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# Hybridized and Isosteric Analogues of $N^1$-Acetyl-$N^4$-dimethyl-piperazinium Iodide (ADMP) and $N^1$-Phenyl-$N^4$-dimethyl-piperazinium Iodide (DMPP) with Central Nicotinic Action

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**Abstract**—A series of piperazine derivatives, obtained by hybridization of $N^1$-acetyl-$N^4$-dimethyl-piperazinium iodide (**1**, ADMP) and $N^1$-phenyl-$N^4$-dimethyl-piperazinium iodide (**3**, DMPP) or of the corresponding tertiary bases (**2**, **4**) with arecoline (**5**) and arecolone (**6**) or by isosteric substitution of the phenyl ring of DMPP, has been synthesized. Hybridization afforded compounds that, both as tertiary bases and as iodomethylates, have no affinity for the nicotinic receptor. On the contrary, isosteric substitution gave compounds that maintain affinity for the receptor; among them, two tertiary bases (**37**, **38**), show affinity in the nanomolar range for the nicotinic receptor. The pharmacological profile of these isomeric compounds is quite interesting as they present differences in their peripheral and central effects, suggesting that they interact with different subtypes of the nicotinic receptor. 

## Introduction

Central nicotinic cholinergic receptors (nAChRs) are an expanding area of research due to their implication in neurodegenerative disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD), where cholinergic transmission seems severely affected.[1,2]

For several years the cholinergic hypothesis of Alzheimer's disease[3] has stimulated intense research efforts, mainly on muscarinic agonists[4–6] while nicotinic agonists were substantially neglected, principally because of the cardiovascular, gastrointestinal and endocrine side effects of nicotine, as well as its negative connotation associated with tobacco smoking. In the past few years, however, thanks also to progress in molecular biology, physiology and pharmacology of central nicotinic receptors,[7,8] the potential of nicotinic agonists for the treatment of neurodegenerative disorders has been recognized and intensive research has been performed with the synthesis and pharmacological evaluation of several classes of drugs that possess nicotinic properties without the undesirable side effects of nicotine.[9–13]

As a matter of fact, the number of nicotinic receptors in patients affected by PD or AD is lower than those present in the brain of healthy individuals;[14] there is an inverse relationship between smoking and the likelihood to contract PD or AD;[1] treatment with nicotine has shown positive effects on memory, both on humans and animal models[15] and, in contrast to other neurotransmitters, the number of nicotinic receptors is increased after administration of nicotine.[16] Finally, the emerging heterogeneity of nicotinic receptors seems to provide an opportunity to discover ligands which would be selective and possibly devoid of nicotine side effects.[8,17]

Continuing our long-term research on cholinergic ligands[18,19] we have recently reported the synthesis of a few nicotinic agonists related to $N^1$-acetyl-$N^4$-dimethyl-piperazine iodide.[20] In the present paper we report the synthesis and preliminary pharmacological evaluation of the compounds with general structure A (see Chart 1), obtained by hybridization of $N^1$-acetyl-$N^4$-dimethyl-piperazinium iodide (**1**, ADMP)[20,21] and the corresponding tertiary base **2**,[20] $N^1$-phenyl-$N^4$-dimethyl-piperazinium iodide (**3**, DMPP)[22] and the corresponding tertiary base **4**, with arecoline (**5**),[23] arecolone (**6**)[24] and by isosteric substitution of the phenyl ring of DMPP. Compounds **1**, **3**, **5** and **6** are endowed with nicotinic activity, even if they present different degrees

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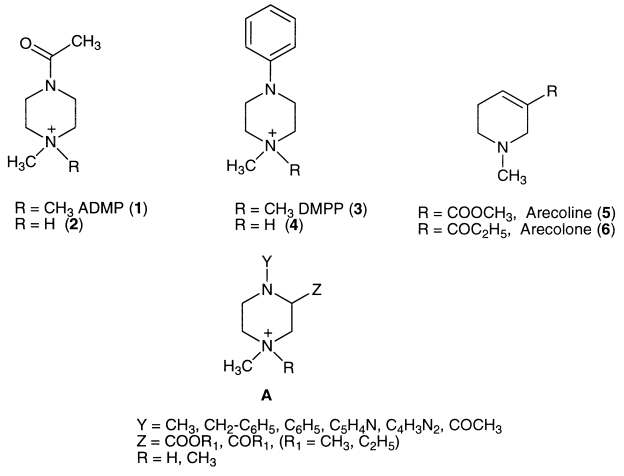



**Chart 1.**

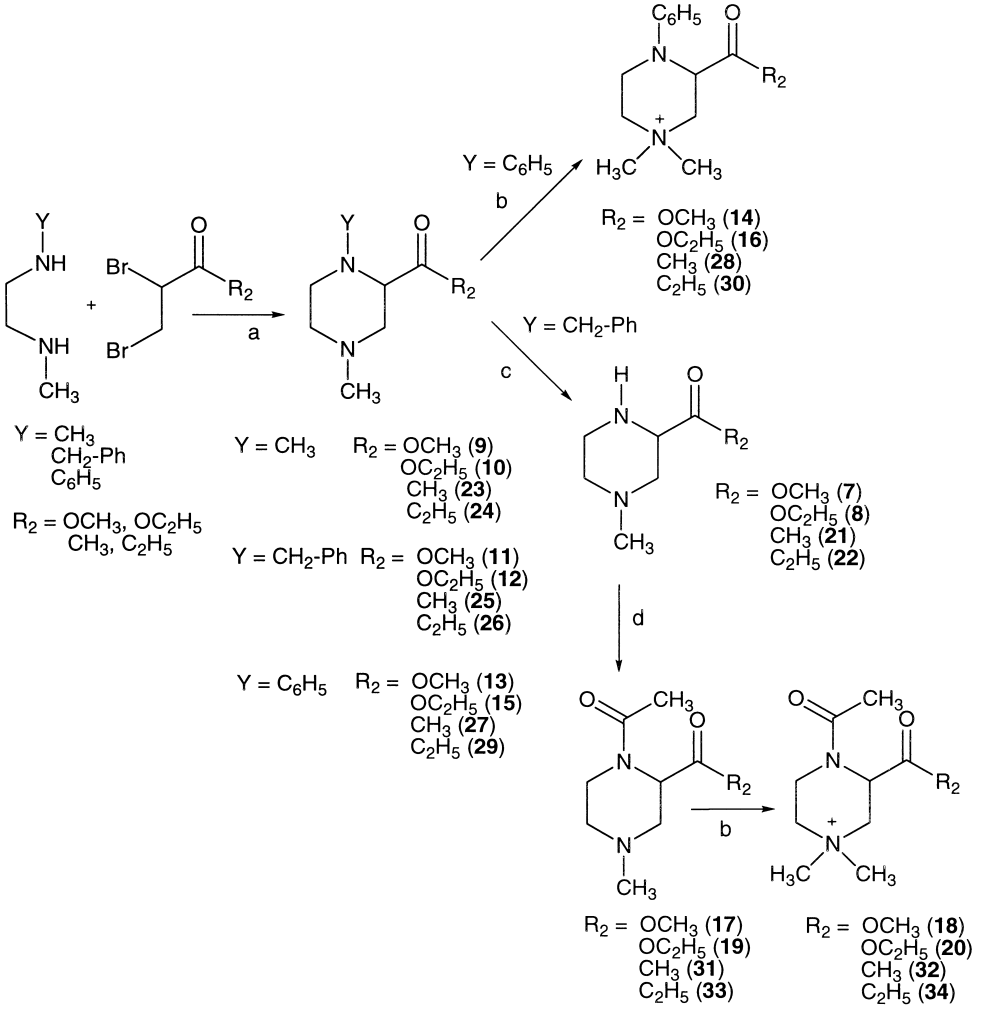
of potency and selectivity toward this kind of cholinergic receptor subtype.

Since the main objective of our work has been that of obtaining nicotinic agonists able to cross the blood–brain barrier (BBB) and therefore be useful as drug candidates for the treatment of neurodegenerative diseases, we have been especially interested in compounds lacking a permanent charge on the nitrogen atom. However, a second goal of the research has been to develop nicotinic agonists useful to characterize nicotinic receptors and possibly to draw up a model of interaction; for this reason we have also synthesized and studied the corresponding methiodide derivatives that are unable to cross the BBB, but are known to interact with high affinity with nicotinic receptors.

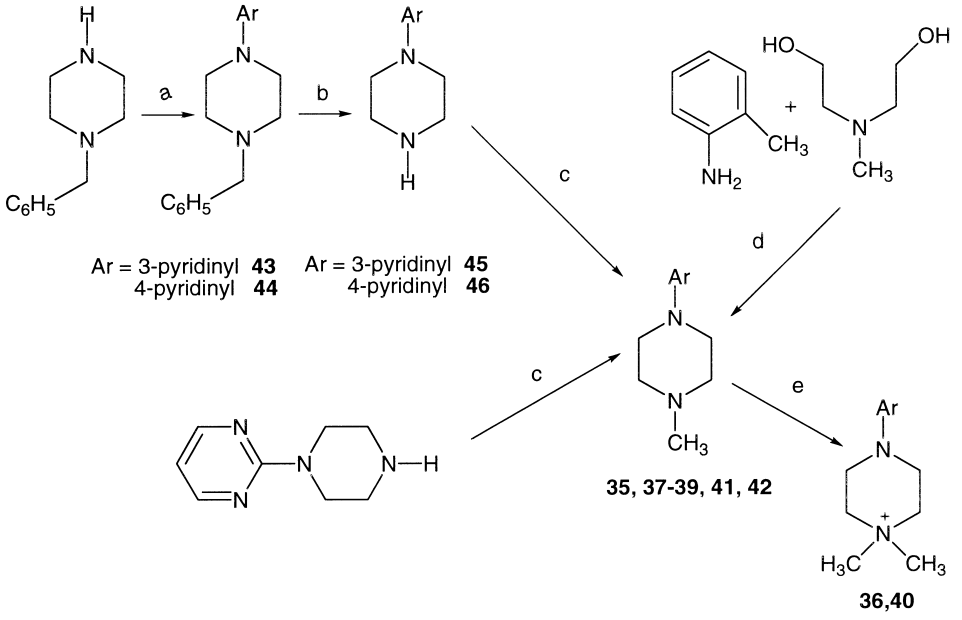
## Chemistry

Synthesis of the compounds designed involved simple chemistry and the use of standard methods. The synthetic pathways used are described in Schemes 1 and 2 and the compounds obtained are reported in Tables 1–3.

The starting material for the piperazino derivatives of Scheme 1 were the previously described *N,N′*-dimethylethylendiamine,[25] *N*-phenyl-*N*-methylethylendiamine[26] and *N*-benzyl-*N*-methylethylendiamine[27] that were condensed with the commercially available methyl and ethyl esters of 2,3-dibromopropionic acid, 3,4-dibromobutan-




**Scheme 1.** (a) Anhyd. benzene/$N(C_2H_5)_3$; (b) $CH_3I$; (c) $Pd/C/H_2$; (d) $CH_3COCl$.




**Scheme 2.** (a) 3- or 4-bromopyridine; (b) $Pd/C/H_2$; (c) $CH_2O/HCOOH$; (d) HCl, high temp. (e) $CH_3I$.

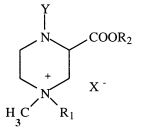
2-one[28] and 4,5-dibromopentan-3-one,[29] respectively. Among the compounds obtained, **9**,[30] **11**[31] and **12**[20,27] have already been reported. Only the series where the nitrogen 4 of piperazine is unequivocally involved in the reaction ($N^1$-phenyl and $N^1$-acetyl series) were treated with methyl iodide to give the quaternary salt. The disappointing pharmacological results obtained, discouraged any further investigation on the stereochemistry of the compounds which are all racemic mixtures.

In Scheme 2 the synthetic pathways used to obtain the compounds reported in Table 3 are reported; of them, compounds **35**,[32] **41**[33] and **42**[34,35] have already been described and **39** was obtained in very low yields. Even in this case, only unequivocally reacting compounds (**35** and **39**) were quaternized to give **36** and **40**.

## Pharmacology

The compounds obtained have been tested in vitro on rat brain homogenates to evaluate their affinities for the central nicotinic receptors labeled by [$^3$H]-cytisine ($2\alpha_4 3\beta_2$ subtype), which represents up >90% of the high-affinity agonist binding sites in rat brain[11,36] and

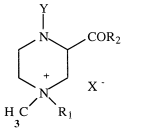
**Table 1.** Compounds obtained by hybridization of **1**, **2**, **3**, **4** and arecoline (**5**)



| N | Y | $R_1$ | $R_2$ | X | Mp °C | Analysis[a] [ref] |
|---|---|---|---|---|---|---|
| **7** | H | — | $CH_3$ | — | b | $C_7H_{14}N_2O_2$ |
| **8** | H | — | $C_2H_5$ | — | b | $C_8H_{16}N_2O_2$ |
| **9** | $CH_3$ | — | $CH_3$ | — | b | [30] |
| **10** | $CH_3$ | — | $C_2H_5$ | — | b | $C_9H_{18}N_2O_2$ |
| **11** | $CH_2C_6H_5$ | — | $CH_3$ | — | b | [31] |
| **12** | $CH_2C_6H_5$ | — | $C_2H_5$ | — | b | [20,27] |
| **13** | $C_6H_5$ | — | $CH_3$ | — | b | $C_{13}H_{18}N_2O_2$ |
| **14** | $C_6H_5$ | $CH_3$ | $CH_3$ | I | 140–142 | $C_{14}H_{21}IN_2O_2$ |
| **15** | $C_6H_5$ | — | $C_2H_5$ | — | b | $C_{14}H_{20}N_2O_2$ |
| **16** | $C_6H_5$ | $CH_3$ | $C_2H_5$ | I | 143–145 | $C_{15}H_{23}IN_2O_2$ |
| **17** | $COCH_3$ | — | $CH_3$ | — | b | $C_9H_{16}N_2O_3$ |
| **18** | $COCH_3$ | $CH_3$ | $CH_3$ | I | 190–192 | $C_{10}H_{19}IN_2O_3$ |
| **19** | $COCH_3$ | — | $C_2H_5$ | — | b | $C_{10}H_{18}N_2O_3$ |
| **20** | $COCH_3$ | $CH_3$ | $C_2H_5$ | I | 193–195 | $C_{11}H_{21}IN_2O_3$ |

[a]All compounds have been analyzed for C, H, N and the results obtained range within ±0.4 of the theoretical values.
[b]Oil (free base).

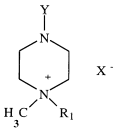
**Table 2.** Compounds obtained by hybridization of **1**, **2**, **3**, **4** and arecolone (**6**)



| N | Y | $R_1$ | $R_2$ | X | Mp °C | Analysis[a] |
|---|---|---|---|---|---|---|
| **21** | H | — | $CH_3$ | — | b | $C_7H_{14}N_2O$ |
| **22** | H | — | $C_2H_5$ | — | b | $C_8H_{16}N_2O$ |
| **23** | $CH_3$ | — | $CH_3$ | — | b | $C_8H_{16}N_2O$ |
| **24** | $CH_3$ | — | $C_2H_5$ | — | b | $C_9H_{18}N_2O$ |
| **25** | $CH_2C_6H_5$ | — | $CH_3$ | — | b | $C_{14}H_{20}N_2O$ |
| **26** | $CH_2C_6H_5$ | — | $C_2H_5$ | — | b | $C_{15}H_{22}N_2O$ |
| **27** | $C_6H_5$ | — | $CH_3$ | — | b | $C_{13}H_{18}N_2O$ |
| **28** | $C_6H_5$ | $CH_3$ | $CH_3$ | I | 135–137 | $C_{14}H_{21}IN_2O$ |
| **29** | $C_6H_5$ | — | $C_2H_5$ | — | b | $C_{14}H_{20}N_2O$ |
| **30** | $C_6H_5$ | $CH_3$ | $C_2H_5$ | I | 133–135 | $C_{15}H_{23}IN_2O$ |
| **31** | $COCH_3$ | — | $CH_3$ | — | b | $C_9H_{16}N_2O_2$ |
| **32** | $COCH_3$ | $CH_3$ | $CH_3$ | I | 180–182 | $C_{10}H_{19}IN_2O_2$ |
| **33** | $COCH_3$ | — | $C_2H_5$ | — | b | $C_{10}H_{18}N_2O_2$ |
| **34** | $COCH_3$ | $CH_3$ | $C_2H_5$ | I | 182–184 | $C_{11}H_{21}IN_2O_2$ |

[a]All compounds have been analyzed for C, H, N and the results obtained range within ±0.4 of the theoretical values.
[b]Oil (free base).

**Table 3.** Compounds obtained by isosteric replacement or substitution of the phenyl ring of **3** and **4**



| N | Y | $R_1$ | X | Mp °C | Analysis[a] [ref] |
|---|---|---|---|---|---|
| **35** | 2-pyridyl | — | — | b | [32] |
| **36** | 2-pyridyl | $CH_3$ | I | 202–204 | $C_{11}H_{18}IN_3$ |
| **37** | 3-pyridyl | — | — | b | $C_{10}H_{15}N_3$ |
| **38** | 4-pyridyl | — | — | b | $C_{10}H_{15}N_3$ |
| **39** | 2-$CH_3$-phenyl | — | — | b | $C_{12}H_{18}N_2$ |
| **40** | 2-$CH_3$-phenyl | $CH_3$ | I | 255–260 | $C_{13}H_{21}IN_2$ |
| **41** | 3-Cl-6-pyridazinyl | — | — | 113–115 | [33] |
| **42** | 2-pyrimidinyl | — | — | b | [34,35] |

[a]All compounds have been analyzed for C, H, N and the results obtained range within ±0.4 of the theoretical values.
[b]Oil (free base).

on guinea pig ileum (ganglionic nicotinic receptors)[37] to evaluate their activity on peripheral receptors. Moreover, since some nicotinic agonists show analgesic activity, which is peculiar of their central action and might be related to their cognitive properties,[2,38] those showing affinity for the central nicotinic receptors have also been tested as analgesics in vivo on mice, using the hot-plate test.[39] Finally, compounds showing both affinity for central nicotinic receptor and analgesic activity were also tested as cognition enhances in the mouse passive avoidance test.[40]

## Results

Molecular hybridization of compounds **1–6**, which gave origin to compounds **7–34** (Tables 1 and 2) completely abolished the nicotinic affinity of the parent compounds. As a matter of fact, these compounds did not show any affinity for central nicotinic receptors up to a concentration of 10 μM and were not investigated any further. Better results were obtained with the isosteric replacement of a carbon atom with a nitrogen in the phenyl ring of compounds **3** and **4** (Table 3).

Central nicotinic binding, peripheral nicotinic activity and analgesic activity of active compounds are reported in Table 4, compared with those of nicotine and of compounds **1–6**. In the evaluation of the results shown in Table 4, it must be considered that the affinity values for nicotine reported in the literature are fairly different[2,24,41,42] and, in our hands, DMPP appears to be somehow less potent for central nicotinic receptors than reported in the literature.[42]

Compounds **36–38** show affinity for the central nicotinic receptors labeled by cytisine in the nanomolar range. As a consequence, these compounds were tested also on ganglionic nicotinic receptors and evaluated in vivo as analgesics. Finally, compounds **37** and **38**, that are tertiary amines and present properties that would make them potential candidates for the treatment of neurodegenerative diseases, were tested also in the passive avoidance test. In Fig. 1, the effect of nicotine and compounds **37** and **38** on the passive avoidance test is reported.

## Discussion

As reported in the previous section, molecular hybridization of compounds **1–6**, to give compounds **7–34** (Tables 1 and 2), completely abolished the nicotinic affinity of the parent compounds: these compounds do not show any affinity for central nicotinic receptors up to a concentration of 10 μM. Apparently the introduction of the carboxylate function of arecoline (**5**) or of the ketonic function of arecolone (**6**) in position 3 of the

**Table 4.** Nicotinic and analgesic activity of compounds **1–6** and **35–38**

| N | Nicotinic activity | | Analgesic activity[c] | |
|---|---|---|---|---|
| | Binding[a] $K_i$ (μM) ±SE | G.p. ileum[b] $ED_{50}$ (μM) ±SE | MAD[d] (μg) | Efficacy[e] (%) |
| Nicotine | 0.0082[f] | 2.13 (0.15) | 0.7 | 71 |
| **1** (ADMP) | 1.07 (0.2) | 3.25 (0.04) | 2.5 | 77 |
| **2** | >100 | >100 | 50 | 69 |
| **3** (DMPP) | 0.25 (0.03)[g] | 3.43 (0.08) | 3 | 67 |
| **4** | >100 | — | — | — |
| **5** (Arecoline) | 0.224[h] | 0.3 (0.01) | 1 | 99 |
| **6** (Arecolone) | 0.0058[h] | n.d. | n.d. | n.d. |
| **35** | >100 | — | — | — |
| **36** | 0.50 (0.03) | 1.25 (0.13) | 100 | 51 |
| **37** | 0.09 (0.01) | 4.67 (0.11) | >300 | — |
| **38** | 0.17 (0.01) | >100 | 100 | 117 |

[a]Binding against [$^3$H]-cytisine on rat cerebral cortices.
[b]Contraction and decontraction of guinea pig ileum in presence of hexamethonium.
[c]Hot-plate test on mice.
[d]MAD = minimum analgesic dose when injected intracerebroventricularly (icv); analgesia was fully reversed by mecamylamine.
[e]Efficacy with respect to morphine (5 μg icv).
[f]See ref. 41; Ward,[24] Decker[2] and Boksa[42] report 8.40, 0.8 and 6.7 nM, respectively.
[g]Ref. 42 reports 0.057 μM.
[h]See ref. 24.
n.d. = not done.

piperazine ring of $N^1$-acetyl-$N^4$-dimethyl-piperazinium iodide (**1**, ADMP) and $N^1$-phenyl-$N^4$-dimethyl-piperazinium iodide (**3**, DMPP) severely affects nicotinic receptor affinity, both for tertiary bases and methiodide derivatives. The same happens when the substituent at nitrogen 1 of the piperazine ring is hydrogen, methyl or benzyl. Interestingly, hybridization has negative consequences also on the muscarinic affinity of arecoline, as compounds **7**, **9**, **11**, **13**, **17** do not show any affinity for muscarinic receptors up to a concentration of 10 μM. These results were quite unexpected but, though negative, could provide useful information for the development of a model of the central nicotinic receptor.

Among the isosteres of DMPP, compound **36** maintains affinity for central nicotinic receptors comparable to that of DMPP. Much like the tertiary base of the parent compound (**4**), the corresponding tertiary base (**35**) lacks affinity for the nicotinic receptor up to a concentration of 100 μM. On the contrary, **37** and **38**, which are tertiary bases, show relevant affinity for the central nicotinic receptors (90 and 170 nM, respectively).

However, the two compounds show different pharmacological profiles. Compound **37** is active on ganglionic nicotinic receptors ($ED_{50} = 4.67$ μM) and lacks analgesic activity; compound **38**, although slightly less potent, has no activity on neuronal ganglia but possess definite analgesic activity in mouse hot-plate test, with a minimum analgesic dose (MAD) of 100 μg, when injected intracerebroventricularly (icv) and higher efficacy (117%) with respect to morphine (5 μg, icv). The analgesic effect was fully reversed by mecamylamine.

Both **37** and **38**, at doses of 100 and 30 mg/kg, respectively, completely prevent the amnesic action of mecamylamine but not that of scopolamine in mouse passive avoidance test, when injected intraperitoneal (ip), thus showing that, as expected, they can cross the blood–brain barrier, they are able to produce the same effects as nicotine on central nicotinic receptors and they do not interact with the muscarinic ones (Fig. 1).

These results suggest that both **37** and **38** interact with the nicotinic receptor subtypes responsible for cognitive effects of nicotine, while only **38** is able to activate the so far unidentified central nicotinic subtype(s) mediating analgesic activity. Compounds that have high affinity for central nicotinic receptors, but do not show analgesic activity are known[43] and the most obvious explanation is that analgesic activity is mediated by nicotinic subtype(s) different from those which predominate in the CNS, like the $\alpha_4\beta_2$ and $\alpha_7$ subtypes, even if other explanations cannot be ruled out.[43]

Whatever the case, compounds **37** and **38** look very promising as leads for the development of new nicotinic ligands that can be useful to characterize central nicotinic receptors, develop models for nicotinic receptor binding sites and design drugs useful in the treatment of neurodegenerative diseases. In this respect, preliminary structure–activity relationship studies have shown that further isosteric substitution of the phenyl

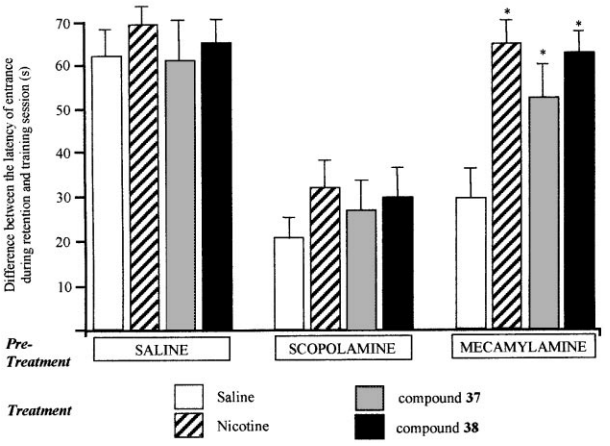



**Figure 1.** Prevention by compounds **37** (100 mg kg$^{-1}$ ip) and **38** (30 mg kg$^{-1}$ ip) of amnesia induced by scopolamine (3 mg kg$^{-1}$ ip) and mecamylamine (20mg kg$^{-1}$ip) in comparison with nicotine (1.5mg kg$^{-1}$ sc) in mouse passive-avoidance test. Compounds **37** and **38** and nicotine were injected 20 min before training session while scopolamine and mecamylamine were administered immediately after the training session. Each column represents the mean of at least 10 mice. $^*P < 0.01$ in comparison with mecamylamine-treated mice.

rings of **36–38** with a second nitrogen atom is detrimental for affinity (**41**, **42**), while introduction of a methyl group in position 2 of the phenyl ring of DMPP completely abolishes nicotinic activity (**39**, **40**). This result can be very informative toward the development of a model of interaction, studies which are in progress and about which we will refer in due time.

## Experimental

### Chemistry

All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin–Elmer 681 spectrophotometer in a Nujol mull for solids and neat for liquids. NMR spectra were recorded on a Gemini 200 spectrometer. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063–0.200 mm, Merck) or flash chromatography (Kieselgel 40, 0.040–0.063 mm, Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. Since the hydrochlorides of tertiary bases are often mixtures of mono- and bis-hydrochlorides (or bis- and tris-hydrochlorides) with variable amounts of water, which makes difficult to obtain good analytical data, final compounds have usually been analyzed as free bases, after column chromatography purification and accurate removal of the solvent.

**1-Methyl-4-phenylpiperazine hydrochloride (3).** Commercially available *N*-phenylpiperazine (0.2 g, 1.23 mmol) was dissolved in 5 mL of abs EtOH and 0.97 g of formic acid (21 mmol) and 0.47 g of formaldehyde (40% in $H_2O$, 6.2 mmol) were added to the solution. The solution was refluxed for 3 h, then evaporated, alkalinized

with $NaHCO_3$ and extracted with $CHCl_3$. Evaporation of the dried solution gave 0.2 g of an oil (93% yield) that was transformed into the hydrochloride with HCl/abs EtOH: mp 190–192 °C; $^1$H NMR (free base, $CDCl_3$) δ 2.35 (s, 3H, $CH_3N$), 2.55–2.60 (m, 4H, $CH_2$), 3.19–3.24 (m, 4H, $CH_2$), 6.85–6.95 (m, 3H, aromatics), 7.23–7.31 (m, 2H, aromatics) ppm; anal. ($C_{11}H_{16}N_2 \cdot 2HCl \cdot H_2O$) C, H, N, Cl.

**1,4-Dimethyl-2-carboxyethylpiperazine (10).** *N,N*-Dimethylethylendiamine[25] (5 mmol) and $NEt_3$ (9 mmol) dissolved in 20 mL of anhyd benzene were added to a solution of commercially available ethyl 2,3-dibromopropionate (5 mmol), dissolved in anhyd benzene (20 mL), kept at 40 °C. The solution was refluxed for 3 h and the solid formed was filtered after cooling. The benzene solution was then washed with $H_2O$, dried and evaporated at reduced pressure. The oily residue was purified by column chromatography, using $CHCl_3$/MeOH (9/1) as eluent: Yield 25%; IR (neat) ν 1720 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 1.27 (t, 3H, $\underline{CH_3}CH_2$); 2.35 (s, 3H, $CH_3N$); 2.36 (s, 3H, $CH_3N$); 2.38–2.53 (m, 3H, CHN); 2.72–3.01 (m, 3H, CHN); 3.06–3.18 (m, 1H, CHN); 4.22 (q, 2H, $\underline{CH_2}CH_3$); $^{13}$C NMR ($CDCl_3$) δ 16.11 (q), 45.45 (q), 47.79 (q), 55.35 (t), 56.45 (t), 59.36 (t), 62.69 (t), 68.17 (d), 173.44 (s) ppm; anal. ($C_9H_{18}N_2O_2$) C, H, N.

In the same way, starting from *N*-methyl-*N*-phenylethylendiamine[26] and the appropriate 2,3-dibromopropionate, the following compounds were obtained:

compound **13**; its hydrochloride was obtained treating the free base with HCl/abs EtOH: mp 75–77 °C from anhyd EtOH/anhyd ether. Analytical data indicate that the salt is the bis-hydrochloride with a small amount of mono-hydrochloride and water;

compound **15**; its hydrochloride was obtained treating the free base with HCl/abs EtOH: mp 74–76 °C from anhyd EtOH/anhyd ether. Analytical data indicate that the salt is the bis-hydrochloride with a small amount of mono-hydrochloride and water.

IR and $^1$H NMR spectra of **13** and **15** are consistent with the proposed structures and their chemical and physical characteristics are reported in Table 1.

**4-Methyl-2-carboxymethylpiperazine (7).** 1-Benzyl-4-methyl-2-carboxymethylpiperazine[31] (**11**, 0.28 g) was dissolved in abs EtOH (20 mL) and hydrogenated at a pressure of 55 psi in presence of 10% Pd/C for 18 h. Filtration and evaporation of the solvent gave an oil that was purified by column chromatography using $CHCl_3$/MeOH (9/1) as eluent: Yield 92%; IR (neat) ν 3200–3400 (NH), 1720 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.30 (s, 3H, $CH_3N$); 2.32 (bs, NH); 2.52–2.68 (m, 1H, CHN); 2.73–3.12 (m, 5H, CHN); 3.58 (dd, 1H, CHCO); 3.73 (s, 3H, $CH_3O$); $^{13}$C NMR ($CDCl_3$) δ 46.16 (t), 48.26 (q), 53.93 (q), 57.14 (t), 59.04 (t), 68.74 (d), 174.35 (s); anal. ($C_7H_{14}N_2O_2$) C, H, N.

In the same way, starting from 1-benzyl-4-methyl-2-carboxyethylpiperazine[20,27] (**12**), **8** was obtained, whose IR and $^1$H NMR spectra are consistent with the proposed structure. Its chemical and physical characteristics are reported in Table 1.

**1-Acetyl-4-methyl-2-carboxymethylpiperazine (17).** 0.316 g of **7** (2 mmol) were dissolved in $CHCl_3$ (5 mL), cooled at 0 °C and added to 0.28 mL of $NEt_3$ (2 mmol) and 0.17 mL of acetylchloride (2 mmol). After 2 h at 0 °C, 10 mL of $CHCl_3$ were added, the solution washed with $H_2O$, dried and evaporated to give an oil that was purified by column chromatography using $CHCl_3$/abs EtOH/petr. ether/$NH_4OH$ (600/225/90/30) as eluent: Yield 40%; IR (neat) ν 1680 (CON), 1720 (COO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.07 (s, 3H, $CH_3CO$), 2.19 (s, 3H, $CH_3N$), 2.21–2.30 (m, 1H, CHN), 2.58–2.85 (m, 2H, $CH_2$), 3.21–3.59 (m, 4H, $CH_2$), 3.66 (s, 3H, $CH_3N$); $^{13}$C NMR ($CDCl_3$) δ 23.30 (q), 45.72 (q), 48.36 (t), 54.16 (q), 56.41 (t), 57.65 (t), 59.15 (d), 172.66 (s), 172.71 (s) ppm; anal. ($C_9H_{16}N_2O_3$) C, H, N.

Similarly, starting from **8**, **19** was obtained, whose IR and $^1$H NMR spectra are consistent with the proposed structure. Its chemical and physical characteristics are reported in Table 1.

**1-Phenyl-4,4-dimethyl-2-carboxymethylpiperazinium iodide (14).** An excess of methyl iodide (2 mL) was added to a solution of **13** (1 mmol) in anhyd ether and the solution kept in the dark at room temperature overnight. The solid obtained was filtered and recrystallized from anhyd EtOH/anhyd ether: mp 140–142; Yield 90%; IR (nujol) ν 1720 (CO) cm$^{-1}$; $^1$H NMR ($D_2O$) δ 3.21 (s, 6H, $CH_3N$), 3.40–3.59 (m, 2H, $CH_2N$), 3.67 (s, 3H, $CH_3O$), 3.63–3.80 (m, 4H, $CH_2N$), 4.49–4.52 (m, 1H, CHCO), 6.83–6.87 (m, 2H, arom.), 7.20–7.29 (m, 3H, arom.); anal. ($C_{14}H_{21}IN_2O_2$) C, H, N.

In the same way, starting from **15**, **17** and **19**, **16**, **18** and **20** were obtained, respectively, whose IR and $^1$H NMR spectra are consistent with the proposed structure. Their chemical and physical characteristics are reported in Table 1.

**2-Acetyl-1,4-dimethylpiperazine (23).** Following the procedure described for **10**, starting from *N,N′*-dimethylethylendiamine[25] and 3,4-dibromo-2-butanone[28] compound **23** was obtained as an oil in 25% yield, after column chromatography purification ($CHCl_3$/MeOH, 9/1 as eluent): IR (neat) ν 1725 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 2.12–2.29 (m, 1H, CHN), 2.20 (s, 3H, $CH_3C$), 2.23 (s, 3H, $CH_3N$), 2.38 (s, 3H, $CH_3N$), 2.43–2.54 (m, 2H, CHN), 2.80–2.93 (m, 3H, CHN), 3.13 (dd, 1H, CHN); $^{13}$C NMR ($CDCl_3$) δ 28.93 (q), 45.62 (q), 47.86 (q), 56.05 (t), 56.45 (t), 58.35 (t), 75.57 (d), 210.68 (s); anal. ($C_8H_{16}N_2O$) C, H, N.

The following compounds were obtained in the same way:

compound **24**, starting from *N,N′*-dimethylethylendiamine and 4,5-dibromo-3-pentanone;[29]

compound **25**, starting from *N*-benzyl-*N′*-methylethylendiamine[27] and 3,4-dibromo-2-butanone;

compound **26**, starting from *N*-benzyl-*N*′-methylethylendiamine and 4,5-dibromo-3-pentanone;

compound **27**, starting from *N*-phenyl-*N*′-methylethylendiamine and 3,4-dibromo-2-butanone; its hydrochloride was obtained treating the free base with HCl/abs EtOH: mp 60–62 °C from anhyd EtOH/anhyd ether. Analytical data indicate that the salt is the bis-hydrochloride with a small amount of mono-hydrochloride and water.

Compound **29**, starting from *N*-phenyl-*N*′-methylethylendiamine and 4,5-dibromo-3-pentanone; its hydrochloride was obtained treating the free base with HCl/abs EtOH: mp 65–67 °C from anhyd EtOH/anhyd ether. Analytical data indicate that the salt is the bis-hydrochloride with a small amount of mono-hydrochloride and water.

IR and $^1$H NMR spectra of **24**, **25**, **26**, **27** and **29** are consistent with the proposed structure. Chemical and physical characteristics are reported in Table 2.

**4-Methyl-2-acetylpiperazine (21).** Following the procedure described for **7** and starting from **25**, the title compound was obtained as an oil in 98% yield: IR (neat) ν 3200–3350 (NH), 1720 (CO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 1.00 (d, 3H, $CH_3CO$), 1.83 (t, 1H, CHN), 2.02–2.15 (m, 1H, CHN), 2.10 (bs, NH), 2.24 (s, 3H, $CH_3N$), 2.37–2.69 (m, 2H, CHN), 2.73–2.86 (m, 2H, CHN), 3.03–3.12 (m, 1H, CHN); $^{13}$C NMR ($CDCl_3$) δ 23.59 (q), 34.50 (t), 45.30 (t), 47.61 (q), 53.40 (t), 72.70 (d), 210.22 (s); anal. ($C_7H_{14}N_2O$) C, H, N.

In the same way, starting from **26**, **22** was obtained whose IR and $^1$H NMR spectra are consistent with the proposed structure. Its chemical and physical characteristics are reported in Table 2.

**1,2-Diacetyl-4-methylpiperazine (31).** Following the procedure described for **17** and starting from **21**, the title compound was obtained as an oil in 14% yield: IR (neat) ν 1680 (CON), 1720 (COO) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 1.17 (d, 3H, $CH_3CO$), 2.05 (s, 3H, $CH_3$ CON), 2.22 (s, 3H, $CH_3N$), 2.75–2.96 (m, 2H, CH), 3.11–3.22 (m, 1H, CH), 3.46–3.77 (m, 2H, CH), 3.98–4.20 (m, 1H, CH), 4.31–4.47 (m, 1H, CH); $^{13}$C NMR ($CDCl_3$) δ 23.92 (q), 24.01 (q), 45.60 (q), 47.83 (t), 55.73 (t), 56.42 (t), 72.71 (d), 171.40 (s), 171.55 (s); anal. ($C_9H_{16}N_2O_2$) C, H, N.

Similarly, starting from **22**, **33** was obtained whose IR and $^1$H NMR spectra are consistent with the proposed structure. Its chemical and physical characteristics are reported in Table 2.

**1-Phenyl-4,4-dimethyl-2-acetylpiperazinium iodide (28).** Following the procedure described for **14** and starting from **27**, the title compound was obtained in 92% yield: mp 135–137 °C; IR (nujol) ν 1720 (CO) cm$^{-1}$; $^1$H NMR ($D_2O$) δ 2.06 (s, 3H, $CH_3CO$), 3.23 (s, 6H, $CH_3N$), 3.47–3.53 (m, 2H, $CH_2N$), 3.68–3.82 (m, 4H, $CH_2N$), 4.32–4.37 (m, 1H, CHCO), 6.83–6.90 (m, 3H, arom.), 7.21–7.29 (m, 2H, arom.); anal. ($C_{14}H_{21}IN_2O$) C, H, N.

The following compounds were obtained similarly: **30** (starting from **29**), **32** (starting from **31**) and **34** (starting from **33**). Their IR and $^1$H NMR spectra are consistent with the proposed structure. Chemical and physical characteristics are reported in Table 2.

**1-Benzyl-4-(3′-pyridinyl)-piperazine (43).** Commercially available 3-bromopyridine (1 g, 6 mmol), *N*-benzylpiperazine (4.5 g, 25 mmol) and $CuSO_4$ (0.130 g) were added to 5 mL of $H_2O$ and heated at 140 °C in a steel bomb for 24 h. Ether extraction of the cooled mixture gave an oily product that was purified by column chromatography (using $CHCl_3$/abs EtOH/petr. ether/$NH_4OH$, 340/65/60/8 as eluent) to give 0.55 g of the title compound with a 35% yield. The compound was used as such in the following reaction. $^1$H NMR ($CDCl_3$) δ 2.62–2.68 (m, 4H, $CH_2N$), 3.10–3.17 (m, 4H, $CH_2N$), 3.57 (s, 2H, $NCH_2Ph$), 7.12–7.15 (m, 2H, CH), 7.25–7.35 (m, 5H, arom), 8.08–8.12 (m, 1H, CH), 8.40–8.44 (m, 1H, CH).

In the same way, starting from 4-bromopyridine, compound **44** was obtained with a yield of 98%, whose $^1$H NMR spectrum is consistent with the proposed structure.

**1-(3′-Pyridinyl)-piperazine (45).** 0.25 g of Pd/C 10% and 0.62 g (10 mmol) of ammonium formiate were added to a solution of **43** (0.55 g, 2 mmol) in MeOH (15 mL) and refluxed for 3 h. The cooled reaction mixture was filtered, the solvent evaporated, and the residue alkalinized with $NaHCO_3$; $CHCl_3$ extraction gave 0.22 g of title compound with a 68% yield. The compound was used as such in the following reaction. $^1$H NMR ($CDCl_3$) δ 2.98–3.06 (m, 4H, $CH_2N$), 3.12–3.19 (m, 4H, $CH_2N$), 7.10–7.14 (m, 2H, CH), 8.02–8.12 (m, 1H, CH), 8.41–8.43 (m, 1H, CH).

In the same way, starting from **44**, compound **46** was obtained, with a yield of 67%, whose $^1$H NMR spectrum is consistent with the proposed structure.

**1-Methyl-4-(3′-pyridinyl)piperazine (37).** Following the procedure described for **3** and starting from **45**, the title compound was obtained as an oil in 46% yield after purification by column chromatography, using $CHCl_3$/abs EtOH/petr. ether/$NH_4OH$ (340/65/60/8) as eluent. $^1$H NMR ($CDCl_3$) δ 2.46 (s, 3H, $CH_3N$), 2.71–2.76 (m, 4H, $CH_2N$), 3.30–3.35 (m, 4H, $CH_2N$), 7.16–7.18 (m, 2H, CH), 8.11–8.12 (m, 1H, CH), 8.29–8.31 (m, 1H, CH); $^{13}$C NMR ($CDCl_3$) δ 48.12 (q), 50.22 (t), 56.70 (t), 124.29 (d), 125.45 (d), 140.31 (d), 142.42 (d), 148.84 (s); anal. ($C_{10}H_{15}N_3$) C, H, N.

The hydrochloride was obtained treating the free base with HCl/abs EtOH: mp >260 °C from anhyd EtOH/anhyd ether; anal. ($C_{10}H_{15}N_3 \cdot 2HCl \cdot H_2O$) C, H, N. Cl% calcd 26.44; found 27.02.

The following compounds were obtained in the same way:

compound **35**, with a yield of 79%, starting from the commercially available 1-(2′-pyridinyl)-piperazine; its

hydrochloride was obtained treating the free base with HCl/abs EtOH. mp 228–230 °C from anhyd EtOH/anhyd ether. Analytical data indicate that the salt is the bis-hydrochloride with a small amount of tris-hydrochloride and water;

compound **38**, with a yield of 81%, starting from **46**; its hydrochloride was obtained treating the free base with HCl/abs EtOH. mp 210–212 °C from anhyd EtOH/anhyd ether (hygroscopic). Anal. ($C_{10}H_{15}N_3 \cdot 2HCl \cdot H_2O$) C, H, N; Cl% calcd 26.44; found 26.92;

compound **42**, which has already been described.[34,35]

$^1$H NMR spectra of **35** and **38** are consistent with the proposed structures; their chemical and physical characteristics are reported in Table 3.

**1-(2′-Methyl-phenyl)-4-methylpiperazine (39).** *o*-Toluidine (4 g, 38 mmol) and *N*-methyl-bis-ethanolamine (4.16 g, 38 mmol) were mixed, cooled with ice, and neutralized with 6.6 mL of 37% HCl. The mixture was then heated at 220 °C for 16 h leaving $H_2O$ to distill off. The cooled mixture was alkalinized with 10% NaOH, extracted with $CHCl_3$ and the residue flash-chromatographed using pet. ether/$CH_2Cl_2$/diethyl ether/abs EtOH/$NH_4OH$ (900/360/360/180/9.9) as eluent, to give 0.2 g (3% yield) of the title compound. $^1$H NMR ($CDCl_3$) δ 2.30 (s, 3H, $CH_3C$), 2.36 (s, 3H, $CH_3N$), 2.56–2.62 (m, 4H, $CH_2N$), 2.92–2.97 (m, 4H, $CH_2N$), 6.97–7.05 (m, 2H, aromatics), 7.13–7.20 (m, 2H, aromatics); anal. ($C_{12}H_{18}N_2$) C, H, N.

The hydrochloride was obtained treating the free base with HCl/abs EtOH. Mp 252–255 °C from anhyd EtOH/anhyd ether. Analytical data indicate that the salt is the bis-hydrochloride with a small amount of mono-hydrochloride and water.

**1,1-Dimethyl-4-(2′-pyridinyl)piperazinium iodide (36).** Following the procedure described for **14**, the title compound was obtained starting from **35**, in 80% yield: mp 202–204 °C from anhyd EtOH/anhyd ether; $^1$H NMR ($CDCl_3$) δ 3.62 (s, 6H, $CH_3N$), 3.79–3.89 (m, 4H, $CH_2N$); 3.91–4.00 (m, 4H, $CH_2N$); 6.72–6.84 (m, 2H, CH pyr), 7.58–7.66 (m, 1H, CH pyr), 8.19–8.25 (m, 1H, CH pyr); anal. ($C_{11}H_{18}IN_3$) C, H, N.

In the same way, starting from **39**, compound **40** was obtained with a yield of 98%, whose $^1$H NMR spectrum is consistent with the proposed structure. Its chemical and physical characteristics are reported in Table 3.

## Pharmacology

**Binding studies.** Cerebral cortices of male Wistar rats (150–200 g) were dissected on ice. The tissue was homogenized in 50 mM Tris–HCl buffer (pH 7.4 at 2 °C) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$. The homogenate was centrifuged at 40,000 *g* for 10 min; the pellet was re-suspended in ice-cold buffer, recentrifuged and re-suspended again in buffer. Binding experiments[44] with [$^3$H]-cytisine (New England Nuclear, Boston, MA; 39.7 Ci/mmol) were performed in 250 μL of buffer which contained 2 nM [$^3$H]-cytisine, membranes from 15 mg (wet weight) of tissue and the compound to be tested. After 75 min of incubation at 2 °C, separation of bound from free radioligand was performed by rapid filtration through Whatman GF/C glass fiber filter, which were washed three times with ice-cold buffer, dried and counted in 5 mL of Aquassure (Packard, Downers Grove, USA). Binding in the presence of 10 mM (−)-nicotine bitartrate was defined unspecific and was, routinely, about 10% of the total binding. $K_i$ values were calculated from the Cheng–Prusoff equation[45] using 1.5 nM as the $K_d$ for [$^3$H]-cytisine, determined by previous saturation experiments.

**Functional studies.** 2 cm segments of the terminal portion of the guinea pig ileum were suspended, under 1 *g* tension, in PSS with the following composition (mM): NaCl (137), KCl (2.7), $CaCl_2$ (1.8), $MgCl_2$ (1.05), $NaH_2PO_4$ (0.42), $NaHCO_3$ (11.9), glucose (5.6). The solution was aerated with a gas mixture of 95% $O_2$, 5% $CO_2$ and maintained at 34 °C. Tension changes were recorded isotonically. After 1 h equilibration, the tissues were exposed to nicotine or to the drug to be tested. Concentration response curves were constructed non-cumulatively. Tissues were exposed to drugs for 30 s with at least 10 min rest between exposures. Effects of the antagonist hexamethonium (30 μM) were investigated after 30 min equilibration.

**Antinociceptive activity.** Analgesic activity was evaluated on mice with the hot-plate test[39] and the results are given as minimum analgesic dose (MAD) when injected intracerebroventricularly (icv) and efficacy (E%) with respect to the analgesic activity of 5 μg of morphine icv. More details on the protocols used can be found in previous publications.[46,47] The nicotinic origin of analgesia was checked by its reversal by mecamylamine.

**Antiamnesic test (passive-avoidance test).** The test was performed according to the step-through method described by Jarvik and Kopp,[40] as we modified it for testing drugs endowed with analgesic properties. The apparatus consists of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. In the original method, mice received a punishing electrical shock as soon as they entered the dark compartment, while in our modified method, after entry into the dark compartment, mice receive a non-painful punishment consisting of a fall into a cold water bath (10 °C). For this purpose, the dark chamber was constructed with a pitfall floor. The latency times for entering the dark compartment were measured in the training test and after 24 h in the retention test. For memory disruption, mice were ip injected with the amnesic drugs scopolamine or mecamylamine. All investigated drugs were injected 20 min before the training session, while scopolamine and mecamylamine were injected immediately after termination of the training session. The maximum entry latency allowed in the retention session was 120 s. The

memory of received punishment (fall into cold water) was expressed as the increase in seconds between training and retention latencies.

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