Supplementary methods

Control compounds for the *in vitro* pharmacology assays of mefloquine are detailed in the Supplemental Table 2.

1. Binding screening for mefloquine targets
According to the CRO (Eurofins CEREP) recommendations, results showing an inhibition (or a stimulation for assays run in basal conditions) higher than 50% were considered to represent significant effects of THN101. This cut-off value was thus considered for further investigation, such as determination of IC_{50} or EC_{50} values from concentration-response curves (see below). Results showing an inhibition (or stimulation) between 25% and 50% were considered as indicative of weak to moderate effects. Results showing an inhibition (or stimulation) lower than 25% were not considered significant, being mostly attributable to the variability of the signal around control levels.

2. Binding assays
Results were expressed as a percent of control specific binding:

$$\frac{\text{measured specific binding}}{\text{control specific binding}} \text{x } 100$$

and as a percent inhibition of control specific binding:

$$100 - \left(\frac{\text{measured specific binding}}{\text{control specific binding}} \text{x } 100\right)$$

obtained in the presence of THN01.
The IC_{50} values (concentration causing a half-maximal inhibition of control specific binding) and Hill coefficients (nH) were determined by non-linear regression analysis of the competition curves generated with mean replicate values using Hill equation curve fitting:

$$Y = D + \left[\frac{A - D}{1 + \left(\frac{C}{C_{50}}\right)^{nH}}\right]$$

where Y = specific binding, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, C_{50} = IC_{50}, and nH = slope factor. This analysis was performed using software developed at CEREP (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot® 4.0 for Windows® (© 1997 by SPSS Inc.).

The inhibition constants (K_i) were calculated using the Cheng Prusoff equation:

$$K_i = \frac{IC_{50}}{\left(1 + \frac{L}{K_D}\right)}$$

where L = concentration of radioligand in the assay, and K_D = affinity of the radioligand for the receptor. A scatchard plot is used to determine the K_D.

3. Receptor Functional Assays
The results are expressed as a percent of control agonist response or inverse agonist response:

$$\frac{\text{measured response}}{\text{control response}} \text{x } 100$$

and as a percent inhibition of control agonist response:

$$100 - \left(\frac{\text{measured response}}{\text{control response}} \text{x } 100\right)$$

obtained in the presence of THN01.

The EC_{50} values (concentration producing a half-maximal response) and IC_{50} values (concentration causing a half-maximal inhibition of the control agonist response) were determined by non-linear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting:

$$Y = D + \left[\frac{\text{A} - \text{D}}{1 + \left(\frac{\text{C}}{\text{C}_{50}}\right)^{\text{nH}}}\right]$$

where Y = response, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, and C_{50} = EC_{50} or IC_{50}, and nH = slope factor.

This analysis was performed using software developed at CEREP (Hill software) and validated by comparison with data generated by the commercial software SigmaPlot ® 4.0 for Windows ® (© 1997 by SPSS Inc.).

For the antagonists, the apparent dissociation constants (KB) were calculated using the modified Cheng Prusoff equation:

$$K_B = \frac{\text{IC}_{50}}{(1 + \text{A}/\text{EC}_{50\text{A}})}]$$

where A = concentration of reference agonist in the assay, and EC50A = EC50 value of the reference agonist.

4. Uptake Assays

The results are expressed as a percent of control specific activity:

$$\frac{\text{measured specific activity}}{\text{control specific activity}} \text{x } 100$$

And as a percent inhibition of control specific activity:

$$100 - \left(\frac{\text{measured specific activity}}{\text{control specific activity}} \text{x } 100\right)$$

obtained in the presence of THN01.

The IC_{50} values (concentration causing a half-maximal inhibition of control specific activity), EC_{50} values (concentration producing a half-maximal increase in control basal activity), and Hill coefficients (nH) were determined by non-linear regression analysis of the inhibition/concentration-response curves generated with mean replicate values using Hill equation curve fitting:

$$Y = D + \left[\frac{\text{A} - \text{D}}{1 + \left(\frac{\text{C}}{\text{C}_{50}}\right)^{\text{nH}}}\right]$$

where Y = specific activity, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, C_{50} = IC_{50} or EC_{50}, and nH = slope factor.

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obtained in the presence of THN01.

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$$Y = D + [\frac{A - D}{1 + \left(\frac{C}{C_{50}}\right)^{nH}}]$$

where Y = response, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, and C_{50} = EC_{50} or IC_{50}, and nH = slope factor.
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$$K_B = \frac{IC_{50}}{(1 + A/EC_{50A})}]$$

where A = concentration of reference agonist in the assay, and EC50A = EC50 value of the reference agonist.

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The results are expressed as a percent of control specific activity:

$$\frac{\text{measured specific activity}}{\text{control specific activity}} \text{x } 100$$

And as a percent inhibition of control specific activity:

$$100 - (\frac{\text{measured specific activity}}{\text{control specific activity}} \text{x } 100)$$

obtained in the presence of THN01.
The IC_{50} values (concentration causing a half-maximal inhibition of control specific activity), EC_{50} values (concentration producing a half-maximal increase in control basal activity), and Hill coefficients (nH) were determined by non-linear regression analysis of the inhibition/concentration-response curves generated with mean replicate values using Hill equation curve fitting:

$$Y = D + [\frac{A - D}{1 + \left(\frac{C}{C_{50}}\right)^{nH}}]$$

where Y = specific activity, A = left asymptote of the curve, D = right asymptote of the curve, C = compound concentration, C_{50} = IC_{50} or EC_{50}, and nH = slope factor.
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1A

Recovery yields of target compounds

	% of recovery (n=3)			
	Brain	**Spinal cord**	**Plasma**	**Blood**
Amitriptyline	69 ± 5%	25.9 ± 3%	37 ± 7%	31 ± 2%
Nortriptyline	94 ± 5%	25.9 ± 3%	37 ± 7%	27 ± 3%
Mefloquine	55 ± 4%	21 ± 2%	31 ± 3%	22 ± 5%

1B

HPLC gradient

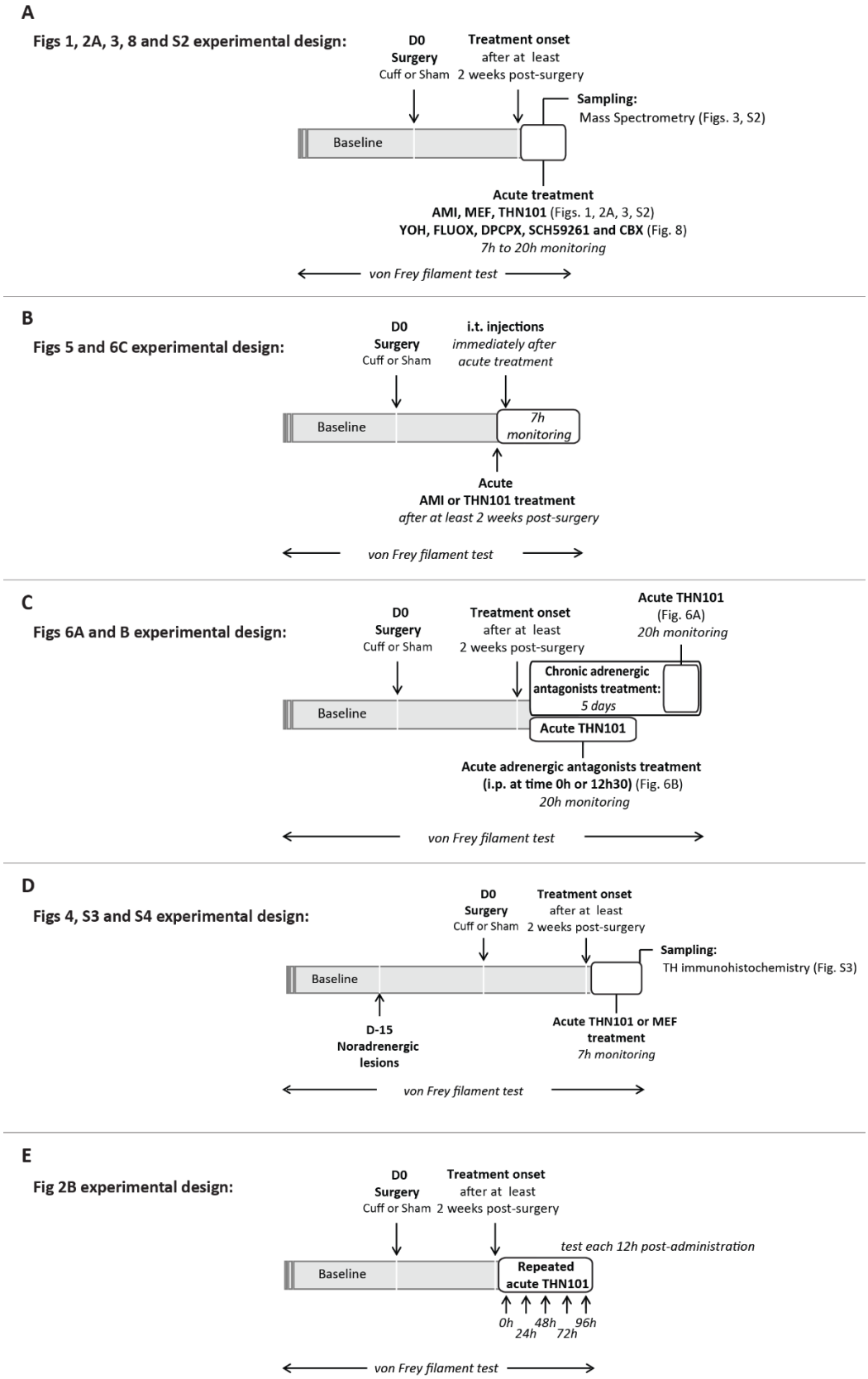
Time (min)	0	2.5	4.5	14.5	16.5	18	22
% B buffer	0	0	30	98	98	0	0

1C

MS ionization, selection, fragmentation and identification parameters

	Precursor (m/z)	**Product (m/z)**	**CE (V)**	**RF Lens (V)**
Amitriptyline	278.18	191.06 202.06 233.11	26.13 55.00 17.99	181.38
D3-amitriptyline	281.20	191.04 202.04 233.11	26.43 53.94 18.44	168.03
Nortriptyline	264.33	117.11 191.06 233.04	20.92 23.75 15.21	164.08
D3-nortriptyline	267.21	117.11 191.04 233.11	21.17 24.41 15.71	162.57
Mefloquine	379.13	321.04 333.06 361.11	28.20 28.91 21.73	260.25
D10-mefloquine	389.20	330.11 339.11 371.09	30.47 31.48 24.11	257.83

Supplementary Table 1. Recovery yields, and LC and MS/MS conditions for the purification, detection and quantification of amitriptyline, nortriptyline and mefloquine and their respective heavy tagged counterparts. Buffer A corresponded to ACN 1% / H_2O 98.9% / AF 0.1% (v/v/v), whereas buffer B was ACN 99.9 % / AF 0.1% (v/v). The flow rate was set at 10 µL/min.

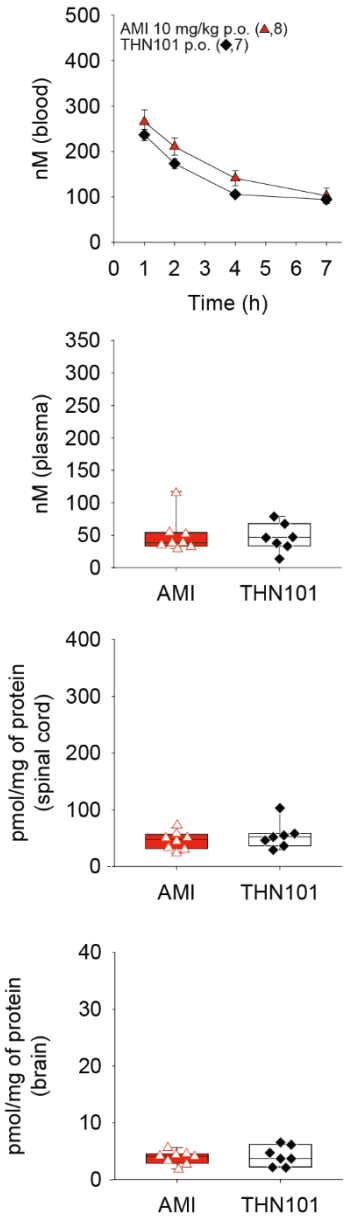


Supplemental Figure 1. Experimental chronograms. For all experiments, a two-week period of habituation to the facilities and of baseline measures for paw withdrawal thresholds was present before the first procedure (either surgery, with cuff-implantation or sham-surgery; or intrathecal procedure for lesions). The drug treatments began at least two weeks after the surgical (cuff/sham) procedure.

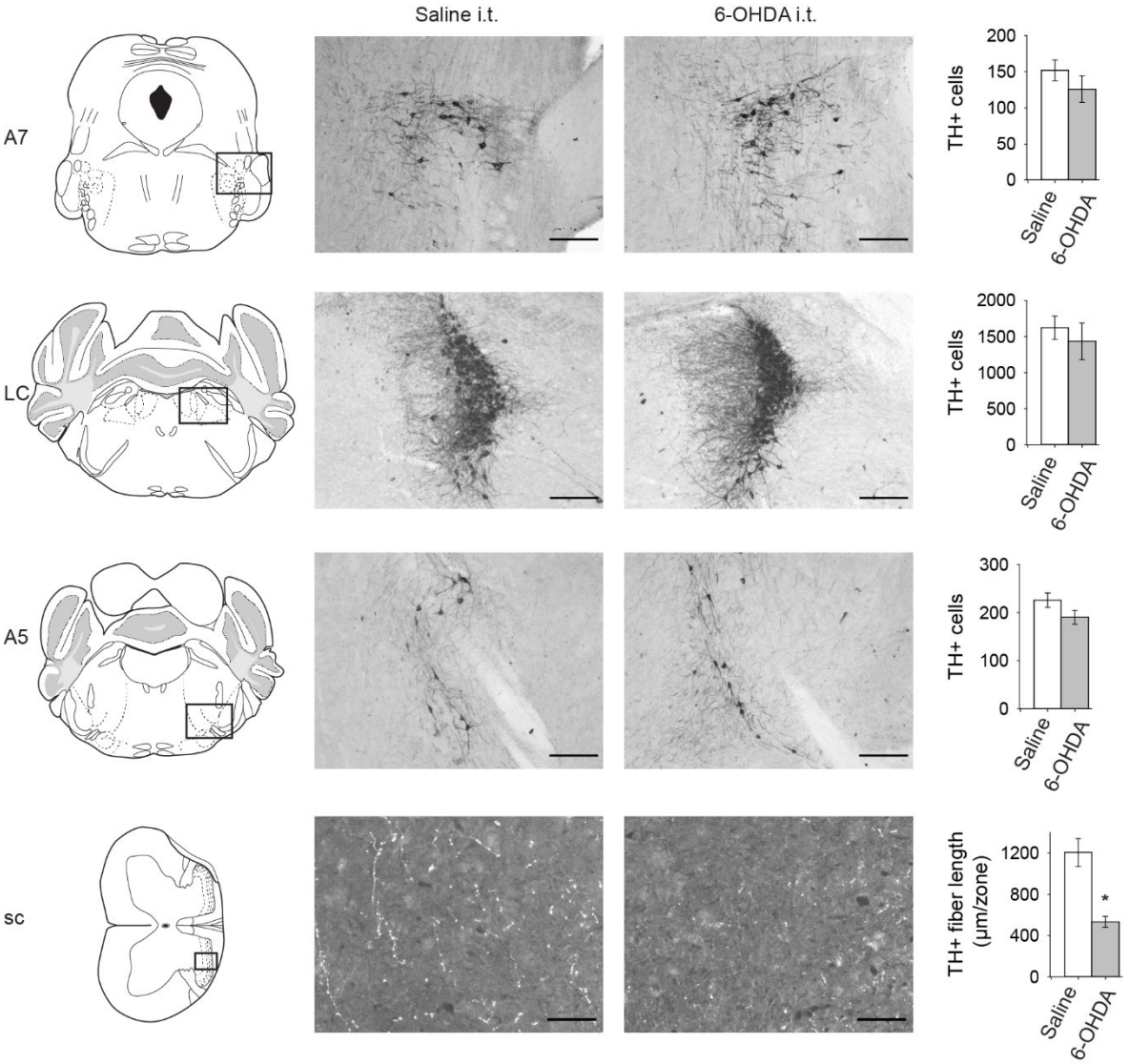
Assay	Source	Ligand	Conc.	Kd	Non Specific	Incubation	Detection Method
Receptors							
A1 *(h)* (antagonist radioligand)	human recombinant (CHO cells)	[3H]DPCPX	1 nM	1.7 nM	DPCPX (1 µM)	60 min RT	Scintillation counting
A2A *(h)* (agonist radioligand)	human recombinant (HEK-293 cells)	[3H]CGS 21680	6 nM	27 nM	NECA (10 µM)	120 min RT	Scintillation counting
A3 *(h)* (agonist radioligand)	human recombinant (HEK-293 cells)	[125I]AB-MECA	0.15 nM	0.22 nM	IB-MECA (1 µM)	120 min RT	Scintillation counting
α1 (non- selective) (antagonist radioligand)	rat cerebral cortex	[3H]prazosin	0.25 nM	0.09 nM	prazosin (0.5 µM)	60 min RT	Scintillation counting
α2 (non- selective) (antagonist radioligand)	rat cerebral cortex	[3H]RX 821002	0.5 nM	0.38 nM	(-)epinephrine (100 µM)	60 min RT	Scintillation counting
β1 *(h)* (agonist radioligand)	human recombinant (HEK-293 cells)	[3H](-)CGP 12177	0.3 nM	0.39 nM	alprenolol (50 µM)	60 min RT	Scintillation counting
β2 *(h)* (agonist radioligand)	human recombinant (CHO cells)	[3H](-)CGP 12177	0.3 nM	0.15 nM	alprenolol (50 µM)	120 min RT	Scintillation counting
CGRP *(h)* (agonist radioligand)	human recombinant (CHO cells)	[125I]hCGRPα	0.03 nM	0.06 nM	hCGRPα (1 µM)	90 min RT	Scintillation counting
GABA (non-selective) (agonist radioligand)	rat cerebral cortex	[3H]GABA	10 nM	15 nM	GABA (100 µM)	60 min RT	Scintillation counting
TNF-α *(h)* (agonist radioligand)	U-937 cells	[125I]TNF-α	0.1 nM	0.05 nM	TNF-α (10 nM)	120 min 4°C	Scintillation counting
δ (DOP) *(h)* (agonist radioligand)	human recombinant (CHO cells)	[3H]DADLE	0.5 nM	0.73 nM	naltrexone (10 µM)	120 min RT	Scintillation counting
κ (KOP) (agonist radioligand)	rat recombinant (CHO cells)	[3H]U 69593	1 nM	2 nM	naloxone (10 µM)	60 min RT	Scintillation counting
µ (MOP) *(h)* (agonist radioligand)	human recombinant (HEK-293 cells)	[3H]DAMGO	0.5 nM	0.35 nM	naloxone (10 µM)	120 min RT	Scintillation counting
P2Y (agonist radioligand)	rat cerebral cortex	[35S]dATPαS	10 nM	10 nM	dATPαS	60 min RT	Scintillation counting
5-HT1A *(h)* (agonist radioligand)	human recombinant (HEK-293 cells)	[3H]8-OH-DPAT	0.3 nM	0.5 nM	8-OH-DPAT (10 µM)	60 min RT	Scintillation counting
5-HT1B (antagonist radioligand)	rat cerebral cortex	[125I]CYP (+ 30 µM isoproterenol)	0.1 nM	0.16 nM	serotonin (10 µM)	120 min 37°C	Scintillation counting

5-HT2A *(h)* (antagonist radioligand)	human recombinant (HEK-293 cells)	[3H]ketanserin	0.5 nM	0.6 nM	ketanserin (1 µM)	60 min RT	Scintillation counting
5-HT2B *(h)* (agonist radioligand)	human recombinant (CHO cells)	[125I](±)DOI	0.2 nM	0.2 nM	(±)DOI (1 µM)	60 min RT	Scintillation counting
5-HT2C *(h)* (antagonist radioligand)	human recombinant (HEK-293 cells)	[3H]mesulergine	1 nM	0.5 nM	RS 102221 (10 µM)	120 min 37°C	Scintillation counting
5-HT5a *(h)* (agonist radioligand)	human recombinant (HEK-293 cells)	[3H]LSD	1.5 nM	1.5 nM	serotonin (100 µM)	120 min 37°C	Scintillation counting
5-HT6 *(h)* (agonist radioligand)	human recombinant (CHO cells)	[3H]LSD	2 nM	1.8 nM	serotonin (100 µM)	120 min 37°C	Scintillation counting
5-HT7 *(h)* (agonist radioligand)	human recombinant	[3H]LSD	4 nM	2.3 nM	serotonin (10 µM)	120 min RT	Scintillation counting
sigma (non-selective) (h) (agonist radioligand)	Jurkat cells (endogenous)	[3H]DTG	10 nM	41 nM	Haloperidol (10 µM)	120 min RT	Scintillation counting
Ion channels							
P2X (agonist radioligand)	rat urinary bladder	[3H]α,β-MeATP	3 nM	2.6 nM	α,β-MeATP (10 µM)	120 min 4°C	Scintillation counting
5-HT3 *(h)* (antagonist radioligand)	human recombinant (CHO cells)	[3H]BRL 43694	0.5 nM	1.15 nM	MDL 72222 (10 µM)	120 min RT	Scintillation counting
Transporters							
norepinephrine transporter *(h)* (antagonist radioligand)	human recombinant (CHO cells)	[3H]nisoxetine	1 nM	2.9 nM	desipramine (1 µM)	120 min 4°C	Scintillation counting
dopamine transporter *(h)* (antagonist radioligand)	human recombinant (CHO cells)	[3H]BTCP	4 nM	4.5 nM	BTCP (10 µM)	120 min 4°C	Scintillation counting
5-HT transporter *(h)* (antagonist radioligand)	human recombinant (CHO cells)	[3H]imipramine	2 nM	1.7 nM	imipramine (10 µM)	60 min RT	Scintillation counting

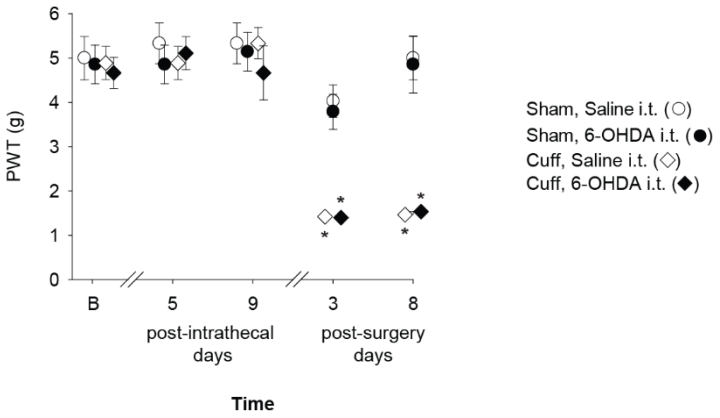
Supplemental Table 2. Control compounds for the *in vitro* pharmacology assays of mefloquine.



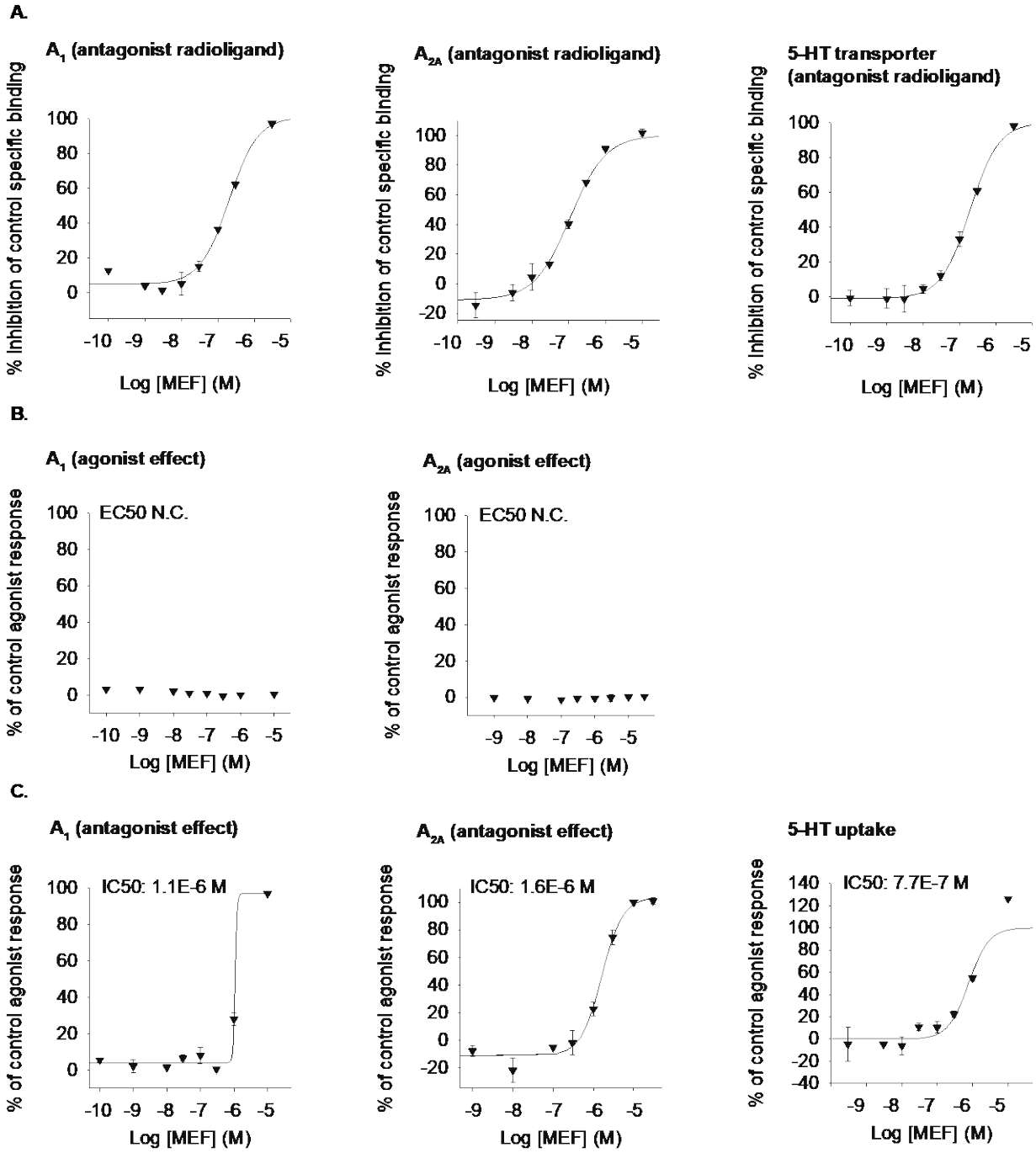
Supplemental Figure 2. Evaluation of amitriptyline concentrations by mass spectrometry after administration of amitriptyline (10 mg/kg, *per os*) or THN101 (amitriptyline 10 mg/kg + mefloquine 0.1 mg/kg, *per os*), without the outlier animal in the THN101 group (*cf.* Figure 3). Measures were performed only in Cuff mice. Amitriptyline concentrations (nM) were measured in blood from 1 to 7 hours after amitriptyline or THN101 *per os* treatment. Results are expressed as mean ± SEM. Amitriptyline plasma concentrations (nM), and spinal cord and brain concentrations (pmol/mg of protein), were also assessed 7 hours after amitriptyline or THN101 *per os* treatment (whisker boxes).



Supplemental Figure 3. Tyrosine hydroxylase (TH) immunostaining in the A5 cell group, locus coeruleus (LC) and A7 cell group (scale bars: 200 µm), and in the dorsal horn of the lumbar spinal cord (SC, scale bar: 50 µm).
The intrathecal 6-OHDA (central lesion, 20 µg, i.t.) led to a loss of TH-positive (TH+) fibers in the spinal cord, without significant change in the overall number of TH+ cells in the considered supraspinal nuclei. Data are expressed as mean ± SEM between animals, and the number of animals *per* group were: A7 n=5/6 (saline/6-OHDA), LC n=5/6, A5 n=6/6, SC n=5/5. For each animal, all TH+ cells were bilaterally counted *per* 40 µm sections every 80 µm (*i.e.* one every other section) over the whole anteroposterior extent of the considered cell group: A7 from -4.64 to -5.04 mm (distance from bregma), LC from -5.36 to -5.84 mm, A5 from -5.12 to -5.92 mm. For the SC, the total TH+ fiber length was measured, using ImageJ software, within 230.10^3 μm^2 areas in the dorsal horn of 3 sections (10 µm thick) *per* animal at lumbar L4-L5 level. Standardization was done for each zone and data are expressed as cumulated fiber length *per* zone. *$p<10^{-6}$ *vs* Saline.



Supplemental Figure 4. Behavioral analyses before and after the noradrenergic lesions and sciatic nerve surgery.
The mechanical paw withdrawal thresholds (PWT) for the right hind paw were assessed using von Frey filaments in mice before the intrathecal injections (B), 5 and 9 days after these injections (*i.e.* 9 and 5 days before the surgery), and 3 and 8 days after the sham/cuff surgeries. The Results are expressed as mean ± SEM. On the graph, a slight horizontal shift was added between the groups' symbols to facilitate vizualization when values overlapped (n=8-6 per group, *$p<10^{-4}$ *vs* baseline values).



Supplemental Figure 5. Binding and functional *in vitro* assays.
Binding radioligands (A), and control agonists for functional assays (B, C), are detailed in the Supplemental Table 2.