Differences in serotonergic metabolism possibly contribute to differences in breathing phenotype of FVB/N and C57BL/6J mice

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FROM BIRTH ONWARDS, THE NEONATAL mammal must be able to breath and adapt its breathing to behavior and environment, which requires correct function and regulations of the medullary respiratory network (RRG). In infants, RRG dysfunctions contribute to distinct syndromes, such as congenital central hypoventilation syndrome (2), sudden infant death syndrome (38), Prader-Willi syndrome (63), and Rett syndrome (30). In aged persons, sleep-disordered breathing and upper airway dysfunctions develop, emerging as risk factors for cardiovascular morbidity (32) and Alzheimer disease (6, 12, 15, 23). RRG dysfunctions remain poorly understood in humans, but mouse readiness for genetic manipulation has allowed the identification of crucial genes for RRG (5) and the production of mutant models for congenital central hypoventilation syndrome, Prader-Willi syndrome and Rett syndrome (1, 2, 55, 58, 59, 64), sleep-disordered breathing (45), and Alzheimer disease (18).

The C57BL/6J and FVB/N inbred strains are often used as background strains to produce mutant models, and the mutant phenotype may be, at least partly, affected by the background strain phenotype. The breathing phenotype of young adults of the C57BL/6J strain has been studied and compared with that of other strains (26, 43, 48–52, 62). However, the breathing phenotype of FVB/N mice has not been extensively documented. In addition, the breathing phenotype of FVB/N and C57BL/6J mice has never been compared at distinct developmental ages. Here, we examined their breathing phenotype from birth up to 1 yr. Using in vitro electrophysiology and in vivo plethysmography, we found interstrain differences in respiratory rhythm robustness, breathing parameters, and breathing regulations. As the neuromodulator/neurotransmitter serotonin (5-HT) is known to affect maturation and function of all central networks, including the RRG (7, 9, 10, 21, 27, 39, 54), we have examined the 5-HT metabolism in the medulla of FVB/N and C57BL/6J neonates and aged mice. For the first time, we found interstrain differences in 5-HT metabolism that could contribute to interstrain differences in breathing. Those interstrain differences in 5-HT and breathing systems have to be taken into account when analyzing the phenotype of mutants produced from FVB/N or C57BL/6J strains.

METHODS

Experiments were performed on 169 mice aged from embryonic day 18 to postnatal (P) day 360 (P360) from FVB/N (n = 82) and C57BL/6J (n = 87) strains (Charles River laboratories, France), in accordance with national legislation (JO 87–848) and European Communities Council Directive of 22 September 2010 (2010/63/EU). Permit numbers A13–505 and 13–426 for C. Menuet and N. Voituron, respectively were approved by Direction Départementale de la Protection des Populations, Préfecture des Bouches du Rhône, France. No follow-up study was performed, and different mice were studied at different ages. All methods and analyses have already been reported in detail elsewhere (14, 18, 55–59).

In Vitro Recordings

In vitro experiments were performed in en bloc preparations at either P1 or embryonic day 18.5 (14, 57). After cold anesthesia, the medulla and cervical spinal cord were dissected out, placed ventral side upward in a 2-ml recording chamber, and superfused with artificial cerebrospinal fluid bubbled with carbogen (in mM: 129.0 NaCl, 3.35 KCl, 1.26 CaCl2, 1.15 MgCl2, 21.0 NaHCO3, 0.58 NaH2PO4, and 30.00 glucose; pH 7.4; 4 ml/min, 27°C). The activity of phrenic motoneuron axons of the C4 ventral root was recorded and integrated, and the phrenic burst (PB) frequency was used to define the activity of the RRG. We analyzed the PB frequency [expressed in cycles per minute (c/min)], the apnea index (AI) (expressed in number of PB cycles/min > 2 mean PB cycles), and the irregularity score (IS) of the PB cycle period (55).
In Vivo Recordings

From birth up to P360, the breathing activity of conscious mice was recorded using noninvasive plethysmographic approaches at 32°C for neonates and 25°C for adults. Before recordings, the mice were habituated to stay in the plethysmograph chamber to reduce stress. Only periods of breathing without body movements were studied. We used a home-made plethysmograph built from a 20-ml syringe, ventilated with air (200 ml/min) at P1, and a constant-flow plethysmograph (EMKA Technologies, Paris, France) with 200-ml animal chamber ventilated with air (600 ml/min) at older ages. A double-chamber plethysmograph was also used in aged mice to simultaneously record the respiratory pressure changes generated by the chest movements in the chest chamber and the resulting airflow in the head chamber (18, 56, 59).

Spirograms were stored and analyzed (Spike 2; Cambridge Electronic Design, Cambridge, UK) to calculate the mean respiratory frequency (RF; expressed in respiratory c/min), tidal volume normalized by the body weight (VT, µl/g), minute ventilation normalized by the body weight (Ve, ml·g⁻¹·min⁻¹), with Ve = RF × VT/l(1,000), the AI defined as the number of apneas per minute > 2 normal respiratory cycles, and the IS. For VT measurements, volume calibrations were performed by injecting small amounts of air in the recording chambers (50–100 µl). As previously reported and discussed (18, 56), whole body plethysmography may lead to relative uncertainty of VT measurements, especially during upper airway dysfunction, with production of enlarged chest respiratory movements to compensate airway narrowing. Therefore, we also used double-chamber plethysmography to examine possible upper airway dysfunction in adult mice. We simultaneously recorded the spiromograms generated by the chest movements in the chest chamber (chest VT) and the resulting airflow in the head chamber (head VT) and calculated the ratio of head VT to chest VT (head VT/ chest VT), with a ratio significantly < 1 being indicative of upper airway dysfunction (18, 56).

We analyzed the effect of imposed hypoxic and hypercapnic challenges by first recording breathing under normal air for 20 min and thereafter under hypoxic (O₂, 10%) or hypercapnic (CO₂, 4%) gas mixture for a 5-min period (58). Changes in RF, VT, and Ve induced by hypoxia or hypercapnia were expressed as increment of control values measured before challenges. We analyzed the effect of sigh on the RF by measuring the RF every second during the 10-s period preceding the sigh (control period) and the 20-s period following the sigh, and expressing RF every second in percentage of the mean RF during the control period (59). For a given mouse, at least seven persigh periods were analyzed, and data were averaged to obtain an individual postsigh time histogram representative of mean RF changes before and after the sigh. The individual postsigh time histograms of different C57BL/6J and FVB/N mice were pooled to build an averaged histogram representative of a given strain.

Biochemical Analysis of Blood-Gas Parameters

Values of pH, P0₂, and Pco₂ levels (Torr) and hematocrit (%) were measured in venous mixed-blood samples of nonanesthetized C57BL/6J and FVB/N aged mice. After tail blood vessel dilatation with water at 40–42°C and small incision at the tip of the tail, 100-µl blood samples were collected in plastic capillaries with electrolyte-balanced heparin (Radiometer, 70 IU heparin/ml) and analyzed with ABL 80 Flex analyzer (Radiometer). Due to blood sample volumes, measurements could not be performed in neonates.

Biochemical Analysis of Brain Stem Serotonergic System

As previously reported (55), the medulla was quickly removed after decapitation and kept at −80°C until measurements. Medullary contents in 5-HT, its precursor L-tryptophan (L-Trp), and its main 5-HT metabolite 5-hydroxy-indol acid acetic (5-HIAA) from the monoamine oxidase A (MAOA) degradation pathway were measured with high-pressure liquid chromatography separation and electrochemical detection (Waters System: pump P510, electrochemical detector EC2465; Atlantis column DC18; mobile phase: citric acid, 50 mM; orthophosphoric acid, 50 mM; sodium octane sulfonic acid, 0.112 mM; EDTA, 0.06 mM; methanol, 5%; NaCl, 2 mM; pH 2.95). Contents were expressed in nanograms per medulla.

Statistical Analysis

Values are given as means ± SE. Statistical comparisons were performed using Student’s unpaired t-test (C57BL/6J VS. FVB/N mice) or Student’s paired t-test (double-chamber plethysmography). Differences were regarded as significant if P < 0.05. In Table 1, significant and nonsignificant differences are indicated as *P < 0.05, **P < 0.01, ***P < 0.001, and nonsignificant (NS), respectively.

RESULTS

Distinct Breathing and Serotonergic Phenotypes in FVB/N and C57BL/6J Neonates

In vivo study of breathing. At P1, whole body plethysmography revealed obvious interstrain differences in the breathing phenotype of conscious, unrestrained FVB/N (n = 8) and C57BL/6J (n = 6) neonates (Table 1), with robust respiratory rhythm in FVB/N vs. unstable in C57BL/6J neonates (Fig. 1A). The duration of consecutive respiratory cycles was stable in FVB/N, but highly variable in C57BL/6J neonates, with significant fivefold lower IS in FVB/N than C57BL/6J neonates (Fig. 2A). In addition, transient apneas were rare in FVB/N, but highly frequent in C57BL/6J neonates, with significant 14-fold lower AI in FVB/N than C57BL/6J neonates. Neonates from both strains had similar VT, but the RF was significantly twofold higher in FVB/N than C57BL/6J neonates, leading to significantly higher Ve in FVB/N than C57BL/6J neonates. Then C57BL/6J compared with FVB/N neonates had lower RF, lower Ve, and unstable breathing pattern at P1.

In vitro study of respiratory-like activity. To examine the origins of the interstrain differences in breathing pattern, in vitro experiments were conducted at P1 using medullary preparations, where the neonatal RRG continued to produce rhythmic PB after total isolation from upper structures and periphery (Fig. 3A). No obvious interstrain difference was observed in the PB amplitude, and the PB frequency was found slightly but nonsignificantly higher in FVB/N (n = 8) than C57BL/6J (n = 14) preparations (10.1 ± 0.8 and 8.8 ± 0.8 c/min, respectively, NS). However, the PB rhythm was robust in FVB/N, but highly unstable in C57BL/6J preparations, with significantly lower IS in FVB/N than C57BL/6J preparations (39 ± 7 and 73 ± 6, respectively, P < 0.01) and AI (0.02 ± 0.01 and 0.38 ± 0.15, respectively, P < 0.05). This revealed an inherent instability of the RRG of C57BL/6J neonates. We then examined whether the RRG instability of C57BL/6J neonates was prenatal in origin by conducting in vitro experiments at gestational day 18.5, 1 day before birth (data not shown). The isolated RRG of C57BL/6J embryos (n = 5) produced rhythmic PB with high IS (69 ± 20) and AI (7.2 ± 0.3). Thus the breathing instability observed in vivo in C57BL/6J neonates originated, at least in part, from a prenatal instability of their RRG.

Different 5-HT Metabolisms in C57BL/6J and FVB/N Neonatal Medullas

Given that endogenous 5-HT affected the RRG maturation and function (7, 27), we analyzed the 5-HT metabolism in the...
medulla of C57BL/6J (n = 10) and FVB/N (n = 10) neonates by measuring contents of 5-HT, its main catabolite 5-HIAA, and its precursor L-Trp. At P1, we found similar 5-HT contents but twofold higher L-Trp contents in FVB/N than C57BL/6J mice. Threefold weaker 5-HIAA contents in FVB/N than C57BL/6J neonates (1.28 ± 0.22 and 0.35 ± 0.48 ng/medulla, P < 0.001) led to a significantly threefold higher 5-HT-to-5-HIAA ratio (5-HT/5-HIAA) in FVB/N than C57BL/6J neonates (1.28 ± 0.22 and 0.35 ± 0.06, P < 0.001). We then examined whether the 5-HT/5-HIAA of C57BL/6J mice was already weak before birth. In C57BL/6J embryos at gestation day 16.5, we found a 5-HT/5-HIAA not significantly different (0.32 ± 0.01) from that of neonates. Then the faster turnover of the neuromodulator/neurotransmitter 5-HT in C57BL/6J than FVB/N medullas could differently affect the prenatal maturation and neonatal function of the RRG.

### Alleviated Breathing Phenotypic Differences in C57BL/6J and FVB/N Young Adults

To examine whether the differences in robustness of the neonatal RRG persisted with aging, plethysmographic recordings were performed in young and aged adult mice (this and next paragraphs, respectively). In young adults, the marked difference in breathing parameters was alleviated (Table 1). At P30, the Rf of FVB/N young adults (n = 8) was slightly reduced compared with that of FVB/N neonates, whereas the Rf of C57BL/6J young adults (n = 10) was significantly increased compared with that of C57BL/6J neonates. This reduced the interstrain difference in Rf, although the mean Rf was slightly but significantly lower in FVB/N than C57BL/6J mice. Neither Vr nor Ve differed between strains, and the rhythm robustness was improved in C57BL/6J mice, with similar weak IS and AI in C57BL/6J and FVB/N mice. Three months later (P120), Rf, Vr, and Ve were decreased in both strains, and no interstrain differences in breathing parameters were obvious, even if Rf, AI, and IS were slightly but significantly lower in FVB/N (n = 10) than C57BL/6J (n = 9) mice. Then the breathing phenotypic differences between C57BL/6J and FVB/N mice were alleviated during the P30–P120 period.

### Marked Breathing Phenotypic Differences in C57BL/6J and FVB/N Aged Mice

We thereafter analyzed the breathing pattern of aged C57BL/6J and FVB/N mice at P240 and P360 (Table 1). At P240 (Figs. 1B and 2B), the Rf was similarly reduced in both strains, but the respiratory rhythm remained robust in FVB/N (n = 14), but not in C57BL/6J (n = 8) mice. C57BL/6J compared with FVB/N mice had significantly higher IS and AI, and their Ve was drastically reduced, leading to a significant reduction of Ve. At P360, the same significant interstrain differences in rhythm robustness, Vr, and Ve were observed.

As C57BL/6J compared with FVB/N mice had significantly reduced Vr at Ve at P240, we examined whether this differently affected their blood parameters. Although less indicative than arterial blood samples, venous mixed-blood samples obtained via a relatively noninvasive way from tails of C57BL/6J (n = 6) and FVB/N (n = 8) conscious mice at P240 revealed interstrain differences. The mixed-venous blood PO2 was similar in both strains (50 ± 6 and 61 ± 5 Torr, NS), but the mixed-venous blood pH was significantly higher in FVB/N than C57BL/6J mice (7.19 ± 0.03 and 7.10 ± 0.04, respectively, P < 0.05), and, correspondingly, the mixed-venous blood PCO2 was significantly lower in FVB/N than C57BL/6J mice (54 ± 5 and 69 ± 6 Torr, respectively, P < 0.05). Hematocrit was significantly lower in FVB/N than C57BL/6J mice (44 ± 2 and 49 ± 1%, respectively, P < 0.05).

### Possible Origins of Ve Reduction in C57BL/6J vs. FVB/N Aged Mice

From birth up to 1 yr, the Ve developmental changes were fairly different in FVB/N and C57BL/6J mice, with a monotonous decrease of Ve in FVB/N mice (maximal Ve at birth and progressive decrease with age) vs. bell-shaped changes of Ve in C57BL/6J mice (weak at birth, marked but transient increase

### Table 1. Breathing parameters of neonatal, young and aged C57BL/6J and FVB/N mice

<table>
<thead>
<tr>
<th>Age/Strain</th>
<th>N</th>
<th>Rf, c/min</th>
<th>Vr, μl/g</th>
<th>V̇e, ml·g⁻¹·min⁻¹</th>
<th>AI, apneas/min</th>
<th>IS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57Bl6</td>
<td>8</td>
<td>94 ± 8</td>
<td>10.0 ± 0.6</td>
<td>0.96 ± 0.11</td>
<td>5.62 ± 0.59</td>
<td>54.5 ± 9.1</td>
</tr>
<tr>
<td>FVB/N</td>
<td>8</td>
<td>213 ± 15</td>
<td>10.0 ± 0.4</td>
<td>2.11 ± 0.14</td>
<td>0.44 ± 0.19</td>
<td>11.1 ± 0.8</td>
</tr>
<tr>
<td>P30</td>
<td>**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57Bl6</td>
<td>10</td>
<td>245 ± 17</td>
<td>10.0 ± 1.2</td>
<td>2.68 ± 0.54</td>
<td>0.20 ± 0.10</td>
<td>11.7 ± 2.7</td>
</tr>
<tr>
<td>FVB/N</td>
<td>8</td>
<td>184 ± 11</td>
<td>9.8 ± 0.9</td>
<td>1.65 ± 0.15</td>
<td>0.30 ± 0.11</td>
<td>8.0 ± 0.7</td>
</tr>
<tr>
<td>P120</td>
<td>**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57Bl6</td>
<td>9</td>
<td>192 ± 4</td>
<td>6.7 ± 0.8</td>
<td>1.30 ± 0.17</td>
<td>0.48 ± 0.08</td>
<td>11.7 ± 0.8</td>
</tr>
<tr>
<td>FVB/N</td>
<td>10</td>
<td>171 ± 6</td>
<td>6.4 ± 0.5</td>
<td>1.10 ± 0.11</td>
<td>0.01 ± 0.01</td>
<td>8.3 ± 1.2</td>
</tr>
<tr>
<td>P240</td>
<td>**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57Bl6</td>
<td>8</td>
<td>146 ± 18</td>
<td>2.7 ± 0.6</td>
<td>0.45 ± 0.15</td>
<td>0.83 ± 0.31</td>
<td>18.3 ± 3.3</td>
</tr>
<tr>
<td>FVB/N</td>
<td>14</td>
<td>148 ± 5</td>
<td>5.6 ± 0.4</td>
<td>0.85 ± 0.08</td>
<td>0.04 ± 0.03</td>
<td>8.5 ± 1.2</td>
</tr>
<tr>
<td>P360</td>
<td>**</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C57Bl6</td>
<td>10</td>
<td>128 ± 8</td>
<td>2.6 ± 0.2</td>
<td>0.33 ± 0.03</td>
<td>0.23 ± 0.07</td>
<td>10.3 ± 1.4</td>
</tr>
<tr>
<td>FVB/N</td>
<td>9</td>
<td>159 ± 10</td>
<td>5.4 ± 0.5</td>
<td>0.75 ± 0.08</td>
<td>0.05 ± 0.03</td>
<td>7.6 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of respiratory frequency (Rf; cycles/min (c/min)), tidal volume (Vr; μl/g), minute ventilation (V̇e; ml·g⁻¹·min⁻¹), apnea index (AI; apneas/min), and irregularity score (IS) of conscious, unrestrained FVB/N and C57BL/6J mice at a given class of age, from postnatal day 1 (P1) up to P360, with N indicating the number of studied animals. Significant differences between mean values at a given class of age (unpaired Student's t-test) are indicated by *P < 0.05, **P < 0.01, and ***P < 0.001 (NS, nonsignificant). Note the significant interstrain difference in breathing phenotype of C57BL/6J and FVB/N neonates (P1) and aged mice (P240 and P360).
at P30–P120, and decrease at P240–P360) mainly caused by a stepped, drastic decrease of VT between P120 and P240. Such VT decrease deserved further examination, and we, therefore, analyzed upper airway function, breathing regulations, and 5-HT metabolism in FVB/N and C57BL/6J mice at P240.

**Lack of Upper Airway Dysfunction in C57BL/6J and FVB/N Aged Mice**

Whole body plethysmography may detect false VT values in case of airways dysfunction (18, 56). To examine whether the VT reduction at P240 observed with whole body plethysmography in C57BL/6J mice was actual or reflected airway dysfunction, we used double-chamber plethysmography. This allowed the simultaneous recordings of chest breathing movements (chest VT) and resulting airflow (head VT) and calculation of the head VT/chest VT. A reduced head VT/chest VT was reported to be the sign of upper airway dysfunction, with mouse producing enlarged chest movements to ventilate against partly obstructed airways. Double-chamber plethysmography in C57BL/6J mice did not reveal significant decreases in the head VT/chest VT from P30 to P240 (1.12 ± 0.22, n = 7, and 0.90 ± 0.15, n = 7, respectively, NS). As no upper airway dysfunction developed with age in C57BL/6J mice, then an actual reduction of VT occurred at P240. Double-chamber plethysmography in FVB/N mice at P30 (n = 8) and P240 (n = 6) similarly showed no reduction of the head VT/chest VT between P30 and P240.

**Differences in VT Responses to O2 and CO2 Drive in C57BL/6J and FVB/N Aged Mice**

To examine whether the VT reduction of C57BL/6J mice at P240 reflected a weak sensitivity of their respiratory centers to O2 and CO2 chemosensory drives, FVB/N and C57BL/6J mice were subjected to identical hypoxic (10% O2, 5 min) or hypercapnic (4% CO2, 5 min) challenges at P240. As illustrated in Fig. 4, hypoxia or hypercapnia significantly increased Rf, VT, and VE in both strains, but the responses were larger in FVB/N than C57BL/6J mice. Hypoxia induced a nonsignificant larger increment of Rf in FVB/N (n = 10) than C57BL/6J (n = 8) mice (56 ± 22 and 28 ± 13 c/min, respectively, NS), a significant fourfold larger increment of VT in FVB/N than C57BL/6J mice (9.2 ± 1.9 and 2.2 ± 0.9 ml·g, respectively, P < 0.01), and, therefore, a significant sixfold larger increment of VE in FVB/N than C57BL/6J mice (2.5 ± 0.5 and 0.4 ± 0.2 ml·g−1·min−1, respectively, P < 0.01). Hypercapnia similarly increased Rf in FVB/N (n = 10) and C57BL/6J (n = 8) mice (94 ± 8 and 89 ± 25 c/min, respectively, NS), but induced a significant threefold larger increment of VT in FVB/N than C57BL/6J mice (14.1 ± 1.5 and 4.0 ± 1.1 ml·g, respectively, P < 0.001), and, therefore, of VE in FVB/N than C57BL/6J mice (3.9 ± 0.4 and 1.3 ± 0.4 ml·g−1·min−1, respectively, P < 0.001). Then C57BL/6J aged mice were less sensitive to chemical drives than FVB/N aged mice, and the weak sensitivity to chemical drives of C57BL/6J mice was highly likely contributing to their reduced VT under basal conditions.

**Different Postsigh Pattern in C57BL/6J vs. FVB/N Aged Mice**

As previously reported (59), sighs are deep inspirations that spontaneously occur at a low frequency (about 0.5 sigh/min) and overinflate the lungs, which prevents lung atelectasis, restores functional residual capacity, and transiently improves alveolar oxygenation. The sigh-induced overinflation of the lungs first induces a brief Rf reduction, reflecting the overactivation of pulmonary stretch receptors (Hering-Breuer reflex) and, second, induces a long-lasting bradypnea, reflecting the improvement of alveolar oxygenation (chemosensory reflex). Then the Rf changes during the post-sigh period reflected the Hering-Breuer and chemosensory regulations in conscious, unrestrained mice (59). In FVB/N (n = 14) and C57BL/6J
(n = 8) mice at P240, we compared the postsigh Rf to the control Rf before the sigh (100%). The Rf was first depressed during the second following the sigh in both strains (Fig. 5), but significantly less in FVB/N (79 ± 3% of the control) than C57BL/6J mice (58 ± 5% of the control), suggesting a stronger Hering-Breuer inhibitory reflex in C57BL/6J than FVB/N mice. Thereafter, a long-lasting post-sigh bradypnea developed in both strains, but the mean Rf during the following 5 s was significantly more reduced in FVB/N (57 ± 2% of the control) than C57BL/6J mice (72 ± 3% of the control), suggesting a weaker responsiveness to sigh-induced improvement of O₂ in C57BL/6J than FVB/N mice, revealing hypoventilation of C57BL/6J mice caused by low Rf in neonates and low Vr in old adults.

Differences in Brain Stem 5-HT Metabolism of C57BL/6J vs. FVB/N Aged Mice

As 5-HT was shown to contribute to respiratory function and dysfunctions in adult mice (14, 27), we analyzed the 5-HT metabolism of C57BL/6J (n = 7) and FVB/N (n = 7) mice at P240. No significant differences were observed in medullary contents of the L-Trp precursor (10 ± 1 and 12 ± 3 ng/medulla, NS) and the 5-HIAA catabolite (1.39 ± 0.09 and 1.79 ± 0.26 ng/medulla, NS), but the 5-HT contents were significantly higher in the medulla of FVB/N than C57BL/6J mice (3.26 ± 0.22 and 2.19 ± 0.18 ng/medulla, respectively, P < 0.01). Therefore, the 5-HT/5-HIAA was significantly higher in the medulla of FVB/N than C57BL/6J mice (2.36 ± 0.13 and 1.35 ± 0.19, respectively, P < 0.001), revealing again differences in 5-HT metabolism that possibly contributed to breathing differences.

DISCUSSION

For the first time, we report phenotypic differences in 5-HT and breathing systems of FVB/N and C57BL/6J mice from birth onwards. Given that 5-HT affects maturation and function of all central networks (21), including the respiratory network (7, 9, 27, 54), then interstrain differences in 5-HT metabolism may contribute to interstrain differences in breathing. As FVB/N and C57BL/6J strains are often used as background strain for producing mutants, and as the background phenotype may affect the mutant phenotype, the 5-HT and breathing differences between FVB/N and C57BL/6J strains should be taken into account for analyzing the mutant phenotype.

RRG Robustness vs. RRG Instability: Possible Role of 5-HT

From birth onwards, robustness vs. instability characterizes the RRG of FVB/N and C57BL/6J mice, respectively. The RRG instability of C57BL/6J mice is, at least partly, central and prenatal in origins, as the isolated RRG of C57BL/6J embryos produces an unstable rhythm. The RRG is composed of two coupled, interacting networks that form and are active before birth: the pre-Bötzinger complex and the retrotrapezoid nucleus/parafacial respiratory group (53). The RRG function relies on pacemaker and synaptic properties (60) and on several...
genes whose expression is crucial for neonatal survival (2, 4, 8, 14). It also relies on 5-HT afferent inputs from raphe neurons (27), affecting the RRG perinatal maturation and neonatal function (7, 9, 17, 21, 27, 64). The different 5-HT/5HIAA in C57BL/6J and FVB/N neonates reveal differences in 5-HT metabolism, which may differentially affect RRG maturation and function at birth, but also induce long-lasting sequelae in adults. Although differences in 5-HT metabolism have not been previously documented in FVB/N and C57BL/6J mice, they have already been described in C57BL/6J and BALBc adults, with lower 5-HT levels in C57BL/6J (16).

RRG instability has been commonly reported in mutant mice where genetic manipulations have altered 5-HT systems. In MAOA-null mice, the lack of 5-HT degradation by MAOA induces excess of 5-HT, altered wiring of the respiratory network, unstable breathing in neonates, and frequent apneas in adults (7, 9, 31, 46). In Necdin-null mice, the 5-HT and RRG systems dysfunction at birth, and unstable respiratory rhythm with frequent apneas persists in adults, despite 5-HT metabolism restoration (64). In Mecp2-null mice, the 5-HT and RRG systems correctly function in neonates, but dysfunction in adults, leading to erratic rhythm, severe apneas, and premature...
death (55). In mutant mice lacking 5-HT2A receptors, a role of 5-HT in sleep apneas has been suggested (42). In mutant mice with 5-HT defects and also in C57BL/6J mice, the RRG instability is affected by 5-HT treatments (1, 7, 46, 48, 49, 64). Indeed, differences in 5-HT metabolism of FVB/N and C57BL/6J mice may contribute to differences in RRG robustness from birth onwards.

Differences in Breathing Regulations: Possible Role of 5-HT

Aged C57BL/6J mice compared with aged FVB/N mice show reduced VT and VE, with reduced pH and increased PCO2 in mixed-venous blood. Double-chamber plethysmography reveals that the weak VT of C57BL/6J mice is not due to upper airway dysfunctions (18, 56). The weak VT of C57BL/6J mice might reflect age-related alteration of respiratory effectors. Lung parenchyma and diaphragm contractile properties degrade with age in rodents, but during the senescent period (28, 29), not as early as P240. The weak VT of C57BL/6J mice more likely reflects altered breathing regulations that impair correct adjustments of the central respiratory drive to physiological needs. In C57BL/6J mice, the marked reduction of Rf just after the sigh suggests a potent respiratory inhibition from pulmonary stretch receptors, whereas their weak postsigh bradypnea confirms weak sensitivity/responsiveness to chemical inputs (59), consistently with weak VT increments during respiratory challenges. Interstrain differences in sensitivity of pulmonary stretch receptors to lung inflation have already been reported between OF1 and C3H/J adults (11), as well as interstrain differences in carotid body size and breathing responses to hypoxia between DBA/2J and A/J mice (61). Both retrotrapezoid nucleus/parafacial respiratory group and 5-HT systems contribute to breathing regulations (20, 24, 25, 27, 36, 37, 39, 47, 65). In C57BL/6J neonates, fluoxetine-induced alteration of the 5-HT metabolism abolishes the RRG response to acidosis (57). In C57BL/6J but not A/J mice, a periodic breathing occurs after hypoxic challenges (26), persists after pretreatment with nitric oxide agent (43), but is abolished by pretreatment with the 5-HT1A receptor agonist buspirone (62). In MAOA-null adults, the genetically induced alteration of 5-HT metabolism reduces the resting VT, the response to hypoxia, and the response to lung inflations, these defects being alleviated after 5-HT treatments (10). In Necdin-null neonates, the altered 5-HT metabolism blunts their breathing responses to hypercapnia and hypoxia (64). In addition, maternal dietary tryptophan deficiency affects the 5-HT system of rat pups and, in turn, alters their breathing pattern and ventilatory responses to hypercapnia (40). Then differences in 5-HT metabolism between FVB/N and C57BL/6J mice may differently affect not only RRG robustness, but also resting ventilation and breathing regulations.

The P30–P120 Intermediate Period

The 5-HT/5-HIAA at P1 and P240 is lower in C57BL/6J than FVB/N medulla. At P1, the low precursor and the high catabolite contents in C57BL/6J medulla are consistent with a faster turnover of 5-HT in C57BL/6J than FVB/N neonates. At P240, although the precursor content is similar in the two strains, the low 5-HT and the high catabolite contents in C57BL/6J medulla also support a fast turnover of 5-HT.
However, further experiments are required to examine the genetic and molecular origins of the interstrain difference in 5-HT metabolism at P1 and P240. From P30 to P120, after the postnatal maturational period during which pups learn to breathe and to manage with peripheral and upper brain inputs (19), FVB/N and C57BL/6J mice display a rather similar breathing pattern, even if slight, but significant, differences persist in Rf, AI, and IS. During this period, the breathing improvement in C57BL/6J mice may be related to postnatal changes in 5-HT metabolism, as those reported in multiple nuclei of rats (34). In C57BL/6J mice, our laboratory previously reported, but did not comment on, weak 5-HT/5HIAA (0.27) at P2, but large 5-HT/5HIAA (1.98) at P30 (64). Such increase in 5-HT/5HIAA from P2 to P30 reveals changes in the 5-HT metabolism of C57BL/6J mice, which correlates well with their breathing improvement at P30–P120. Interestingly, the inactivation of the 5-HT transporter has age-related effects on the Ve of mutant mice: no effect at P8, reduction at P15, and increase at 5–6 mo (33, 41), which further suggests a complex link between 5-HT and respiratory systems. However, besides 5-HT inputs, the mature RRG receives a plethora of inputs, including those from upper structures, to adapt its activity to mood, motor activity, temperature, etc. FVB/N mice are more anxious, more active, and have a slightly higher central temperature than C57BL/6J mice (44), and this may also contribute to breathing differences, although the 5-HT system affects anxiety, motor activity, and thermoregulation.

As the distinct brain stem raphe nuclei differ in terms of rostrocaudal projections, targeted structures, and modulated functions, it may be important to further examine which mechanisms and raphe nuclei sustain the 5-HT and breathing differences between FVB/N and C57BL/6J mice.

Selecting the Background Strain for Producing Mutant Models

Both FVB/N and C57BL/6J strains are suitable for producing mutants, but their distinct 5-HT and breathing phenotypes may affect the mutant phenotype. On the one hand, the inherent RRG instability of the C57BL/6J background may worsen the mutant breathing phenotype. Teashirt3 inactivation on the C57BL/6J background is 100% lethal for neonatal monoamine oxidase A-deficient transgenic mice: possible role of a serotonin excess. J Neurosci 20: 4646–4656, 2000.


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