



**HAL**  
open science

## Antidepressant-like effect of low dose of scopolamine in the H/Rouen genetic mouse model of depression

Malika El Yacoubi, Jean-Marie Vaugeois, Stéphane Jamain

### ► To cite this version:

Malika El Yacoubi, Jean-Marie Vaugeois, Stéphane Jamain. Antidepressant-like effect of low dose of scopolamine in the H/Rouen genetic mouse model of depression. *Fundamental & Clinical Pharmacology*, 2020, Online ahead of print. 10.1111/fcp.12639 . inserm-03198220

**HAL Id: inserm-03198220**

**<https://inserm.hal.science/inserm-03198220>**

Submitted on 14 Apr 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Antidepressant-like effect of low dose of scopolamine in the H/Rouen genetic**  
2 **mouse model of depression**

3

4 Running title: Scopolamine in H/Rouen mice.

5

6 Malika El Yacoubi<sup>1</sup>, Jean-Marie Vaugeois<sup>2</sup>, Stéphane Jamain<sup>1,\*</sup>

7

8 1) Univ Paris Est Créteil, INSERM, IMRB, Translational Neuropsychiatry, Fondation  
9 FondaMental, F-94010, Créteil, France

10 2) Normandie Univ, UNIROUEN, UNICAEN, ABTE, Rouen, F-76000, France

11

12 \*Corresponding author:

13 Dr Stéphane Jamain

14 Inserm U955, Psychiatrie Translationnelle

15 Faculté de Médecine, 8 rue du Général Sarrail, Créteil, 94000, France.

16 Phone: +33 1 49 81 37 75

17 E-mail: stephane.jamain@inserm.fr

18

19

20 **Abstract**

21 Rodent models of depression are useful for the investigation of cellular and neuronal  
22 mechanisms of antidepressant drugs and for the discovery of potential new targets. In  
23 this study, we examined the antidepressant-like effect of scopolamine, a non-selective  
24 muscarinic antagonist, in a genetic mouse model of depression obtained through a  
25 selective breeding strategy and called H/Rouen. In this model, we observed that  
26 scopolamine was active both in males and females at a lower dose (0.03 mg/kg) in the  
27 tail suspension test, 30 min following its administration, than observed in CD-1 mice.  
28 In addition, we showed this antidepressant-like effect was partly inhibited by an  
29 injection of 10 mg/kg of the AMPA receptor antagonist NBQX in both males and  
30 females, suggesting the anti-depressant like effect of scopolamine was mainly driven  
31 by AMPA receptors in the H/Rouen mouse line. Altogether, our results showed the  
32 high sensitivity of the H/Rouen mouse model of depression to study the  
33 antidepressant-like effects of pharmacological compounds.

34

35

36 **Keywords:** scopolamine; NBQX; tail suspension test; mouse model; depression, male  
37 and female

38

39

## 40 **Introduction**

41 Most of the antidepressants available on the market are based on the monoamine  
42 hypothesis of depression and aim at increasing monoamine neurotransmission in  
43 patients with a major depressive disorder [1]. However, these drugs are not efficient  
44 for all patients and one-third of them still manifest symptoms after 4 different  
45 medication courses [2]. Evidence has been obtained for a rapid effect of the non-  
46 selective muscarinic receptors antagonist scopolamine in depressed patients [for  
47 reviews see 3, 4, 5]. In preclinical studies performed in rodents, high doses of  
48 scopolamine have revealed a good efficiency to decrease the immobility time in the tail  
49 suspension test and the forced swim test [6, 7], two tests particularly relevant to study  
50 antidepressant-like effects [8, 9]. One major hypothesis concerning the rapid effects of  
51 scopolamine concerns the involvement of a glutamatergic burst [10].

52 Using CD-1 outbred mice selective breeding-based on behavioral reaction after an  
53 acute mild stress, we previously generated a mouse model, called H/Rouen [11]. This  
54 model was obtained after mating animals with the lowest mobility in the tail suspension  
55 test (TST). In addition to the low mobility observed in the TST, these animals  
56 manifested depressive-like behaviors, anhedonia-like behaviors, anxiety-like  
57 behaviors, impairment in sleep patterns, and a high basal corticosterone level [11-13],  
58 which were reversed by chronic treatment with serotonin reuptake inhibitors [11]. Here  
59 we investigated the effects of scopolamine, a rapid-acting antidepressant on this  
60 mouse model. We first demonstrated that scopolamine is active at very low doses in  
61 the tail suspension test (TST) following its administration to both male and female  
62 H/Rouen mice. Next, we showed that the antidepressant-like effect of scopolamine  
63 was significantly reduced by a pretreatment with the 2,3-dihydroxy-6-nitro-7-sulfamoyl-

64 benzo[f]quinoxaline (NBQX), an  $\alpha$ -amino-3-hydroxy-5-méthylisoazol-4-propionate  
65 acid (AMPA) receptor antagonist.

66

## 67 **Experimental procedures**

### 68 **Animals**

69 H/Rouen mice have been bred in the University Lyon 1 animal facility and selected for  
70 their low mobility in the TST as previously described [11]. They were kept on a 7 a.m.-  
71 7 p.m. light cycle with food and water ad libitum. Testing was performed between 9  
72 a.m. and 5 p.m.. All experiments were conducted in accordance with the regulations  
73 of the local ethical committee (Lyon 1 University CE2A- UCBL BH 2009-03) and the  
74 European Community Council Directive of November the 24<sup>th</sup>, 1986 (86/609/EEC).

75

### 76 **Drugs**

77 Scopolamine hydrochloride (Sigma-Aldrich, St Louis, MO, U.S.A.) and NBQX disodium  
78 salt hydrate (Sigma-Aldrich) were dissolved daily in vehicle. Doses always refer to the  
79 free bases.

80

### 81 **Tail Suspension Test**

82 The TST was performed with a computerized device. Mice were suspended by the tail  
83 with adhesive tape to a hook connected to a strain gauge. This gauge transmitted  
84 movements to a computer calculating total duration of immobility during a 6-min test.  
85 Mice that climbed up their tail during the test session were excluded from the study.

86

87

88

## 89 **Drugs treatments**

90 Acute treatments were performed on adult mice (10 to 14-week-old). Drug  
91 administration were performed intraperitoneally. Mice received either an acute  
92 injection of scopolamine or of a vehicle. All mice were tested with the TST 30 minutes  
93 after injection. When tested, NBQX was administered 45 min before the test, i.e. 15  
94 minutes before the injection of scopolamine or its vehicle.

95

## 96 **Statistical Analysis**

97 Results were expressed as median  $\pm$  interquartile range. Statistical analysis was  
98 performed using non-parametric Mann-Whitney or Kruskal-Wallis test. Significance  
99 levels were set at  $P < 0.05$ .

100

## 101 **Results**

102 Scopolamine has been previously reported to produce a dose-dependent  
103 antidepressant-like effects in the forced-swim test (FST) in both rats and mice [7].  
104 Here, we observed in CD-1 male mice such a dose-dependent efficiency in the TST  
105 (Kruskal-Wallis test,  $K=8.17$ ,  $P=0.04$ ; Figure 1A) with the only significant effect at a  
106 dose of 0.3 mg/kg (Mann-Whitney test,  $U=56.5$ ,  $P=0.02$ ). As observed for CD-1 mice,  
107 scopolamine significantly reduced in a dose-dependent manner the immobility of  
108 H/Rouen males (Kruskal-Wallis test,  $K=20.48$ ,  $P=0.0001$ ; Figure 1B) and females  
109 (Kruskal-Wallis test,  $K=18$ ,  $P=0.0004$ ; Figure 1C) in the TST. Interestingly, the effect  
110 was observed for both male and female mice at a dose as low as 0.03 mg/kg (Mann-  
111 Whitney test,  $U_{\text{males}}=7$ ,  $P_{\text{males}}=0.007$  and  $U_{\text{females}}=0$ ,  $P_{\text{females}}=0.002$ ), suggesting a  
112 higher sensitivity of H/Rouen mice than CD-1 mice to test antidepressant medications.

113 We then used NBQX to antagonize the effect of scopolamine in H/Rouen male mice.  
114 Again, scopolamine significantly decreased the immobility time in the TST at a dose of  
115 0.1 mg/kg both in males (Mann-Whitney test,  $U=7$ ;  $P<0.0001$ ; Figure 2A) and females  
116 (Mann-Whitney test,  $U=3$ ;  $P<0.0001$ ; Figure 2B). Although a small effect still persisted  
117 when male and female mice first received an injection of NBQX before the injection of  
118 scopolamine (Mann-Whitney test,  $U_{\text{males}}=17$ ;  $P_{\text{males}}=0.01$ ;  $U_{\text{females}}=11$ ;  $P_{\text{females}}=0.001$ ),  
119 this effect was reduced for both sexes (Mann-Whitney test,  $U_{\text{males}}=19$ ;  $P_{\text{males}}=0.007$ ;  
120  $U_{\text{females}}=26$ ;  $P_{\text{females}}=0.04$ ) when compared with treatment with scopolamine alone. As  
121 expected, no effect was observed after injection of NBQX alone for none of the genders  
122 (Mann-Whitney test,  $U_{\text{males}}=36$ ;  $P_{\text{males}}=0.53$ ;  $U_{\text{females}}=23$ ;  $P_{\text{females}}=0.15$ ; Figure 2).

123

## 124 **Discussion**

125 In this study, we examined the effect of scopolamine on mice with an extreme  
126 phenotype in the TST. We confirmed that scopolamine is effective in our mouse model  
127 of depression at very low doses. Scopolamine has previously been shown to increase  
128 the mobility time in rats and mice during FST [7, 10] and TST [7, 14]. Here, we  
129 observed, both in males and females, an effect on depressive-like phenotype at a dose  
130 ten folds lower than those previously published, suggesting that our H/Rouen model  
131 was a more sensitive model than commercial mice. In basal conditions, H/Rouen mice  
132 display an extremely long immobility duration in the TST with a low variability between  
133 individuals. In contrast, CD-1 mice are a heterogenous group of animals, including  
134 some having depressive-like behaviors whereas others have not, and for which  
135 scopolamine will have no antidepressant-like effect at low dose. The extreme  
136 homogeneous behaviors observed in our H/Rouen model is closer to a subgroup of

137 patients sharing similar phenotypes, as those observed in patients with major  
138 depressive disorders, and thus more appropriate to test antidepressant medications.  
139 The antidepressant effect of scopolamine has been suggested to act through a rapid  
140 increase of the mammalian target of rapamycin (mTOR) complex 1 (mTORC1)  
141 signaling after a glutamate burst in the prefrontal cortex, as observed after the  
142 ketamine injection [10]. Consistent with previous reports [4, 10, 15], we observed that  
143 NBQX reduced the effect of scopolamine in resigned animals. This confirms that the  
144 antidepressant-like effects of scopolamine resulted from a glutamatergic burst [4].  
145 However, this effect was not fully reversed by NBQX, in contrast to what has been  
146 previously reported in rats [10]. This suggests that in H/Rouen mice scopolamine might  
147 act through more than one mechanism. An additional action on noradrenergic  
148 transmission has been previously proposed [14] and may explain this partial effect.  
149 Indeed, a 50% reduction of noradrenaline in the prefrontal cortex, while not changing  
150 the mouse behavior in the TST, significantly reduce the higher mobility observed after  
151 scopolamine injection. It has also been proposed that dopamine D2/D3 receptors are  
152 involved in the rapid antidepressant-like effects of ketamine in the forced-swim test,  
153 since this effect was blocked by dopamine D2/D3 receptor antagonists [16]. Recently,  
154 Addy and colleagues showed that injection of scopolamine in the ventral tegmental  
155 area (VTA) decreased the immobility time in the FST [17], strongly supporting a role of  
156 VTA cholinergic activity in depressive-like behaviors. The blockade of the muscarinic  
157 acetylcholine receptors in the VTA by scopolamine may thus reduce the phasic  
158 dopamine release in the nucleus accumbens and modulate depressive-like behaviors  
159 through dopamine D2/D3 receptors. Scopolamine has also been shown to rescue  
160 depressive-like behaviors and increase the brain derived neurotrophic factor (BDNF),  
161 the serotonin transporter and the tryptophan hydroxylase 1 levels in reserpine-induced

162 mouse model of depression [18]. The BDNF increase after low dose of scopolamine  
163 injection is inhibited by L-Type Voltage-Dependent Calcium Channel antagonists and  
164 prevent the antidepressant-like effect of scopolamine in the FST [19]. These calcium  
165 channels have been repeatedly associated with mood disorders in human and are  
166 involved in the release of many neurotransmitters, as well as in the activity of midbrain  
167 dopaminergic neurons and adrenal chromaffin cells [20]. These mechanisms might  
168 thus explain the remaining antidepressant-like effect of scopolamine after NBQX  
169 injection observed in H/Rouen animals and need further experiments to decipher the  
170 molecular mechanisms involved in the antidepressant-like effect of scopolamine.

171

## 172 **Conclusion**

173 Overall, the present study shows evidence that the H/Rouen male and female mice  
174 are relevant models for preclinical studies to test new drugs alternative to standard  
175 antidepressant in treatment of major depressive disorder. In the future, such models  
176 should help in a better comprehension of the pathophysiology of depression.

177

178

179 **Acknowledgments**

180 This work was supported by the *Fondation de France* under reference 00081240. This  
181 work also received financial support from the *Institut National pour la Santé et la*  
182 *Recherche Médicale* (Inserm) and the *Centre National pour la Recherche Scientifique*  
183 (CNRS). The Jamain's team is affiliated to the Paris School of Neuroscience (ENP)  
184 and the Bio-Psy Laboratory of Excellence.

185

186 **Conflict of interest**

187 All other authors declare that they have no conflicts of interest.

188

189 **References**

- 190 [1] Slattery DA, Cryan JF. The ups and downs of modelling mood disorders in  
191 rodents. *ILAR J.* (2014) **55** 297-309.
- 192 [2] Malhi GS, Mann JJ. Depression. *Lancet.* (2018) **392** 2299-312.
- 193 [3] O'Leary OF, Dinan TG, Cryan JF. Faster, better, stronger: towards new  
194 antidepressant therapeutic strategies. *Eur J Pharmacol.* (2015) **753** 32-50.
- 195 [4] Duman RS. Ketamine and rapid-acting antidepressants: a new era in the battle  
196 against depression and suicide. *F1000Res.* (2018) **7**.
- 197 [5] Witkin JM, Martin AE, Golani LK, Xu NZ, Smith JL. Rapid-acting  
198 antidepressants. *Adv Pharmacol.* (2019) **86** 47-96.
- 199 [6] Podkowa K, Podkowa A, Salat K, Lenda T, Pilc A, Palucha-Poniewiera A.  
200 Antidepressant-like effects of scopolamine in mice are enhanced by the group II mGlu  
201 receptor antagonist LY341495. *Neuropharmacology.* (2016) **111** 169-79.
- 202 [7] Witkin JM, Overshiner C, Li X, Catlow JT, Wishart GN, Schober DA, et al. M1  
203 and m2 muscarinic receptor subtypes regulate antidepressant-like effects of the rapidly  
204 acting antidepressant scopolamine. *J Pharmacol Exp Ther.* (2014) **351** 448-56.
- 205 [8] Cryan JF, Holmes A. The ascent of mouse: advances in modelling human  
206 depression and anxiety. *Nat Rev Drug Discov.* (2005) **4** 775-90.

- 207 [9] Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for  
208 assessing antidepressant activity: review of pharmacological and genetic studies in  
209 mice. *Neurosci Biobehav Rev.* (2005) **29** 571-625.
- 210 [10] Voleti B, Navarria A, Liu RJ, Banasr M, Li N, Terwilliger R, et al. Scopolamine  
211 rapidly increases mammalian target of rapamycin complex 1 signaling,  
212 synaptogenesis, and antidepressant behavioral responses. *Biol Psychiatry.* (2013) **74**  
213 742-9.
- 214 [11] El Yacoubi M, Bouali S, Popa D, Naudon L, Leroux-Nicollet I, Hamon M, et al.  
215 Behavioral, neurochemical, and electrophysiological characterization of a genetic  
216 mouse model of depression. *Proc Natl Acad Sci U S A.* (2003) **100** 6227-32.
- 217 [12] El Yacoubi M, Vaugeois JM. Genetic rodent models of depression. *Curr Opin*  
218 *Pharmacol.* (2007) **7** 3-7.
- 219 [13] El Yacoubi M, Rappeneau V, Champion E, Malleret G, Vaugeois JM. The  
220 H/Rouen mouse model displays depression-like and anxiety-like behaviors. *Behav*  
221 *Brain Res.* (2013) **256** 43-50.
- 222 [14] Palucha-Poniewiera A, Podkowa K, Lenda T, Pilc A. The involvement of  
223 monoaminergic neurotransmission in the antidepressant-like action of scopolamine in  
224 the tail suspension test. *Prog Neuropsychopharmacol Biol Psychiatry.* (2017) **79** 155-  
225 61.
- 226 [15] Martin AE, Schober DA, Nikolayev A, Tolstikov VV, Anderson WH, Higgs RE,  
227 et al. Further Evaluation of Mechanisms Associated with the Antidepressantlike

228 Signature of Scopolamine in Mice. *CNS Neurol Disord Drug Targets*. (2017) **16** 492-  
229 500.

230 [16] Li Y, Zhu ZR, Ou BC, Wang YQ, Tan ZB, Deng CM, et al. Dopamine D2/D3 but  
231 not dopamine D1 receptors are involved in the rapid antidepressant-like effects of  
232 ketamine in the forced swim test. *Behav Brain Res*. (2015) **279** 100-5.

233 [17] Addy NA, Nunes EJ, Wickham RJ. Ventral tegmental area cholinergic  
234 mechanisms mediate behavioral responses in the forced swim test. *Behav Brain Res*.  
235 (2015) **288** 54-62.

236 [18] Yu H, Lv D, Shen M, Zhang Y, Zhou D, Chen Z, et al. BDNF mediates the  
237 protective effects of scopolamine in reserpine-induced depression-like behaviors via  
238 up-regulation of 5-HTT and TPH1. *Psychiatry Res*. (2019) **271** 328-34.

239 [19] Yu H, Li M, Shen X, Lv D, Sun X, Wang J, et al. The Requirement of L-Type  
240 Voltage-Dependent Calcium Channel (L-VDCC) in the Rapid-Acting Antidepressant-  
241 Like Effects of Scopolamine in Mice. *Int J Neuropsychopharmacol*. (2018) **21** 175-86.

242 [20] Andrade A, Brennecke A, Mallat S, Brown J, Gomez-Rivadeneira J, Czepiel N,  
243 et al. Genetic Associations between Voltage-Gated Calcium Channels and Psychiatric  
244 Disorders. *Int J Mol Sci*. (2019) **20**.

245

246

247 **Figure legends**

248 **Figure 1. Dose-dependent effect of scopolamine.** Increasing doses of scopolamine  
249 (0.03, 0.1 and 0.3 mg/kg) was injected intraperitoneally in 15 CD-1 males (A), as well  
250 as in 8 male (B) and 6 female (C) H/Rouen mice and compare to the vehicle injection.  
251 Immobility time during the tail suspension test was measure for 6 min, 30 min after  
252 injection. Individual measures are shown with black circles. Boxplots represent  
253 median  $\pm$  interquartile range. \*\*P<0.01, \*\*\*P<0.001.

254

255 **Figure 2. Inhibition of the scopolamine antidepressant-like effect using the**  
256 **AMPA receptor antagonist NBQX.** NBQX was injected or not in at least 7 male (A)  
257 and 8 female (B) H/Rouen mice at a dose of 10 mg/kg, 15 min before injection  
258 scopolamine (scop). Immobility times in a 6 min session of the tail suspension test was  
259 compared to vehicle injection (stars) or after injection of scopolamine (hashes).  
260 Individual measures are shown with black circles. Boxplots represent  
261 median  $\pm$  interquartile range. \*P<0.05, \*\*\*P<0.001, ##P<0.01.

262