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Short communication

Ethanol intake is not elevated in male 5-HT_{1B} receptor knockout mice

J. Adriaan Bouwknecht [a,*], Theo H. Hijzen [a], Jan van der Gugten [a], Robert A.A. Maes [b], René Hen [c], Berend Olivier [a,d,e]

[a] *Department of Psychopharmacology, Faculty of Pharmacy, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, Netherlands*
[b] *Department of Analysis and Toxicology, Faculty of Pharmacy, Utrecht University, Sorbonnelaan 16, 3584 CA Utrecht, Netherlands*
[c] *Center for Neurobiology and Behavior, Columbia University, New York, NY 10032, USA*
[d] *PsychoGenics Inc., 4 Skyline Drive, Hawthorne, NY 10532, USA*
[e] *Department of Psychiatry, Yale University School of Medicine, 34 Park Street, New Haven, CT 06508, USA*

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Abstract

Recently, the phenotype of increased ethanol intake in mice lacking 5-HT_{1B} receptors could not be replicated. We assessed ethanol consumption in male wildtype and 5-HT_{1B} receptor knockout mice derived from the original population. Intake of water and ethanol (0%, 3%, 6%, 10% and 20% v/v) from two pipettes was determined daily for 40 days. Ethanol intake (g/kg body weight) did not differ between genotypes, while body weights (20–25%) and water intake (50%) were elevated in 5-HT_{1B} receptor knockout mice. Hence, the initial finding of elevated ethanol intake in 5-HT_{1B} receptor knockout mice may have been due to phenotypic differences in fluid intake.

Keywords: 5-HT_{1B} receptor; Knockout mouse; Ethanol intake; Water intake; Food intake

1. Introduction

Crabbe et al. (1996) reported that 5-HT_{1B} receptor knockout mice drank more ethanol than wildtype mice did. However, recent studies involving several laboratories consistently failed to reproduce this phenotypic effect in 5-HT_{1B} receptor knockout (Crabbe et al., 1999; Risinger et al., 1999). Such controversial findings led to discussion about phenotypic differences in mutants (Gerlai, 1996; Phillips et al., 1999). Explanations mentioned are genetic drift across generations and introduction of different genes from multiple substrains of 129/Sv mice during the course of breeding (Phillips et al., 1999). Alternatively, experimental differences might affect the outcome. In a multiple-laboratory study (Crabbe et al., 1999), ethanol intake (6% v/v) was determined for only 4 days in mice that had been tested previously. The original study used naive mice (Crabbe et al., 1996), which were trained and tested during much longer periods using multiple concentrations of ethanol.

In our institute, lines of wildtype and 5-HT_{1B} receptor knockout mice have been bred for several years under well-controlled conditions without crossing with any other (sub)strain. These mice were obtained from heterozygote breeding pairs in Dr. Hen's laboratory and originate from the same population as those used in the early Crabbe study (1996). The present study investigated ethanol intake in male wildtype and 5-HT_{1B} receptor knockout mice following the original procedure (Crabbe et al., 1996) in mice with a similar genetic makeup as in the original study.

2. Material and methods

2.1. Animals and maintenance

The founders of our wildtype and 5-HT_{1B} receptor knockout populations (129/Sv strain) were derived from heterozygote breeding at Dr. Hen's laboratory. Both lines have been bred as homozygotes at the Central Animal Facility (Utrecht University, The Netherlands) since December 1995. Animals were kept under a controlled light–dark cycle (lights on: 7:00 a.m.–7:00 p.m.). Socially

* Corresponding author. Tel.: +31-30-2536708; fax: +31-30-2537387.
E-mail address: j.a.bouwknecht@pharm.uu.nl (J.A. Bouwknecht).

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reared males ($n = 16$ per genotype; age-matched (9–11 weeks)) were derived from these colonies and housed singly in Macrolon® Type 2 cages ($22 \times 16 \times 14$ cm) at the start of the experiment. Two pipettes (10 ml) were placed on each cage, one containing water and the other varying concentrations of ethanol. Mice had continuous access to both pipette tips, which were suspended 6 cm above the sawdust. The ethical committee of the Faculty of Pharmacy at Utrecht University approved this experiment.

2.2. Experimental protocol

We followed the original procedure as closely as possible (Crabbe et al., 1996), except that we used males only. Fluid intake was determined daily between 3:00 and 4:00 p.m. for 40 days. During the first 4 days, both pipettes were filled with tap water, followed by four blocks of eight consecutive days per ethanol concentration (3%, 6%, 10% or 20% ethanol v/v, respectively). A final block of 4 days, with both pipettes containing water assessed putative effects of ethanol pre-exposure on drinking. Order of ethanol concentrations was not balanced to minimize ethanol avoidance in naive mice when exposed to the highest concentration (Crabbe, personal communication). Pipettes were filled daily with freshly prepared fluids. Every second day, the positions of the ethanol and water pipettes were switched and body weight was determined. Food intake was measured by weighing racks every 4 days. Cages were cleaned at 8-day intervals in the middle of each ethanol block to avoid interaction with changes in ethanol concentrations.

2.3. Data analysis

We analyzed: body weight (g); total volume (water plus ethanol volume; ml/mouse); relative volume (ratio = [water plus ethanol volume]/body weight; ml/g body weight); ethanol intake (ratio = [ethanol volume (ml) $\times$ (Y/100) $\times$ 0.79 (g/ml)]/kg body weight, in which Y = concentration ethanol and 0.79 is specific gravity of 100% ethanol; g/kg body weight); ethanol volume (volume ethanol intake; ml/mouse); ethanol preference (ratio = [ethanol volume/water plus ethanol volume] $\times$ 100; %); and food intake (ratio = food intake/body weight; g/g body weight). Data were averaged per concentration (8 days) and analyzed by repeated measures analysis of variance (ANOVA) with Greenhouse–Geisser epsilon (ε) corrections. Ethanol concentration was a within-subject factor and genotype a between-subjects factor. When appropriate, post-hoc comparisons were made using *T*-tests with Bonferroni corrections for repeated comparisons. Significance was accepted at $P < 0.05$. All statistical analyses were performed using SPSS (Windows v.9.0, Chicago, Ill., USA).

3. Results

Data are presented in Table 1. Body weight showed main effects of genotype ($F_{(1,30)} = 87.7$, $P < 0.001$) and ethanol concentration ($F_{(4,120)} = 39.1$, $P < 0.001$, $\varepsilon = 0.43$), but no interaction. During the entire experiment, body weight was about 20–25% higher in 5-HT_{1B} receptor knockout than in wildtype mice. The small elevation in

Table 1
Ethanol, water, and food intake, and body weight of male 5-HT_{1B} receptor knockout and wildtype mice (mean ± S.E.M, $n = 16$ per genotype) at varying ethanol concentrations.
See Section 2.3 for calculation of parameters.

Parameter	Genotype	0%	3%	6%	10%	20%
Body weight (g)	KO	$27.4^{a} \pm 0.3$	$28.1^{a,b} \pm 0.4$	$28.3^{a,b} \pm 0.4$	$28.5^{a,b} \pm 0.4$	$28.7^{a,b} \pm 0.4$
	WT	21.8 ± 0.5	$22.6^{b} \pm 0.5$	$23.0^{b} \pm 0.4$	$23.3^{b} \pm 0.5$	$23.6^{b} \pm 0.5$
Total volume (ml/mouse)	KO	$6.1^{a} \pm 0.2$	$6.2^{a} \pm 0.2$	$5.7^{a,b} \pm 0.2$	$6.4^{a} \pm 0.2$	$6.1^{a} \pm 0.2$
(*Ethanol + water*)	WT	3.8 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	4.0 ± 0.1
Relative volume (ml/g body weight)	KO	$0.22^{a} \pm 0.01$	$0.22^{a} \pm 0.01$	$0.20^{a,b} \pm 0.01$	$0.22^{a} \pm 0.01$	$0.21^{a} \pm 0.01$
(*Ethanol + water*)	WT	0.17 ± 0.00	$0.18^{b} \pm 0.00$	0.17 ± 0.00	0.17 ± 0.00	0.17 ± 0.00
Ethanol (g/kg body weight)	KO	–	0.74 ± 0.13	0.69 ± 0.13	1.14 ± 0.23	$2.5^{b} \pm 0.43$
	WT	–	0.49 ± 0.04	0.63 ± 0.07	$1.11^{b} \pm 0.13$	$2.8^{b} \pm 0.28$
Ethanol volume (ml)	KO	–	0.89 ± 0.17	$0.42^{b} \pm 0.09$	$0.40^{b} \pm 0.08$	0.47 ± 0.09
	WT	–	0.47 ± 0.05	$0.30^{b} \pm 0.04$	$0.32^{b} \pm 0.04$	0.42 ± 0.04
Ethanol preference (%)	KO	–	14 ± 3	9 ± 2	8 ± 1	9 ± 1
	WT	–	12 ± 1	$6^{b} \pm 0$	$7^{b} \pm 1$	$9^{b} \pm 1$
Food intake (g/g body weight)	KO	0.27 ± 0.01	$0.21^{b} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.21^{b} \pm 0.02$	$0.19^{b} \pm 0.01$
	WT	0.28 ± 0.01	$0.22^{b} \pm 0.01$	$0.18^{b} \pm 0.01$	$0.20^{b} \pm 0.01$	$0.18^{b} \pm 0.01$

[a] Indicates $P < 0.05$ versus wildtype mice.
[b] Indicates $P < 0.05$ versus control treatment (0% or 3%).

body weight for both genotypes with increasing ethanol concentrations seemed to reflect normal growth, due to the non-balanced order of exposure to ethanol concentrations. The total volume of fluid intake (water plus ethanol) was about 50% higher in 5-HT_{1B} receptor knockout than in wildtype mice ($F_{(1,30)} = 124.4$, $P < 0.001$) for all concentrations. Compared to the first 4-days, at the end of the experiment water intake was not affected by ethanol-exposure (data not shown). Total fluid intake showed a significant interaction of genotype and concentration ($F_{(4,120)} = 4.5$, $P < 0.05$, $\varepsilon = 0.44$), which was mainly due to decreased intake in 5-HT_{1B} receptor knockout mice during the 6% ethanol block. Even after correction for body weight differences, the relative measure showed similar effects. Per gram body weight 5-HT_{1B} receptor knockout drank more than wildtype mice ($F_{(1,30)} = 43.7$, $P < 0.001$). The interaction between genotype and concentration ($F_{(4,120)} = 5.5$, $P < 0.01$, $\varepsilon = 0.54$) could be explained by the decreased intake in 5-HT_{1B} receptor knockout mice during one ethanol block (6%).

Ethanol consumption, as g/kg body weight, showed no difference between genotypes ($F_{(1,30)} = 0.01$, $P = 0.92$), but increased at higher concentrations ($F_{(3,90)} = 63.6$, $P < 0.001$, $\varepsilon = 0.39$). While total fluid intake was increased in 5-HT_{1B} receptor knockout mice, the volume of ethanol drunk was not different from wildtype mice. However, this measure was not stable across concentrations ($F_{(3,90)} = 9.9$, $P < 0.005$, $\varepsilon = 0.44$); 6% and 10% ethanol concentrations showed reduced drinking versus 3% ethanol.

Food intake, after correction for body weight differences, showed no genotype effect. There was a main effect of concentration ($F_{(4,120)} = 82.6$, $P < 0.001$, $\varepsilon = 0.46$); food intake was reduced when ethanol was present.

4. Discussion

Our data show that ethanol intake, expressed as g/kg body weight, was not different in male wildtype and 5-HT_{1B} receptor knockout mice. Ethanol consumption increased with increasing concentrations, although the absolute volume of ethanol intake remained constant or was even reduced. A sevenfold increase in concentration (from 3% to 20%) led to a less than sevenfold increase in ethanol intake (g/kg body weight). Thus, concentration effects per se can explain elevated ethanol intake at higher concentrations. In the present study, levels of ethanol consumption in wildtype mice were somewhat lower than those reported in the original paper (Crabbe et al., 1996), although the pattern is similar.

Across inbred mouse strains, large differences have been described in ethanol intake and ethanol preference, in which 129/J mice appeared moderate (Belknap et al., 1993). Our 129/Sv mice show very low ratios (< 50%) of ethanol preference indicating ethanol avoidance at any concentration. During the first days of the 3% concentration period (data not shown in detail), ethanol intake and preference ratios were somewhat higher, which suggests that it takes about 3 days for naive mice to distinguish the preferred water pipette from ethanol. This finding might explain the higher levels of ethanol consumption observed in the laboratory comparison study (Crabbe et al., 1999), in which mice were exposed to ethanol (6% v/v) for only 4 days. An alternative explanation is that 3% ethanol is close to the concentration that our mice can discriminate at all, making it difficult to choose accurately. In a conditioned-taste-aversion paradigm, no difference in ethanol aversion was found between wildtype and 5-HT_{1B} receptor knockout mice, while 5-HT_{1B} receptor knockout mice showed reduced sensitivity to ethanol reward (Risinger et al., 1996). It was argued that high ethanol intake might reflect reduced sensitivity to aversive effects of ethanol. However, ethanol aversion seems to be genotype independent, because similar increases of ethanol intake were found after 0.15% saccharin sweetening (Risinger et al., 1999).

In the present study, body weight was about 20–25% higher in male 5-HT_{1B} receptor knockout mice as found repeatedly in independent samples of mice from our colony (Bouwknecht et al., submitted). In the latter study, this phenotype was more evident in males than in females. Moreover, gender is an important factor in body weight regulation, because males were about 35–40% heavier than females.

The present study showed that total as well as relative fluid intake was rather stable over ethanol concentrations, suggesting that ingesting sufficient fluid to attain homeostasis was unaffected by ethanol. The total fluid intake was about 50% higher in 5-HT_{1B} receptor knockout than in wildtype mice, indicating that this phenotypic difference cannot be explained solely by body weight differences. Also, this phenotype of elevated fluid intake was found consistently across generations within our breeding colony (Bouwknecht et al., submitted). Moreover, our data confirm another study on 5-HT_{1B} receptor knockout mice in an operant situation, where absolute levels of water intake were similar to our data (Risinger et al., 1999).

In contrast to water intake, relative food intake (g/g body weight) did not differ between genotypes. These data confirm our previous findings, suggesting a discrepancy between regulation of food intake, which is related to body weight, and water intake in 5-HT_{1B} receptor knockout mice (Bouwknecht et al., submitted). In general, phenotypic differences were more evident in males than in females. Most of the studies used mixtures of males and females (Crabbe et al., 1996; Risinger et al., 1996, 1999), which might explain why differences in body weight and fluid intake have seldom been reported. However, a recent study separating males and females confirms our data (Brunner et al., 1999).

In the present study, the reduction in food intake when ethanol was present suggests an effect of ethanol exposure, but the relatively low amount of ethanol consumption

makes that rather unlikely. This finding could also be a protocol-related artifact instead of ethanol-induced hypophagia, because elevation of food intake was seen during the first days that mice were housed singly (data not shown in detail).

In conclusion, this study like others failed to replicate elevated ethanol intake in 5-HT_{1B} receptor knockout mice. We used the original protocol, excluding an explanation by differences in experimental setup. Other phenotypic effects on body weight and fluid intake were stable across generations, which suggests a limited role of genetic drift. To explain the lack of elevated ethanol intake in 5-HT_{1B} receptor knockout mice by genetic drift in multiple, independent studies would imply that in separate populations adaptation over generations is reflected as regression to mean levels. However, a final conclusion about the influence of genetic drift can be drawn only when the phenotype has been conserved in rederived mice from cryo-preserved embryos from the original population, as suggested recently (Crabbe, 1999). Unfortunately, rederiving such populations is very time consuming.

5-HT_{1B} receptors play an important role in body weight regulation and food and fluid intake. Activation of this receptor induces anorexia and leads to a general decrease of consummatory behavior (Kennett et al., 1987; Maurel et al., 1999). The present study in 5-HT_{1B} receptor knockout mice provides further evidence that 5-HT_{1B} receptors play an inhibitory role in regulation of body weight and fluid intake in particular. The inconsistency in ethanol intake, expressed as gram per kg body weight, in 5-HT_{1B} receptor knockout mice might be explained by the fact that two parameters involved in this measure, i.e. body weight and fluid intake, are affected themselves by the absence of 5-HT_{1B} receptors.

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