RAPID REPORT

Serotonin transporter knockout mice have a reduced ventilatory response to hypercapnia (predominantly in males) but not to hypoxia

Aihua Li and Eugene Nattie

Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756-0001, USA

Medullary serotonergic (5-HT) neurons are implicated in central chemoreception and 5-HT abnormalities are present in many cases of the sudden infant death syndrome (SIDS). Mice with a targeted disruption of the serotonin transporter (5-HTT) develop in the presence of excess 5-HT in brain extracellular fluid (ECF). As adults they exhibit reduced 5-HT neuron activity and 5-HT_{1A} receptor binding with varying changes in postsynaptic 5-HT receptor function. They exhibit behavioural phenotypes (anxiety, reduced aggression) but little is known about their control of breathing. We show that conscious adult male and female 5-HTT knockout mice breathing air at room temperature have a higher resting $\dot{V}_{O_2}$, breathing frequency and $\dot{V}_E$ but a normal body temperature and $\dot{V}_E/\dot{V}_{O_2}$ ratio (the ventilatory equivalent) compared to wild-type (WT) controls. In hypercapnia, there is a reduced ventilatory response (expressed as the $\dot{V}_E/\dot{V}_{O_2}$ ratio) that is much more prominent in males (−68%) than females (−22%). In hypoxia, both males and females exhibit a higher $\dot{V}_E$, $\dot{V}_{O_2}$ and body temperature but their $\dot{V}_E/\dot{V}_{O_2}$ ratio is normal. We conclude that 5-HTT knockout mice have a diminished function of the medullary 5-HT system, which is manifest most remarkably in a substantial loss of CO_2 sensitivity predominantly in males. This finding supports the importance of medullary 5-HT neurons in central chemoreception. Females either rely less on 5-HT neurons in chemoreception or adapt more readily to the loss of 5-HT function. This genetic model allows examination of the role of excess 5-HT in ECF in the development of the control of breathing and central chemoreception, which may be pertinent to SIDS.

(Received 7 February 2008; accepted after revision 20 March 2008; first published online 20 March 2008)
Corresponding authors A. Li and E. Nattie: Department of Physiology, Dartmouth Medical School, Lebanon, NH 03756-0001, USA. Email: aihua.li@dartmouth.edu and eugene.nattie@dartmouth.edu

Central chemoreception, the process by which the brainstem detects changes in pH or P_{CO_2} and increases breathing, is present at many locations and involves more than one type of neuron (Nattie & Li, 2001, 2006; Feldman *et al.* 2003; Hodges *et al.* 2004, 2008; Nattie *et al.* 2004; Guyenet *et al.* 2005; Richerson *et al.* 2005; Taylor *et al.* 2005; Li *et al.* 2006; Mulkey *et al.* 2007). Medullary serotonergic (5-HT) neurons have been proposed as important participants in central chemoreception as CO_2 detectors (Richerson *et al.* 2005) and as modulators of other detector neurons (Li *et al.* 2006; Mulkey *et al.* 2007; Hodges *et al.* 2008). It is not clear which type of neuron or which chemoreceptor location is of greatest importance in normal physiology (Feldman *et al.* 2003; Guyenet *et al.* 2005; Nattie & Li, 2006; Mulkey *et al.* 2007).

Striking abnormalities in the medullary 5-HT system have been reported in cases of sudden infant death syndrome (SIDS) (Paterson *et al.* 2006). Many SIDS cases have an increased number of 5-HT neurons and exhibit decreased binding of the 5-HT_{1A} autoreceptor and serotonin transporter (5-HTT) (Paterson *et al.* 2006). How such abnormalities translate into a physiological mechanism for sudden and unexpected death in an apparently healthy infant remains a mystery (Paterson *et al.* 2006; Thach, 2008).

We hypothesize that medullary 5-HT neurons are of special importance in central chemoreception, in the development of the control of breathing and in the pathogenesis of SIDS. As one experimental model in the examination of this hypothesis we employed mice with a targeted disruption of the serotonin transporter

 DOI: 10.1113/jphysiol.2008.152231

(5-HTT) (Holmes *et al.* 2003). These mice develop in the presence of excess 5-HT in brain extracellular fluid (ECF). As adults they exhibit increased 5-HT in ECF (Mathews *et al.* 2004; Kim *et al.* 2005), decreased tissue 5-HT (Kim *et al.* 2005), increased 5-HT synthesis (Kim *et al.* 2005), reduced 5-HT neuron activity (Gobbi *et al.* 2001), reduced 5-HT_{1A} receptor binding (Li *et al.* 2000; Bouali *et al.* 2003) and have varying changes in postsynaptic 5-HT receptor function (Qu *et al.* 2005). This neurochemical phenotype includes two of the three abnormalities described in the brainstem of the SIDS cases, decreased 5-HT_{1A} and 5-HTT binding. The 5-HTT knockout mice exhibit behavioural phenotypes: increased anxiety-like behaviours, reduced aggression, and exaggerated stress responses (Holmes *et al.* 2003; Adamec *et al.* 2006) but little is known about their physiology. There are known sex differences in the 5-HTT knockout. Female as compared to male adult 5-HTT knockout mice appear to have a greater desensitization of dorsal raphe 5-HT neurons (Bouali *et al.* 2003) and a greater increase in brain 5-HT synthesis (Kim *et al.* 2005) and there are sex differences in the incidence of SIDS (60 : 40; male predominance; see Centers for Disease Control and Prevention, 2008). In this study we examine breathing, body temperature and oxygen consumption in air and in response to increased CO_2 or decreased O_2 in unanaesthetized adult male and female 5-HTT knockout and wild-type mice. Our hypothesis is that with long-term excess of 5-HT in ECF these 5-HTT knockout mice will have a brainstem 5-HT system that is 'turned down', i.e. is less responsive. We expect to see a decreased CO_2 response of a degree commensurate with the importance of 5-HT neurons in central chemoreception.

Methods

Ethical approval

All experimentation procedures and protocols were within the guidelines of the National Institutes of Health for animal use and care and were approved by the Dartmouth College Institutional Animal Use and Care Committee. The mice were never anaesthetized nor were they killed. The 5-HTT knockout and the WT control mice both of the C57BL/6 strain were acquired from Taconic Farm and tested at age 5–6 months. We verified their genotype before the experiments. In brief, the tail samples were digested and subjected to polymerase chain reaction (PCR) with three primers for 5-HTT (5-HTT-A primer: 5′-TCT ATG GGA AGG CTG ACA GGT-3′; 5-HTT-B primer: 5′-TTG CTG ACT GGA GTA CAG GCT A-3′; and NEO primer: 5′-TCG ACG TTG TCA CTG AAG CGG-3′). The PCR product was analysed by electrophoresis. The 5-HTT wild-type allele is identified at 1.4 kb, and the 5-HTT knockout allele is identified at 1 kb. The mice were housed in a room in the Animal Resource Center with a light, rest period from midnight to noon and a dark, active period from noon to midnight. Food and water were available *ad libitum*. All the experiments were performed between 8 am and 3 pm. Ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (f), and oxygen consumption ($\dot{V}_{O_2}$) were measured in non-instrumented WT and 5-HTT knockout mice of both sexes using whole-body plethysmography during wakefulness while breathing room air, 5% CO_2 or 10% O_2. We studied a total of 24 mice; five and six WT males and females, respectively, and seven and six 5-HTT knockout males and females, respectively. The rectal temperature was measured before and after each experiment using a small thermistor probe. The volume of the plethysmograph was ∼214 ml (5.5 mm diameter, 9 mm long cylinder). The inflow gas for the plethysmograph chamber was humidified and controlled by a flowmeter at a minimum of 0.4 l min^{-1}. The outflow was matched to the inflow via a flowmeter connected to a vacuum system. Approximately 100 ml min^{-1} of outflow gas served O_2 and CO_2 analysers (Applied Electrochemistry). We measured chamber pressure by transducer and calibrated the plethysmograph with multiple 0.1 ml injections. The chamber temperature was measured by a thermometer continuously. After the mouse was acclimatized to the plethysmograph chamber (usually ∼30 min), data were obtained over 20–30 min of breathing air and during the last 5 min of a 15–20 min period of exposure to 5% CO_2 (5% CO_2, 21% O_2, remainder N_2) or 10% O_2 (10% O_2, remainder N_2). These two responses were tested on separate days. Tidal volume (V_T) was calculated using plethysmograph temperature at that time and mouse temperature measured closest to that time (Li *et al.* 2006), and breathing frequency (f) per breath to estimate ventilation ($\dot{V}_E$) per breath.

Statistics

All data analysis was performed within sexes to avoid complications of normalization due to sex-related differences in age and body weight. Baseline age, body weight, body temperature, $\dot{V}_E$ and $\dot{V}_{O_2}$ values were compared within each sex by t test. We analysed the responses to hypercapnia or hypoxia using a repeated measures one-way ANOVA with the ventilatory variable, body temperature or $\dot{V}_{O_2}$ as the repeated measure and the treatment being wild-type *versus* 5-HTT knockout. *Post hoc* Tukey's test was applied as indicated by a significant interactive term in the ANOVA. Data for breathing room air at rest are pooled from data before the CO_2 and before the hypoxic tests.

Results

Male WT and 5-HTT knockouts were the same age and body weight on average at the time of these

Table 1. Age, body weight, baseline ventilation and oxygen consumption of wild-type control and 5-HTT knockout mice

	Age (days)	Body weight (g)	f (min^{-1})	V_T (ml g^{-1})	$\dot{V}_E$ (ml min^{-1} g^{-1})	$\dot{V}_{O_2}$ (ml min^{-1} g^{-1})	T_{body} (°C)	$\dot{V}_E/\dot{V}_{O_2}$
Male WT ($n = 5$)	163 ± 12	31 ± 2	205 ± 5	0.012 ± 0.001	2.31 ± 0.09	0.060 ± 0.002	36.6 ± 0.2	39 ± 2
Male KO ($n = 7$)	165 ± 7	29 ± 1	254 ± 9	0.011 ± 0.0002	2.90 ± 0.11	0.080 ± 0.002	37.0 ± 0.2	37 ± 2
	n.s.	n.s.	*	n.s.	*	+	n.s.	n.s.
Female WT ($n = 6$)	181 ± 10	24 ± 1	192 ± 9	0.015 ± 0.001	2.83 ± 0.21	0.078 ± 0.003	36.5 ± 0.2	37 ± 2
Female KO ($n = 6$)	168 ± 8	25 ± 1	235 ± 6	0.014 ± 0.001	3.35 ± 0.18	0.093 ± 0.005	36.8 ± 0.2	36 ± 2
	n.s.	n.s.	*	n.s.	n.s.	+	n.s.	n.s.

Values are means ± S.E.M. $^*P < 0.01$; $^+P < 0.05$; t test applied within each sex. f, breathing frequency; V_T, tidal volume; $\dot{V}_E$, ventilation; $\dot{V}_{O_2}$, oxygen consumption; T_{body}, body temperature; $\dot{V}_E/\dot{V}_{O_2}$, ventilatory equivalent.

studies. Female mice were older but of lower body weight than males but there was no difference between female WT and 5-HTT knockouts (Table 1). While breathing air (Table 1), male 5-HTT knockout mice had a faster breathing frequency ($P < 0.01$) and a greater $\dot{V}_E$ ($P < 0.01$) compared to male WT. Resting $\dot{V}_{O_2}$ was also greater ($P < 0.05$) such that the ventilatory equivalent ($\dot{V}_E/\dot{V}_{O_2}$) was not affected by the 5-HTT knockout. While breathing air (Table 1), female 5-HTT knockout mice had a faster breathing frequency ($P < 0.01$) and a greater $\dot{V}_E$ compared to female WT although this did not reach statistical significance. Resting $\dot{V}_{O_2}$ was also greater ($P < 0.05$) and the ventilatory equivalent ($\dot{V}_E/\dot{V}_{O_2}$) was not affected by the 5-HTT knockout. Body temperature did not differ between WT and 5-HTT knockout mice of either sex at room temperature during air breathing (Table 1).

We evaluated the ventilatory response to increased CO_2 first by normalizing the data to body weight. Figure 1 shows that the $\dot{V}_E$ response to CO_2 was markedly reduced in male 5-HTT knockout mice compared to wild-type ($P < 0.001$, treatment effect and interactive term of one-way repeated measures ANOVA; $P < 0.01$, *post hoc* comparison at 5% CO_2), an effect entirely due to a reduced V_T response ($P < 0.001$, treatment effect and interactive term of one-way repeated measures ANOVA; $P < 0.01$, *post hoc* comparison at 5% CO_2). $\dot{V}_{O_2}$ and body temperature did not change during exposure to 5% CO_2 in any group (data not shown). In females the $\dot{V}_E$ response to CO_2 was also reduced ($P < 0.001$, treatment effect and $P < 0.03$ interactive term of one-way repeated measures ANOVA). If we define the CO_2 response as the percentage increase in $\dot{V}_E$ from the air breathing value, the WT males have a 183% increase compared to an 81% increase in the 5-HTT knockout mice. The male knockouts have a 56% reduction in the CO_2 response so defined; the females a 34% reduction. If we define the CO_2 response as the change in $\dot{V}_E$ from air to 5% CO_2 breathing, in WT male mice Δ is 4.56 ml min^{-1} g^{-1} while in 5-HTT knockouts Δ is 2.3 ml min^{-1} g^{-1}, a reduction of 48% in this measure of the CO_2 response; the females have a 21% reduction.

To take into consideration the large difference in resting $\dot{V}_{O_2}$ between the knockout and WT mice, we also normalized the ventilatory output as $\dot{V}_E/\dot{V}_{O_2}$. The CO_2 response was again markedly reduced in male 5-HTT knockout mice compared to male wild-type (Fig. 2) ($P < 0.001$, treatment effect and interactive term of one-way repeated measures ANOVA). In female mice the CO_2 response was also significantly reduced in the 5-HTT knockout mice compared to male WT (Fig. 2) ($P < 0.05$, treatment effect but no interactive term of one-way repeated measures ANOVA) but the degree of the reduction was less. In the male mice, the reduction in the $\dot{V}_E/\dot{V}_{O_2}$ ratio while breathing 5% CO_2 was 50%. If we define the CO_2 response as the percentage increase in $\dot{V}_E/\dot{V}_{O_2}$ from the air breathing value, the WT males have a 252% increase compared to an 88% increase in the 5-HTT knockout mice. The male knockouts have a 65% reduction in the CO_2 response so defined; the females have a 15% reduction. If we define the CO_2 response as the change in the $\dot{V}_E/\dot{V}_{O_2}$ ratio from air to 5% CO_2 breathing, in WT male mice Δ is 96 while in the 5-HTT knockouts Δ is 31, a reduction of 68% in this measure of the CO_2 response; the females have a 22% reduction.

The response to breathing 10% O_2 in small rodents includes changes in body temperature, $\dot{V}_E$, $\dot{V}_{O_2}$ and $\dot{V}_E/\dot{V}_{O_2}$, which are shown in Figs 3 (males) and 4 (females). The male 5-HTT knockout mice compared to the wild-type (Fig. 3) have a significantly greater $\dot{V}_E$ ($P < 0.001$ for treatment effect; no significant interaction) and $\dot{V}_{O_2}$ ($P < 0.001$ for treatment effect; no significant interaction) such that the response of $\dot{V}_E/\dot{V}_{O_2}$ is the same as in controls. The female 5-HTT knockout mice compared to the wild-type (Fig. 4) have a significantly greater $\dot{V}_E$ ($P < 0.001$ for treatment effect; $P < 0.02$ for the interactive effect) and $\dot{V}_{O_2}$ ($P < 0.001$ for treatment effect; no significant interaction) such that the response of $\dot{V}_E/\dot{V}_{O_2}$ is the same as in controls. In both sexes the 5-HTT

knockout mice have a smaller drop in body temperature as a result of hypoxic exposure compared to the wild-type controls ($P < 0.05$ treatment effect; $P < 0.05$ for the interactive effect).

Discussion

Our hypothesis was supported by the major finding of the study, a substantial decrease in the CO_2 response in the 5-HTT knockout mice compared to WT controls. Our findings complement the recent observation that mice with total absence of brainstem 5-HT neurons due to conditional knockout of the transcription factor *Lmx1b*, which is necessary for their final determination, also have a markedly reduced CO_2 response (Hodges *et al.* 2008). However, in our experiments there is excess 5-HT in ECF while in the *Lmx1b* conditional knockout there is an absence of 5-HT neurons. These findings indicate that 5-HT neurons play an important role in central chemoreception but do not show whether this 5-HT effect is via a direct chemosensing property of 5-HT neurons (Richerson *et al.* 2005) or via an indirect effect by which medullary 5-HT neuronal output can alter the chemosensitivity of other medullary neurons, e.g. in the retrotrapezoid nucleus (Li *et al.* 2006; Mulkey *et al.* 2007). As 5-HT participates in carotid body function (Jacono *et al.* 2005) the 5-HTT

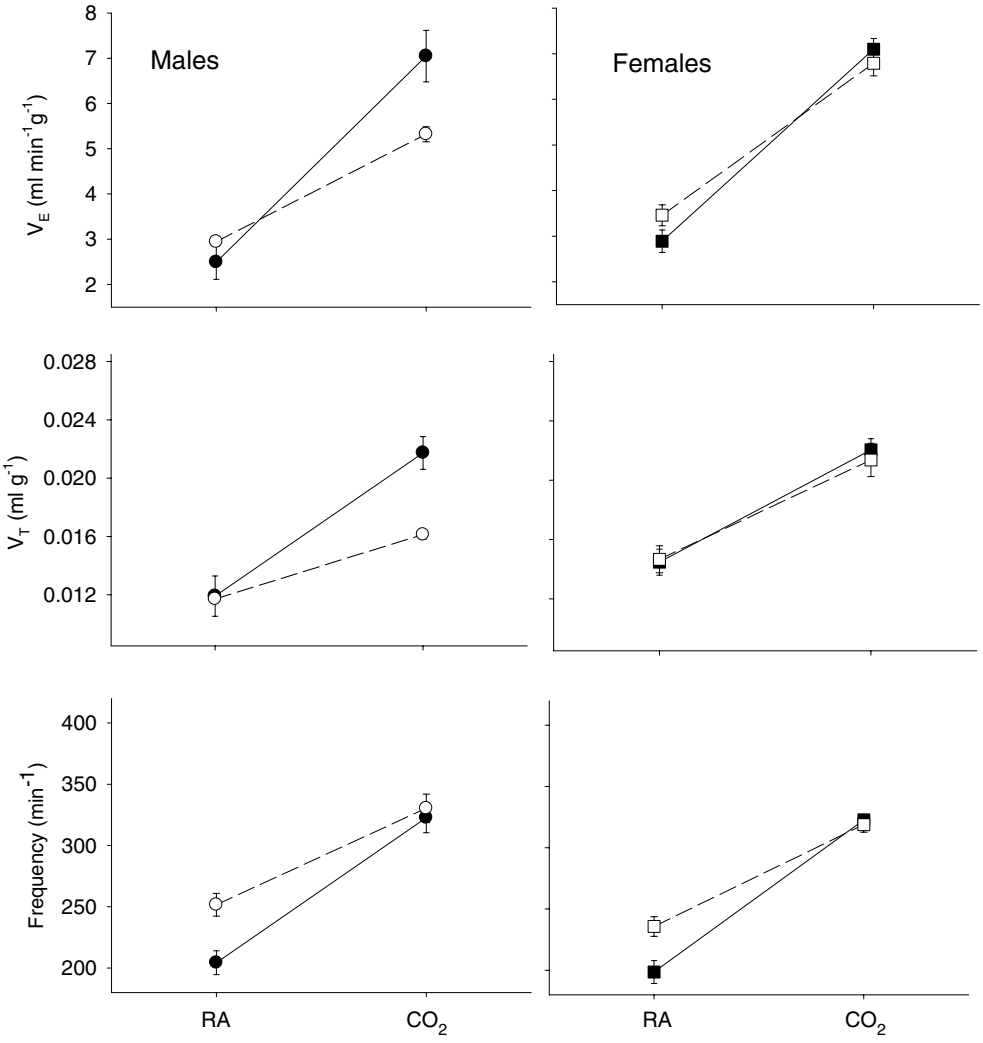

Figure 1
The CO_2 responses in unanaesthetized, unrestrained male (left) and female (right) wild-type (●; $n = 5$, 6) and 5-HTT knockout mice (○; $n = 7$, 6) are shown as the ventilation $\dot{V}_E$, tidal volume V_T, and breathing frequency, while breathing room air (RA) or 5% CO_2 in air (CO_2) during wakefulness. The symbols show the mean values and error bars show S.E.M. At 5% CO_2, $\dot{V}_E$ is significantly less in the 5-HTT *versus* wild-type in males as is V_T ($P < 0.01$, *post hoc* test with significant interactive term of ANOVA). Breathing frequency is significantly greater during air breathing in the 5-HTT mice of both sexes ($P < 0.01$, *post hoc* test with significant interactive term of ANOVA).

knockout could, in addition, have altered the peripheral chemoreceptor response to CO_2. The absence of an effect on the response of the $\dot{V}_E/\dot{V}_{O_2}$ ratio to hypoxia provides some evidence that carotid body function is relatively normal in the 5-HTT knockout.

The male predominance of the 5-HTT knockout effects on the CO_2 response is unexpected. Other studies of central chemoreception involving 5-HT neurons have predominantly been in males (Nattie & Li, 2001; Nattie *et al.* 2004; Taylor *et al.* 2004, 2005; Li *et al.* 2006; Hodges *et al.* 2008). One study that did examine both sexes after lesions of 5-HT neurons in newborn piglets did find a reduced CO_2 response only in males and only in NREM sleep (Penatti *et al.* 2006). These data show that the role of 5-HT neurons in chemoreception varies with sex and raise the provocative issue of whether chemoreception *per se* involves different central chemosensitive neurons or regions in males and females.

One possible explanation for the lesser effect of the 5-HTT knockout on the CO_2 response in females is a sex difference in adaptability or plasticity. Behan *et al.* (2002) found that long-term facilitation (LTF), a form of respiratory plasticity in which repeated exposures to hypoxic stimulation result in a lasting increase in respiratory output, was greater in older (13 months) than in younger females (3–4 months) while in males it was reversed. In our mice, the relative absence of any reduction in CO_2 sensitivity in adult females could represent more effective adaptation, perhaps analogous to LTF.

Other studies of the 5-HTT knockout mouse have uncovered sex differences in phenotype. Administration of the 5-HT_{1A} agonist 8-OH-DPAT to small rodents causes hypothermia due to inhibition of brainstem 5-HT neurons that (a) stimulate brown fat metabolism to generate heat, and (b) vasoconstrict blood vessels to conserve heat (Morrison, 2004; Ootsuka & Blessing, 2006). The 5-HT neurons in 5-HTT knockout mice have been chronically exposed to excess 5-HT and their 5-HT_{1A} receptors are down-regulated by adulthood; the neurons exhibit less inhibition of firing rates and the mice less hypothermia when exposed to 8-OH-DPAT (Bouali *et al.* 2003) and this down-regulation is greater in females than in males (Li *et al.* 2000; Bouali *et al.* 2003). Perhaps in males the 5-HT_{1A}

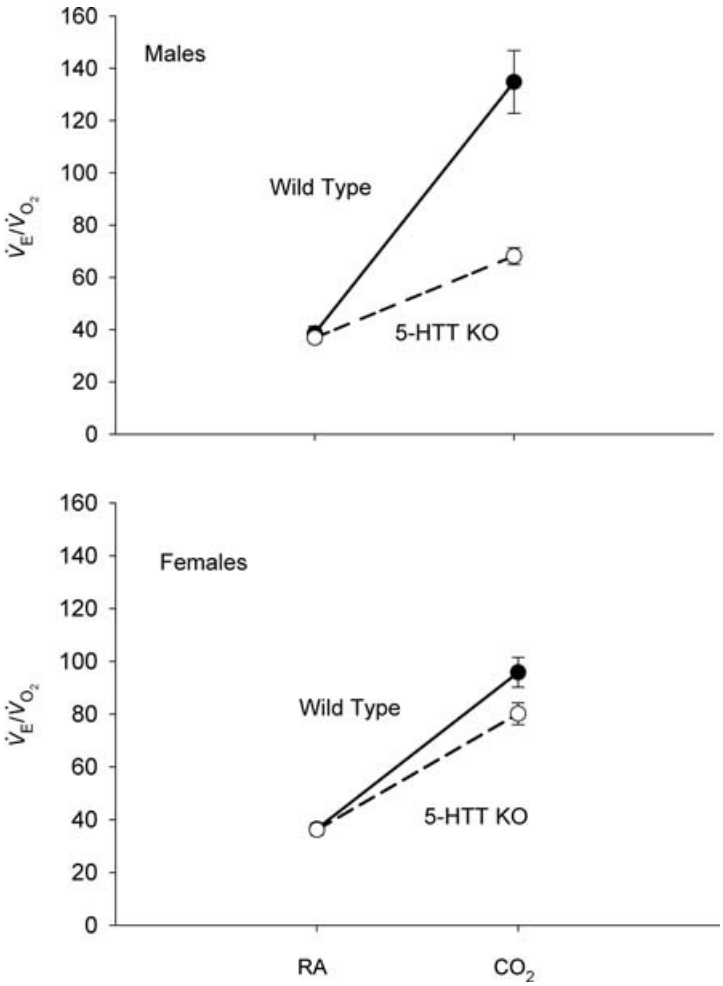

Figure 2
The CO_2 responses in unanaesthetized, unrestrained male (top) and female (bottom) wild-type (●; $n = 5, 6$) and 5-HTT knockout mice (○; $n = 7, 6$) are shown as the ventilatory equivalent, $\dot{V}_E/\dot{V}_{O_2}$, while breathing room air (RA) or 5% CO_2 in air (CO_2) during wakefulness. The symbols show the mean values and error bars show S.E.M. At 5% CO_2, $\dot{V}_E/\dot{V}_{O_2}$ is significantly less in the 5-HTT *versus* wild-type in both sexes; $P < 0.01$, *post hoc* test with significant interactive term of ANOVA.

autoreceptors are less affected by the excess 5-HT in ECF, a conclusion supported by the observation that castration in male 5-HTT knockout mice resulted in female-like down-regulation of $5HT_{1A}$ function (Bouali *et al.* 2003). Or males could be exposed to smaller increases of 5-HT in ECF as the increased brain 5-HT synthesis observed in 5-HTT knockout mice is greater in females than males (Kim *et al.* 2005). Both of these explanations presume that 5-HT neurons in males have been less desensitized, which does not explain the decreased CO_2 response. However, a reduction in postsynaptic 5-HT receptor function has been described in the 5-HTT knockout (Qu *et al.* 2005). Were this greater in males, it could account for the reduced CO_2 response, e.g. chemoreceptor neurons in the retrotrapezoid nucleus that express $5\text{-}HT_{2A}$ receptors could be less responsive to 5-HT release (Li *et al.* 2006; Mulkey *et al.* 2007).

Another unexpected observation is the greater $\dot{V}_{O_2}$ present at rest in 5-HTT knockout mice of both sexes. For this we have no clear explanation. The experiments were conducted at 24°C, which is below the thermoneutral zone of the mouse and should increase $\dot{V}_{O_2}$ but in WT as well as knockout. Noradrenaline (NA) levels are unchanged in 5-HTT knockout mice under resting conditions but can show an enhanced response to stress (Tjurmina *et al.* 2002; Kim *et al.* 2005). Our mice are studied unrestrained in a chamber in which they can freely move about, certainly a stress much less than that reported to affect NA levels (Tjurmina *et al.* 2002) although predator odours alone can produce stress in 5-HTT knockouts (Adamec *et al.* 2006). The 5-HTT knockout mice could also have a thermoregulatory defect as shown, for example, in the *Lmx1b* conditional knockout mice (Hodges *et al.* 2008). Whatever the cause of the increased $\dot{V}_{O_2}$, the 5-HTT knockout mice respond with an appropriate increase in $\dot{V}_E$ such that the ventilatory equivalent, the $\dot{V}_E/\dot{V}_{O_2}$ ratio, is unchanged from control. In a similar fashion, in response to the stress of hypoxia, the

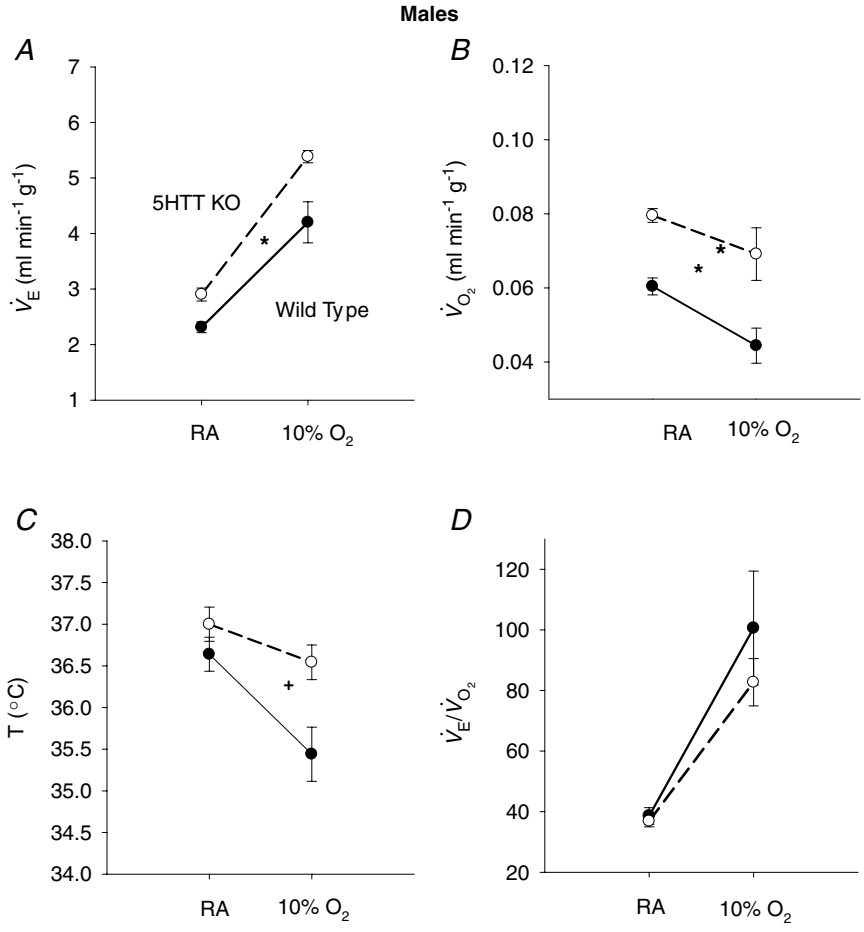

Figure 3
The responses of $\dot{V}_E$ (*A*), $\dot{V}_{O_2}$ (*B*), body temperature (*C*), and $\dot{V}_E/\dot{V}_{O_2}$ (*D*) in unanaesthetized, unrestrained male wild-type (●; $n = 5$) and 5-HTT knockout mice (○; $n = 7$) are shown while breathing room air (RA) or 10% O_2 in nitrogen during wakefulness. The symbols show the mean values and error bars show S.E.M. $^*P < 0.001$; $+P < 0.05$, for main treatment and interactive effect.

5-HTT knockout mice responded with a normal $\dot{V}_E/\dot{V}_{O_2}$ even though they had greater $\dot{V}_E$ and $\dot{V}_{O_2}$ than did the WT controls. The 5-HTT knockout mice have a normal overall response to hypoxia.

Conclusions

The 5-HTT knockout mice develop in the presence of excess ECF 5-HT. Initially this causes an excess of 5-HT 'tone'; with time, there are adaptations that include down-regulation of $5\text{-}HT_{1A}$ autoreceptors and variable adaptations in postsynaptic receptors. The time course of this switch from excess 5-HT function to reduced 5-HT function is unclear and may vary by sex, age and brain location. In adult male rats, daily microdialysis of the 5-HTT inhibitor fluoxetine into the medullary raphe for 3 weeks enhanced the CO_2 response (Taylor *et al.* 2004) suggesting that with this dose and duration of treatment, the excess 5-HT was excitatory. With absence of 5-HTT function since early development, by adulthood there is most clearly a dramatic reduction of the CO_2 response that is more prominent in males indicating that over this time period the net effect was a down-regulation of 5-HT function in chemoreception. This has relevance for SIDS in which (1) there are abnormalities in brainstem 5-HT neurons, (2) there is a male predominance, and (3) an important hypothesis for cause of death involves inadequate responses to asphyxia, which involve chemoreception (Thach, 2008). Our findings may also have relevance to the behavioural phenotype of the 5-HTT knockout mice which includes increased anxiety, lowered aggression and enhanced stress responses (Tjurmina *et al.*

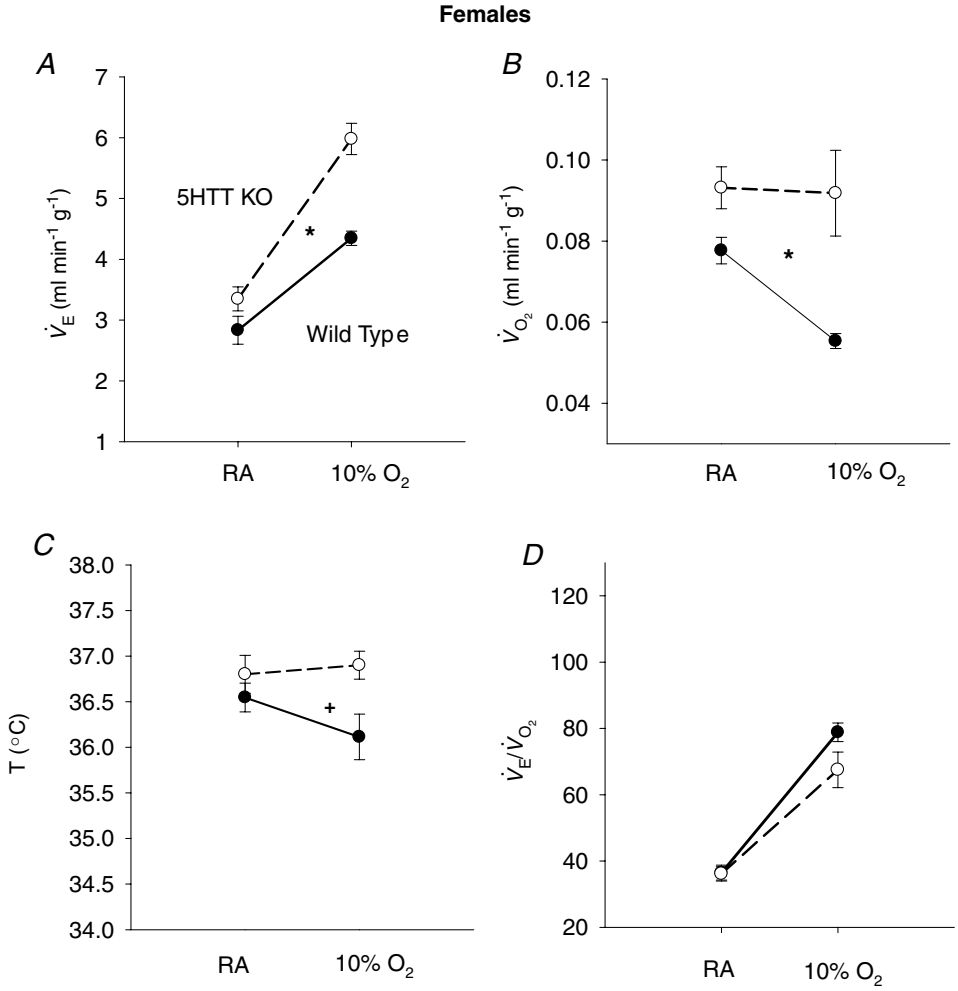

Figure 4
The responses of $\dot{V}_E$ (*A*), $\dot{V}_{O_2}$ (*B*), body temperature (*C*), and $\dot{V}_E/\dot{V}_{O_2}$ (*D*) in unanaesthetized, unrestrained female wild-type (●; $n = 6$) and 5-HTT knockout mice (○; $n = 6$) are shown while breathing room air (RA) or 10% O_2 in nitrogen during wakefulness. The symbols show the mean values and error bars show S.E.M. $*P < 0.001$; $+P < 0.05$, for main treatment and interactive effect.

2002; Holmes *et al.* 2003). One cannot study 5-HT function in physiology without reference to sex.

References

Adamec R, Burton P, Blundell J, Murphy DL & Holmes A (2006). Vulnerability to mild predator stress in serotonin transporter knockout mice. *Behav Brain Res* **170**, 126–140.

Behan M, Zabka AG & Mitchell GS (2002). Age and gender effects on serotonin-dependent plasticity in respiratory motor control. *Respir Physiol Neurobiol* **131**, 65–77.

Bouali S, Evrard A, Chastanet M, Lesch KP, Hamon M & Adrien J (2003). Sex hormone-dependent desensitization of 5-HT_{1A} autoreceptors in knockout mice deficient in the 5-HT transporter. *Eur J Neurosci* **18**, 2203–2212.

Centers for Disease Control and Prevention (2008). National Center for Health Statistics. CDC WONDER On-line Database, compiled from Compressed Mortality File 1999–2005 Series 20. No. 2K. Accessed at http://wonder.cdc.gov/cmf-icd10.html on March 17, 2008.

Feldman JR, Mitchell GS & Nattie EE (2003). Breathing: rhythmicity, plasticity, chemosensitivity. *Annu Rev Neurosci* **26**, 239–266.

Gobbi G, Murphey DL, Lesch KP & Blier P (2001). Modifications of the serotonergic system in mice lacking serotonin transporters: An *in vivo* electrophysiological study. *J Pharm Exper Ther* **296**, 987–995.

Guyenet PG, Stornetta RL, Bayliss DA & Mulkey DK (2005). Retrotrapezoid nucleus: a litmus test for the identification of central chemoreceptors. *Exp Physiol* **90**, 247–253.

Hodges MR, Martino P, Davis S, Opansky C, Pan LG & Forster HV (2004). Effects on breathing of focal acidosis at multiple medullary raphe sites in awake goats. *J Appl Physiol* **97**, 2303–2309.

Hodges MR, Tattersall GT, Harris MB, McEvoy SD, Richerson DN, Deneris ES, Johnson RL, Chen Z-F & Richerson GB (2008). Defects in breathing and thermoregulation in mice with near complete absence of central serotonin neurons. *J Neurosci* **28**, 2495–2505.

Holmes A, Murphy DL & Crawley JN (2003). Abnormal behavioral phenotypes of serotonin transporter knockout mice: parallels with human anxiety and depression. *Biol Psychiatry* **54**, 953–959.

Jacono FJ, Peng YJ, Kumar GK & Prabhakar NR (2005). Modulation of the hypoxic sensory response of the carotid body by 5-hydroxytryptamine: role of the 5-HT_2 receptor. *Respir Physiol Neurobiol* **145**, 135–142.

Kim DK, Tolliver TJ, Huang SJ, Martin BJ, Andrews AM, Wichems C, Holmes A, Lesch KP & Murphy DL (2005). Altered serotonin synthesis, turnover and dynamic regulation in multiple brain regions of mice lacking the serotonin transporter. *Neuropharmacology* **49**, 798–810.

Li A, Zhou S & Nattie E (2006). Simultaneous inhibition of caudal medullary raphe and retrotrapezoid nucleus decreases breathing and the CO_2 response in conscious rats. *J Physiol* **577**, 307–318.

Li Q, Wichems C, Heils A, Lesch KP & Murphy DL (2000). Reduction in the density and expression, but not G-protein coupling, of serotonin receptors (5-HT_{1A}) in 5-HT transporter knock-out mice: gender and brain region differences. *J Neurosci* **20**, 7868–7895.

Mathews TA, Fedele DE, Coppelli FM, Avila AM, Murphy DL & Andrews AM (2004). Gene dose-dependent alterations in extraneuronal serotonin but not dopamine in mice with reduced serotonin transporter expression. *J Neurosci Methods* **140**, 169–181.

Morrison SF (2004). Central pathways controlling brown adipose tissue thermogenesis. *News Physiol Sci* **19**, 67–74.

Mulkey DK, Rosin DL, West G, Takakura AC, Moreira TS, Bayliss DA & Guyenet PG (2007). Serotonergic neurons activate chemosensitive retrotrapezoid nucleus neurons by a pH-independent mechanism. *J Neurosci* **27**, 14128–14138.

Nattie EE & Li A (2001). CO_2 dialysis in the medullary raphe of the rat increases ventilation in sleep. *J Appl Physiol* **90**, 1247–1257.

Nattie EE & Li A (2006). Central chemoreception 2005: a brief review. *Auton Neurosci* **126–127**, 332–338.

Nattie EE, Li A, Richerson G & Lappi DA (2004). Medullary serotonergic neurons and adjacent neurons that express neurokinin-1 receptors are both involved in chemoreception *in vivo*. *J Physiol* **556**, 235–253.

Ootsuka Y & Blessing WW (2006). Activation of 5-HT_{1A} receptors in rostral medullary raphe inhibits cutaneous vasoconstriction elicited by cold exposure in rabbits. *Brain Res* **1073–1074**, 252–261.

Paterson DS, Trachtenberg FL, Thompson EG, Belliveau RA, Beggs AH, Darnall R, Chadwick AE, Krous HF & Kinney HC (2006). Multiple serotonergic brainstem abnormalities in sudden infant death syndrome. *JAMA* **296**, 2124–2132.

Penatti EM, Berniker AV, Kereshi B, Cafaro C, Kelly ML, Niblock MM, Gao HG, Kinney HC, Li A & Nattie EE (2006). Ventilatory response to hypercapnia and hypoxia after extensive lesion of medullary serotonergic neurons in newborn conscious piglets. *J Appl Physiol* **101**, 1177–1188.

Qu Y, Villacreses N, Murphy DL & Rapoport SI (2005). 5-$HT_{2A/2C}$ receptor signaling via phospholipase A_2 and arachidonic acid is attenuated in mice lacking the serotonin reuptake transporter. *Psychopharmacology (Berl)* **180**, 12–20.

Richerson GB, Wang W, Hodges MR, Dohle CI & Diez-Samprado A (2005). Homing in on the specific phenotype (s) of central respiratory chemoreceptors. *Exp Physiol* **90**, 259–266.

Taylor NC, Li A, Green A, Kinney HC & Nattie EE (2004). Chronic fluoxetine microdialysis into the medullary raphe nuclei of the rat, but not systemic administration increases the ventilatory response to CO_2. *J Appl Physiol* **97**, 1763–1773.

Taylor NC, Li A & Nattie EE (2005). Medullary serotonergic neurones modulate the ventilatory response to hypercapnia, but not hypoxia in conscious rats. *J Physiol* **566**, 543–557.

Thach B (2008). Tragic and sudden death. Potential and proven mechanisms causing sudden infant death syndrome. *EMBO Rep* **9**, 114–118.

Tjurmina OA, Armando I, Saavedra JM, Goldstein DS & Murphey DL (2002). Exaggerated adrenomedullary response to immobilization in mice with targeted disruption of the serotonin transporter gene. *Endocrinology* **143**, 4520–4526.

Acknowledgements

This work was supported by NHLBI grant R37 HL 28066.