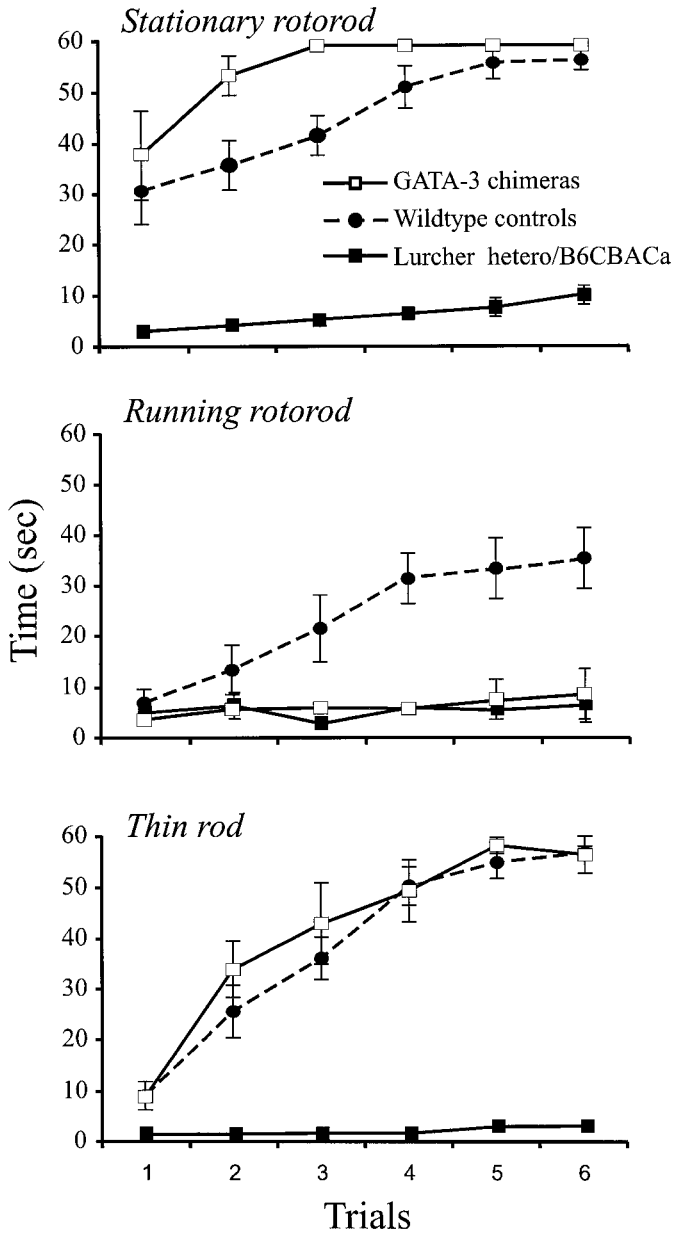


*Figure 5.* GATA-3 chimeric mice show locomotion deficits but no balance problems. GATA-3 chimeric mice and wild-type controls were put on the stationary rotorod (*top*), the running rotorod (*middle*), and the stationary horizontal thin rod (*bottom*) for a maximum of 60 sec for each trial. Note that the GATA-3 chimeras perform normally except on the running rotorod. For comparison, the staying duration times of lurcher mice, which show severe impairment of motor coordination, have been added (De Zeeuw et al., 1998). Error bars indicate SD.

development. We showed that the location of the dorsally located GATA-3-positive cells in the hindbrain probably corresponds to that of raphe nuclei neurons, which express serotonin transporter and serotonin at approximately embryonic days 10 and 13, respectively (Hansson et al., 1998). The complex of raphe nuclei neurons, which as a whole is probably derived from multiple rhombomeres, including r2, r3, r4, r5, r6, and r7 (Marín and Puelles, 1995), develops from two separate cell clusters (Aitken and Törk, 1988; Törk, 1990). In the rat the rostral raphe nuclei (PnR, DR, DRVL, and B9), which give rise to ascending serotonergic projections, are derived from a rostral cluster, whereas the caudal raphe nuclei (ROb, RPa, RMg, and B4), which give rise to descending serotonergic projections, are derived 2 d later from a caudal cell cluster (Aitken and Törk, 1988; Törk, 1990). Thus, because this differential development of the caudal and rostral cell groups can also be identified in our analysis of GATA-3-positive neurons in the hindbrain, it would indeed be possible that GATA-3 has a more prominent developmental role in the formation of the serotonergic neurons in the caudal raphe nuclei than those in the rostral raphe nuclei.

Taken together, our data indicate that GATA-3 is involved in the development of serotonergic neurons in the caudal raphe nuclei neurons and that it may be necessary for the role of these neurons in locomotion control during adulthood.

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