Blockade of MK-801-induced heat shock protein 72/73 in rat brain by antipsychotic and monoaminergic agents targeting D2, 5-HT$_{1A}$, 5-HT$_{2A}$ and α_1-adrenergic receptors

Tamara Romón,[1,2] Anna M. Planas[3] and Albert Adell[1,2*]

[1]*Department of Neurochemistry and Neuropharmacology, Instituto de Investigaciones Biomédicas de Barcelona, CSIC, IDIBAPS, Barcelona, Spain*

[2]*Centro de Investigación Biomédica en Red de Salud Mental (CIBERSAM)*

[3]*Department of Brain Ischemia and Neurodegeneration, Instituto de Investigaciones Biomédicas de Barcelona, CSIC, IDIBAPS, Barcelona, Spain*

*Corresponding author:

Dr. Albert Adell

Department of Neurochemistry and Neuropharmacology

Instituto de Investigaciones Biomédicas de Barcelona, CSIC, IDIBAPS

Carrer Rosselló 161, 6th floor, room 630

08036 Barcelona, Spain

Phone: +34-93-3638321

Fax: +34-93-3638301

E-mail: albert.adell@iibb.csic.es

Abstract

Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists can produce positive and negative symptomatology as well as impairment of cognitive function that closely resemble those present in schizophrenia. In rats, these drugs induce a behavioral syndrome (characterized by hyperlocomotion and stereotypies), an enhanced glutamatergic transmission in the medial prefrontal cortex, and damage to retrosplenial cortical neurons in adult rats, which was measured as the induction of the stress protein 72/73 kDa heat shock protein (Hsp72/73). In the present work we have examined the existence of possible differences among different antipsychotic drugs in their capacity to block immunolabeling of Hsp72/73 in the retrosplenial cortex of the rat induced by the potent NMDA receptor antagonist, MK-801. In addition, the effects of selective monoaminergic agents were also studied to delineate the particular receptors responsible for the actions of antipsychotic drugs. Pretreatment with clozapine, chlorpromazine, olanzapine, ziprasidone -and to a lesser extent haloperidol- reduced the formation of Hsp72/73 protein in the rat retrosplenial cortex after the administration of MK-801. In addition, antagonism at dopamine D2 (raclopride), 5-HT_{2A} (M100907) and α_1-adrenoceptors (prazosin) as well as agonism at 5-HT_{1A} receptors (BAY x 3702) also diminished the MK-801-induced number of cells labeled with Hsp72/73. Each of these effects may contribute to antipsychotic action. The results suggest that the efficacy of atypical antipsychotic drugs in the clinic may result from a combined effect on 5-HT_{2A}, 5-HT_{1A} and α_1-adrenergic receptors added to the classical dopamine D2 receptor antagonism.

Keywords:

MK-801; antipsychotic drugs; dopamine D2 receptor; 5-HT_{2A} receptor; 5-HT_{1A} receptor; α_1-adrenoceptor

INTRODUCTION

Noncompetitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as phencyclidine (PCP) and ketamine, can produce positive and negative symptomatology as well as impairment of cognitive function in humans that closely resemble those present in schizophrenia [1,2]. MK-801 is a noncompetitive NMDA receptor antagonist that produces hyperlocomotion and stereotypies in rodents [3,4], behaviors that are regarded as representative of the positive symptoms of schizophrenia [5,6]. In addition, noncompetitive NMDA receptor antagonists such as phencyclidine (PCP) and MK-801 also produce neuronal vacuolization in rats that was confined to layer III of retrosplenial cortex [7,8]. To explain the paradoxical effect of these agents, it was hypothesized that NMDA receptor antagonists would predominantly reduce GABAergic influence to pyramidal neurons in the retrosplenial cortex, thus activating major excitatory output that would ultimately lead to excitotoxicity induced by excessive release of glutamate [9]. This process of glutamatergic disinhibition has been recently confirmed in the prefrontal cortex [10]. As a likely response to the overexcitability of glutamatergic transmission, PCP, ketamine and MK-801 also trigger the synthesis of 70 KDa heat shock protein (Hsp70), which constitutes a reliable marker of reversible neuronal damage [11-14]. This Hsp70 response is also limited to putative pyramidal neurons localized to layer III of retrosplenial cortex [15]. It is possible that the same neurotoxic mechanism that caused vacuolization at earlier stages (4-12 hours) was also responsible for the later formation of Hsp72/73 (24-48 hours) after NMDA receptor blockade. The retrosplenial cortex corresponds to Brodmann's areas 26/29/30 and is heavily connected to orbital and dorsolateral prefrontal cortex. It is the cortical region most consistently activated by emotionally salient stimuli. In addition, abnormal activity of the retrosplenial cortex has been observed in neuropsychiatric diseases that are associated with impairment of emotional responses [16].

Although it is not known whether these neuropathological alterations evoked by NMDA receptor antagonists take place in the human brain, the observation that these agents recreate schizophrenia symptoms [1,2] led some

investigators to postulate that such changes may also occur in NMDA receptor antagonist-induced psychosis and perhaps in the schizophrenia brain [7,17].

Heat shock proteins are a group of highly conserved proteins that have a strong cytoprotective action and are inducible under different stressful conditions. As a matter of fact, Hsp72 is the most important inducible form of Hsp70 family. In previous studies we have shown that MK-801 enhanced glutamate release in the prefrontal cortex [18,19]. Thus, in the conditions of the present study, an enhanced Hsp72/73 synthesis would protect neurons from the neuronal injury caused by an excessive glutamate release after NMDA receptor blockade. We have also evidenced that classical and atypical antipsychotic drugs blocked the increase in glutamate efflux in the medial prefrontal cortex (mPFC) caused by MK-801, whereas only the latter class of drugs was able to additionally block the increased efflux of serotonin (5-HT) [18,20]. We proposed [19,20] that typical antipsychotics would prevent the MK-801-induced excessive glutamatergic transmission by increasing GABAergic transmission via a mechanism based on their D2 antagonism properties. On the other hand, it has been shown that atypical antipsychotic drugs may further act on 5-HT_{2A}, 5-HT_{1A} and α_1-adrenoceptors present in pyramidal cells (including those projecting to the dorsal raphe nucleus), which would inhibit an excessive activation of these cells.

The ability of NMDA receptor antagonists to induce Hsp72/73 and exert neurotoxic effects in the retrosplenial cortex has been known for some time, as has the ability of certain antipsychotic drugs to prevent such damage. However, the receptor mechanisms explaining the prevention of this toxicity by such drugs have not been examined. Therefore, the aim of the present work was to examine the existence of possible differences among various antipsychotic drugs in their capacity to block the formation of Hsp72/73 in the retrosplenial cortex of the rat induced by MK-801. In addition, the effects of selective monoaminergic agents were also studied to delineate the particular receptors implicated in the action of antipsychotic drugs. Haloperidol and chlorpromazine were used as typical antipsychotics. Clozapine, olanzapine and ziprasidone were used as atypical antipsychotics. The selective monoaminergic agents

used were raclopride (dopamine D2-like receptor antagonist), M100907 (5-HT_{2A} receptor antagonist), BAY x 3702 (5-HT_{1A} receptor agonist) and prazosin (α_1-adrenoceptor antagonist). The doses of these compounds were selected according to previous work in the literature [21] and taking into consideration the in vivo occupancy of the monoamine receptors studied.

MATERIALS AND METHODS

Animals

Fifty two male Wistar rats (Charles River Laboratories, Spain) weighing 300 ± 15 g were used (four animals in each group). They were kept on a 12 h light/dark cycle (lights on at 07:00) and housed three per cage with food and water available *ad libitum*. All experimental procedures were carried out in strict accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Institutional Animal Care and Use Committees.

Drug treatments

Dizocilpine maleate (MK-801), chlorpromazine hydrochloride, and M100907 were purchased from Sigma-Aldrich (Tres Cantos, Spain). Clozapine, olanzapine, haloperidol, ziprasidone hydrochloride, raclopride, and prazosin hydrochloride were from Tocris (Bristol, UK). BAY x 3702 was generously donated by Bayer AG (Wuppertal, Germany). MK-801, prazosin and BAY x 3702 were dissolved in saline. All other drugs were dissolved in a few drops of glacial acetic acid and then diluted with saline, which was used as vehicle for systemic administration. When it was necessary, the pH of the final concentrations was adjusted to ~7.0 with $NaHCO_3$. All antipsychotic drugs and monoaminergic agents (or vehicle) were administered subcutaneously 1 h before MK-801 that was injected intraperitoneally. Control rats received vehicle followed 1 h later by saline. The dose of MK-801 (1 mg/kg) was chosen

because it produced maximal Hsp72/73 immunoreactivity when measured 24 h later [11].

Hsp72/73 immunohistochemistry

All rats were deeply anesthetized with 60 mg/kg sodium pentobarbital 24 h after the injection of MK-801 or saline, and perfused transcardially with cold heparinized saline followed by 4% paraformaldehyde in 0.1 M pH = 7.4 phosphate buffered saline (PBS, 100 ml/100 g body weight). Each brain was removed immediately, post-fixed in the same fixative for 24 h at 4 °C, and then coronal sections of 30 μm were cut in a vibratome. Three sequential sections for each rat were collected free floating. Immunohistochemistry was performed according to Planas et al. [22]. Briefly, sections were incubated free floating in PBS including 0.2% Triton X-100, 0.2% gelatin and 3% horse serum for 2 h at room temperature followed by overnight incubation with mouse monoclonal anti-Hsp72/73 antibody (Cat No HSP01, Clone W27, Calbiochem, CA, USA) diluted 1:500 in PBS containing 0.2% Triton X-100, 0.2% gelatin and 1% horse serum. Hsp72 is the most prominent inducible form of Hsp70 family. Sections were then incubated in rat absorbed biotinylated horse anti-mouse IgG antibody (Vector Laboratories, CA, USA) diluted 1:200 for 2 h, followed by the avidin-biotin complex at a dilution of 1:100 for 1 h (ABC kit Vectastain®, Vector Laboratories). The antibody reaction was carried out with 0.05% diaminobenzidine in 0.01% hydrogen peroxide. Several sections were incubated in the absence of the primary antibody as a false positive immunoreaction control. Sections were mounted onto gelatin-coated slides with Entellan®.

Cell counting and statistical analysis

The total number of Hsp72/73 positive cells was counted bilaterally in the entire retrosplenial cortex of the sections, using an Olympus BX51 Stereo Microscope (Tokio, Japan). Cell counting was performed blind to treatment,

using Visiopharm Integrator System software (Visiopharm A/S, Denmark). Measurements were performed in triplicate and the values are expressed as mean ± SEM of four animals per group. Statistical differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by post hoc Newman–Keuls multiple comparison tests. Statistical calculations were made using GraphPad Prism software (La Jolla, USA). The level of significance was set at $p < 0.05$.

RESULTS

Representative photomicrographs of the effects of antipsychotic drugs and monoaminergic agents on the MK-801-induced formation of Hsp72/73 protein in the retrosplenial cortex are depicted in figures 1 and 3, respectively. A thorough analysis of brain tissue sections showed that, under the conditions of the present study, no Hsp72/73 immunolabeling was detected after MK-801 in any other brain area (data not shown). Control rats that were not given MK-801 did not show any cell positive for Hsp72/73 (Fig. 1C).

Effects of antipsychotic drugs

The doses of antipsychotic drugs were chosen from an excellent previous work in the literature that showed a clinically relevant occupancy of several monoaminergic receptors [21]. This occupancy data is depicted in Table 1. The administration of antipsychotic drugs effectively blocked the formation of Hsp72/73 protein induced by MK-801 (Fig. 2), as demonstrated by the significant main effect of treatment ($F_{6,28} = 39.01$, $p < 0.0001$). Newman-Keuls post-hoc tests revealed that the effect of haloperidol was significantly lower than that of the rest of antipsychotic drugs ($p < 0.001$). It is of note that no single cell positive for Hsp72/73 was found after MK-801 in rats pretreated with clozapine, olanzapine or ziprasidone.

Effects of monoaminergic agents

The administration of raclopride (1 mg/kg), M100907 (0.3 mg/kg), BAY x 3702 (0.1 mg/kg), and prazosin (0.3 mg/kg) effectively blocked the formation of Hsp72/73 protein induced by MK-801 (Fig. 4), as demonstrated by the significant main effect of treatment ($F_{5,25}$ = 44.50, p < 0.0001). Newman-Keuls post-hoc tests revealed no significant differences in the suppression of MK-801-induced accumulation of Hsp72/73 protein among the four agents tested (p > 0.05).

DISCUSSION

In previous work, we demonstrated that the systemic administration of MK-801 produced an enhanced efflux of glutamate in the prefrontal cortex [18], which is likely the result of an increase in the firing rate of prefrontal, pyramidal neurons [23]. This excessive glutamatergic transmission is responsible for the induction of the cytoprotective Hsp70 family proteins [7]. However, MK-801-induced immunolabeling of Hsp70 is restricted to retrosplenial cortex as observed earlier [7,15,17]. Interestingly, MK-801 also caused a pronounced neuronal vacuolization and elevation of glucose utilization in the same brain area [7,24,25]. However, this excitotoxic cell damage is transient at doses of MK-801 ranging from 0.6 to 2.5 mg/kg and irreversible necrotic changes are only observed after 5 mg/kg of MK-801 [26]. In the prefrontal cortex, induction of Hsp70 mRNA after PCP is detected only following amplification of the signal using reverse transcription-polymerase chain reaction [27]. It is possible that such a low level of Hsp70 induction, which is not sufficient for immunohistochemical protein detection, was related to the reported higher vulnerability of prefrontal cortex to neuronal injury [28,29]. Nonetheless, it has to be noticed that the retrosplenial cortex is a main hub of the so-called default mode network [30], the dysfunction of which may relate to impairments in attention and working memory seen in schizophrenia [31]. There are also connections of the retrosplenial cortex with prefrontal cortex, which may influence executive function. In fact, increased activity [32,33], and grey matter

volume deficits [34] in the retrosplenial cortex have been observed in schizophrenia.

From a pharmacological point of view, the main finding of the present study is that systemic administration of typical and atypical antipsychotics was able to reduce the neuronal injury (assessed by the expression of Hsp72/73) evoked by the NMDA receptor antagonist, MK-801. Previous work has shown that different antipsychotic drugs prevented the formation of Hsp70 induced by NMDA receptor antagonists (see below). However, to the best of our knowledge, this is the first study that compares the suppression of MK-801-induced synthesis of Hsp72/73 produced by different antipsychotic drugs and selective monoaminergic agents acting at receptors for which such antipsychotic drugs exhibit affinity. It is of note that only the atypical antipsychotic drugs and chlorpromazine (see below) were able to totally abolish the appearance of Hsp72/73 immunoreactive cells in the retrosplenial cortex following MK-801. These results are in line with previous work using similar concentrations of the atypical drugs [21], and other studies using higher dosages [35] in female rats. In addition, clozapine and olanzapine also show superior efficacy than haloperidol in preventing MK-801-induced neurotoxicity measured as the number of vacuolized neurons [36-38]. In the present study, haloperidol reduced the number of Hsp72/73 positive cells, but to a lesser extent than the atypical antipsychotics tested, which also agrees with the observations reported above. Similar to our results, a reduced effect of haloperidol (compared to atypical antipsychotic drugs) in the expression of Hsp72/73 after PCP has been observed previously [39]. The effect of haloperidol is likely attributable to its blockade of dopamine D2-like receptors because a similar effect was obtained with a dose of raclopride that has a comparable occupancy of such receptors [40]. The case of chlorpromazine merits a comment since, although it is considered a typical antipsychotic with a characteristic high dopamine D2-like antagonistic activity, it also blocks a substantial proportion of 5-HT_{2A} receptors at the dose administered in the present study [41]. Thus, it is conceivable that the effect of chlorpromazine resembled that of atypical antipsychotics drugs due to its blockade of 5-HT_{2A}

receptors. Given that the doses of clozapine, olanzapine and ziprasidone appear to occupy a similar number of dopamine D2-like receptors in the brain, their superior efficacy in preventing MK-801-induced formation of Hsp72/73 protein must likely be accounted for by actions at other monoaminergic receptors. Clearly, their comparable occupancy of 5-HT_{2A} receptors, similar to that of M100907 [42,43], underscores the importance of 5-HT_{2A} receptor blockade in these effects. BAY x 3702 is a potent 5-HT_{1A} receptor agonist, which is purported to have a 100% occupancy at the dose used [44]. It effectively reduced the number of Hsp72/73-labeled neurons induced by MK-801. Among the atypical antipsychotics tested, only clozapine and ziprasidone possess 5-HT_{1A} agonistic properties [45]. However, in the present conditions the occupancy of 5-HT_{1A} receptors by ziprasidone is less than 5% [46]. Consequently, an action on 5-HT_{1A} receptors can be only attributable to clozapine. In a similar way, prazosin, which is a very potent antagonist that has a ~60% occupancy of α_1-adrenoceptors in the present conditions [47], almost fully suppress the number of Hsp72/73 positive cells elicited by the administration of MK-801. Haloperidol, chlorpromazine, and clozapine have nanomolar affinity for α_1-adrenoceptors [48,49]. However, in the experimental conditions of the present study, the protective effect of α_1-adrenoceptor antagonism can only be attributed to clozapine (there is no data available on chlorpromazine) because it blocks a similar amount of α_1-adrenoceptors than prazosin [47]. Interestingly, the blockade of α_1-adrenoceptors by the antagonist prazosin potentiated the antipsychotic-like effect of the dopamine D2 receptor antagonist, raclopride, [50]. Finally, the action of the drugs and agents described herein can be easily explained by the presence of 5-HT_{2A} [51], 5-HT_{1A} [52], dopamine D2 [53], and α_1-adrenergic receptors [54] in the retrosplenial cortex.

In summary, we have shown that antagonism at dopamine D2, 5-HT_{2A} and α_1-adrenoceptors as well as agonism at 5-HT_{1A} receptors seem to be crucial for conferring protection against the neurotoxicity of MK-801. Our results indicate that similar to clozapine, pretreatment with chlorpromazine, olanzapine, ziprasidone -and to a lesser extent haloperidol- protects against the formation of

Hsp72/73 protein in the rat retrosplenial cortex after the administration of MK-801. The suppression of the expression of Hsp72/73 induced by MK-801 may be implicated in the ability of antipsychotic drugs to prevent similar changes that may occur in the schizophrenia brain. It is possible that the efficacy of antipsychotic drugs in the clinic may result from a combined effect on dopamine D2, 5-HT_{2A}, 5-HT_{1A} and α_1-adrenergic receptors.

ABBREVIATIONS

ANOVA = Analysis of Variance

GABA = ɣ-Aminobutyric acid

Hsp = Heat shock protein

5-HT = Serotonin

IgG = Immunoglobulin G

MK-801 = Dizocilpine

mPFC = Medial prefrontal cortex

NMDA = *N*-methyl-D-aspartate

PBS = Phosphate-buffered saline

PCP = Phencyclidine

CONFLICT OF INTEREST

The authors declare that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by the Instituto de Salud Carlos III, Subdirección General de Evaluación y Fomento de la Investigación (FIS Grants PI070111 and PI1001103), Centro de Investigación Biomédica en Red de Salud Mental, CIBERSAM, and by the Innovative Medicine Initiative Joint Undertaking under grant agreement no. 115008, of which resources are composed of EFPIA in-kind contribution and financial contribution from the European Union's Seventh Framework Programme (FP7/2007-2013). Funding by the Generalitat de Catalunya (2009SGR220) and co-funding by the European Regional Development Fund (a way to build Europe) is also acknowledged. Thanks are given to Bayer (Wuppertal, Germany) for the generous gift of BAY x 3702.

REFERENCES

[1] Javitt, D.C.; Zukin, S.R. Recent advances in the phencyclidine model of schizophrenia. *Am. J. Psychiatry*, **1991,** *148,* 1301-1308.

[2] Krystal, J.H.; Karper, L.P.; Seibyl, J.P.; Freeman, G.K.; Delaney, R.; Bremner, J.D.; Heninger, G.R.; Bowers, M.B., Jr; Charney, D.S. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. *Arch. Gen. Psychiatry*, **1994,** *51,* 199-214.

[3] Homayoun, H.; Stefani, M.R.; Adams, B.W.; Tamagan, G.D.; Moghaddam, B. Functional interaction between NMDA and mGlu5 receptors: effects on working memory, instrumental learning, motor behaviors, and dopamine release. *Neuropsychopharmacology*, **2004,** *29*, 1259-1269.

[4] López-Gil, X.; Jiménez-Sánchez, L.; Romón, T.; Campa, L.; Artigas, F.; Adell, A. Importance of inter-hemispheric prefrontal connection in the effects of non-competitive NMDA receptor antagonists. *Int. J. Neuropsychopharmacol.*, **2012,** *15*, 945-956.

[5] Sams-Dodd, F. Phencyclidine-induced stereotyped behaviour and social isolation in rats: a possible animal model of schizophrenia. *Behav. Pharmacol.*, **1996,** *7*, 3-23.

[6] Lipska, B.K.; Weinberger, D.R. Animal models of schizophrenia; In: *Schizophrenia*; Hirsch, S.R.; Weinberger, D.R., Eds.; Blackwell Science: Malden, **2003**; pp. 388–402.

[7] Olney, J.W.; Labruyere, J.; Wang, G.; Wozniak, D.F.; Price, M.T.; Sesma, M.A. NMDA antagonist neurotoxicity: mechanism and prevention. *Science*, **1991,** *254*, 1515-1518.

[8] Okamura, N.; Reinscheid, R.K.; Ohgake, S.; Iyo, M.; Hashimoto, K. Neuropeptide S attenuates neuropathological, neurochemical and behavioral changes induced by the NMDA receptor antagonist MK-801. *Neuropharmacology*, **2010,** *58,* 166-172.

[9] Olney, J.W.; Farber, N.B. Glutamate receptor dysfunction and schizophrenia. *Arch. Gen. Psychiatry*, **1995,** *52,* 998-1007.

[10] Homayoun, H.; Moghaddam, B. NMDA receptor hypofunction produces opposite effects on prefrontal cortex interneurons and pyramidal neurons. *J. Neurosci.*, **2007,** *27,* 11496-11500.

[11] Sharp, F.R.; Jasper, P.; Hall, J.; Noble, L.; Sagar, S.M. MK-801 and ketamine induce heat shock protein HSP72 in injured neurons in posterior cingulate and retrosplenial cortex. *Ann. Neurol.*, **1991,** *30,* 801-809.

[12] Sharp, F.R.; Butman, M.; Koistinaho, J.; Aardalen, K.; Näkki, R.; Massa, S.M.; Swanson, R.A.; Sagar, S.M. Phencyclidine induction of the Hsp 70 stress gene in injured pyramidal neurons is mediated via multiple receptors and voltage gated calcium channels. *Neuroscience*, **1994,** *62,* 1079-1092.

[13] Hashimoto, K.; Tomitaka, S.; Bi, Y.; Narita, N.; Minabe, Y.; Iyo, M. Rolipram, a selective phosphodiesterase type-IV inhibitor, prevents induction of heat shock protein HSP-70 and Hsp-70 mRNA in rat retrosplenial cortex by the NMDA receptor antagonist dizocilpine. *Eur. J. Neurosci.*, **1997,** *9,* 1891-1901.

[14] O'Neill, M.F.; Hicks, C.A.; Shaw, G.; Parameswaran, T.; Cardwell, G.P.; O'Neill, M.J. Effects of 5-hydroxytryptamine$_2$ receptor antagonism on the behavioral activation and immediate early gene expression induced by dizocilpine. *J. Pharmacol. Exp. Ther.*, **1998,** *287,* 839-846.

[15] Tomitaka, S.; Tomitaka, M.; Tolliver, B.K.; Sharp, F.R. Bilateral blockade of NMDA receptors in anterior thalamus by dizocilpine (MK-801) injures pyramidal neurons in rat retrosplenial cortex. *Eur. J. Neurosci.*, **2000,** *12,* 1420-1430.

[16] Maddock, R.J. The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain. *Trends Neurosci.*, **1999,** 310-316.

[17] Sharp, F.R.; Tomitaka, M.; Bernaudin, M.; Tomitaka, S. Psychosis: pathological activation of limbic thalamocortical circuits by psychomimetics and schizophrenia? *Trends Neurosci.*, **2001,** *24,* 330-334.

[18] López-Gil, X.; Babot, Z.; Amargós-Bosch, M.; Suñol, C.; Artigas, F.; Adell, A. Clozapine and haloperidol differently suppress the MK-801-increased glutamatergic and serotonergic transmission in the medial prefrontal cortex of the rat. *Neuropsychopharmacology*, **2007,** *32,* 2087-2097.

[19] López-Gil, X.; Artigas, F.; Adell, A. Role of different monoamine receptors controlling MK-801-induced release of serotonin and glutamate in the medial prefrontal cortex: relevance for antipsychotic action. *Int. J. Neuropsychopharmacol.*, **2009,** *12,* 487-499.

[20] López-Gil, X.; Artigas, F.; Adell, A. Unraveling monoamine receptors involved in the action of typical and atypical antipsychotics on glutamatergic and serotonergic transmission in prefrontal cortex. *Curr. Pharm. Des.*, **2010,** *16,* 502-515.

[21] Dickerson, J.; Sharp, F.R. Atypical antipsychotics and a Src kinase inhibitor (PP1) prevent cortical injury produced by the psychomimetic, noncompetitive NMDA receptor antagonist MK-801. *Neuropsychopharmacology*, **2006,** *31,* 1420-1430.

[22] Planas, A.M.; Soriano, M.A.; Ferrer, I., Rodriguez-Farré, E. Regional expression of inducible heat shock protein-70 mRNA in the rat brain following administration of convulsant drugs. *Brain Res. Mol. Brain Res.*, **1994,** *27,* 127-137.

[23] Jackson, M.E.; Homayoun, H.; Moghaddam, B. NMDA receptor hypofunction produces concomitant firing rate potentiation and burst activity reduction in the prefrontal cortex. *Proc. Natl. Acad. Sci. USA*, **2004,** *101,* 8467-8472.

[24] Nehls, D.G.; Kurumaji, A.; Park, C.K.; McCulloch, J. Differential effects of competitive and non-competitive N-methyl-D-aspartate antagonists on glucose use in the limbic system. *Neurosci. Lett.*, **1988,** *91,* 204-210.

[25] Piercey, M.F.; Hoffmann, W.E.; Kaczkofsky, P. Functional evidence for PCP-like effects of the anti-stroke candidate MK-801. *Psychopharmacology*, **1988,** *96,* 561-562.

[26] Allen, H.L.; Iversen, L.L. Phencyclidine, dizocilpine, and cerebrocortical neurons. *Science*, **1990,** *247,* 221.

[27] García-Osta, A.; Frechilla, D.; Del Rio, J. Reduced basal and phencyclidine-induced expression of heat shock protein-70 in rat prefrontal cortex by the atypical antipsychotic abaperidone. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, **2003,** *27,* 31-36.

[28] Arnsten, A.F. Stress signalling pathways that impair prefrontal cortex structure and function. *Nat. Rev. Neurosci.*, **2009,** *10,* 410-422.

[29] Holmes, A.; Wellman, C.L. Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neurosci. Biobehav. Rev.*, **2009,** *33,* 773-783.

[30] Vann, S.D.; Aggleton, J.P.; Maguire, E.A. What does the retrosplenial cortex do? *Nat. Rev. Neurosci.*, **2009,** *10,* 792-802.

[31] Bluhm, R.L.; Miller, J.; Lanius, R.A.; Osuch, E.A.; Boksman, K.; Neufeld, R.W.; Théberge, J.; Schaefer, B.; Williamson, P.C. Retrosplenial cortex connectivity in schizophrenia. *Psychiatry Res.*, **2009,** *174,* 17-23.

[32] Andreasen, N.C.; O'Leary, D.S.; Flaum, M.; Nopoulos, P.; Watkins, G.L.; Boles Ponto, L.L.; Hichwa, R.D. Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naive patients. *Lancet*, **1997,** *349,* 1730-1734.

[33] Haznedar, M.M.; Buchsbaum, M.S.; Luu, C.; Hazlett, E.A.; Siegel, B.V., Jr; Lohr, J.; Wu, J.; Haier, R.J.; Bunney, W.E. Jr. Decreased anterior

cingulate gyrus metabolic rate in schizophrenia. *Am. J. Psychiatry*, **1997,** *154,* 682-684.

[34] Mitelman, S.A.; Shihabuddin, L.; Brickman, A.M.; Hazlett, E.A.; Buchsbaum, M.S. Volume of the cingulate and outcome in schizophrenia. *Schizophr. Res.*, **2005,** *72,* 91-108.

[35] Okamura, N.; Hashimoto, K.; Kanahara, N.; Shimizu, E.; Kumakiri, C.; Komatsu, N.; Iyo, M. Protective effect of the antipsychotic drug zotepine on dizocilpine-induced neuropathological changes in rat retrosplenial cortex. *Eur. J. Pharmacol.*, **2003,** *461,* 93-98.

[36] Farber, N.B.; Price, M.T.; Labruyere, J.; Nemnich, J., St. Peter, H.; Wozniak, D.F.; Olney, J.W. Antipsychotic drugs block phencyclidine receptor-mediated neurotoxicity. *Biol. Psychiatry*, **1993,** *34,* 119-121.

[37] Farber, N.B.; Foster, J.; Duhan, N.L.; Olney, J.W. Olanzapine and fluperlapine mimic clozapine in preventing MK-801 neurotoxicity. *Schizophr. Res.*, **1996,** *21:*33-37.

[38] Fujimura, M.; Hashimoto, K.; Yamagami, K. Effects of antipsychotic drugs on neurotoxicity, expression of fos-like protein and c-*fos* mRNA in the retrosplenial cortex after administration of dizocilpine. *Eur. J. Pharmacol.*, **2000,** *398,* 1-10.

[39] Nakahara, T.; Kuroki, T.; Hondo, H.; Tsutsumi, T.; Fukuda, K.; Yao, H.; Uchimura, H. Effects of atypical antipsychotic drugs vs. haloperidol on expression of heat shock protein in the discrete brain regions of phencyclidine-treated rats. Brain Res. Mol. Brain Res., **1999,** *73,* 193-197.

[40] Assié, M.B.; Dominguez, H.; Consul-Denjean, N.; Newman-Tancredi, A. In vivo occupancy of dopamine D_2 receptors by antipsychotic drugs and novel compounds in the mouse striatum and olfactory tubercles. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **2006,** *373,* 441-450.

[41] Kusumi, I.; Takahashi, Y.; Suzuki, K., Kameda, K.; Koyama, T. Differential effects of subchronic treatments with atypical antipsychotic drugs on dopamine D_2 and serotonin 5-HT_{2A} receptors in the rat brain. *J. Neural Transm.*, **2000,** *107,* 295-302.

[42] Watabe, H.; Channing, M.A.; Der, M.G.; Adams, H.R.; Jagoda, E.; Herscovitch, P.; Eckelman, W.C.; Carson, R.E. Kinetic analysis of the 5-HT_{2A} ligand [^{11}C]MDL 100,907. *J. Cereb. Blood Flow Metab.*, **2000,** *20,* 899-909.

[43] Hirani, E.; Sharp, T., Sprakes, M.; Grasby, P.; Hume, S. Fenfluramine evokes 5-HT_{2A} receptor-mediated responses but does not displace [^{11}C]MDL 100907: small animal PET and gene expression studies. *Synapse*, **2003,** *50,* 251-260.

[44] De Vry, J.; Schohe-Loop, R.; Heine, H.G.; Greuel, J.M.; Mauler, F.; Schmidt, B.; Sommermeyer, H.; Glaser, T. Characterization of the aminomethylchroman derivative BAY x 3702 as a highly potent 5-hydroxytryptamine$_{1A}$ receptor agonist. *J. Pharmacol. Exp. Ther.*, **1998,** *284,* 1082-1094.

[45] Newman-Tancredi, A.; Assié, M.B.; Leduc, N.; Ormière, A.M.; Danty, N.; Cosi, C. Novel antipsychotics activate recombinant human and native rat serotonin 5-HT1A receptors: affinity, efficacy and potential implications for treatment of schizophrenia. *Int. J. Neuropsychopharmacol.*, **2005,** *8,* 341-356.

[46] Bantick, R.A.; Rabiner, E.A.; Hirani, E.; de Vries, M.H.; Hume, S.P.; Grasby, P.M. Occupancy of agonist drugs at the 5-HT_{1A} receptor. *Neuropsychopharmacology*, **2004,** *29,* 847-859.

[47] Patel, S.; Fernandez-Garcia, E.; Hutson, P.H.; Patel, S. An in vivo binding assay to determine central α_1-adrenoceptor occupancy using [^{3}H]prazosin. *Brain Res. Brain Res. Protoc.*, **2001,** *8,* 191-198.

[48] Bymaster, F.P.; Calligaro, D.O.; Falcone, J.F.; Marsh, R.D.; Moore, N.A.; Tye, N.C.; Seeman, P.; Wong, D.T. Radioreceptor binding profile of the atypical antipsychotic olanzapine. *Neuropsychopharmacology*, **1996,** *14,* 87-96.

[49] Arnt, J.; Skarsfeldt, T. Do novel antipsychotics have similar pharmacological characteristics? A review of the evidence. *Neuropsychopharmacology*, **1998,** *18,* 63-101.

[50] Wadenberg, M.L.; Hertel, P.; Fernholm, R.; Hygge Blakeman, K.; Ahlenius, S.; Svensson, T.H. Enhancement of antipsychotic-like effects

by combined treatment with the antagonist α_1-adrenoceptor prazosin and the dopamine D_2 receptor antagonist raclopride in rats. *J. Neural Transm.*, **2000,** *107,* 1229-1238.

[51] Cornea-Hébert, V.; Riad, M.; Wu, C.; Singh, S.K.; Descarries, L. Cellular and subcellular distribution of the serotonin 5-HT2A receptor in the central nervous system of adult rat. *J. Comp. Neurol.*, **1999,** *409,* 187-209.

[52] Pompeiano, M.; Palacios, J.M.; Mengod, G. Distribution and cellular localization of mRNA coding for 5-HT_{1A} receptor in the rat brain: correlation with receptor binding. *J. Neurosci.*, **1992,** *12,* 440-453.

[53] Khan, Z.U.; Gutiérrez, A.; Martín, R.; Peñafiel, A.; Rivera, A.; De La Calle, A. Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. *J. Comp. Neurol.*, **1998,** *402,* 353-371.

[54] Palacios, J.M.; Hoyer, D.; Cortés, R. α_1-Adrenoceptors in the mammalian brain: similar pharmacology but different distribution in rodents and primates. *Brain Res.*, **1987,** *419,* 65-75.

[55] Schotte, A.; Janssen, P.F.; Megens, A.A.; Leysen, J.E. Occupancy of central neurotransmitter receptors by risperidone, clozapine and haloperidol, measured ex vivo by quantitative autoradiography. *Brain Res.*, **1993,** *631,* 191-202.

[56] Chou, Y.H.; Halldin, C.; Farde, L. Occupancy of 5-HT_{1A} receptors by clozapine in the primate brain: a PET study. *Psychopharmacology (Berl.)*, **2003,** *166,* 234-240.

[57] Matsui-Sakata, A.; Ohtani, H.; Sawada, Y. Pharmacokinetic-pharmacodynamic analysis of antipsychotics-induced extrapyramidal symptoms based on receptor occupancy theory incorporating endogenous dopamine release. *Drug Metab. Pharmacokinet.*, **2005,** *20,* 187-199.

[58] Mamo, D.; Kapur, S.; Shammi, C.M.; Papatheodorou, G.; Mann, S.; Therrien, F.; Remington, G. A PET study of dopamine D_2 and serotonin 5-HT_2 receptor occupancy in patients with schizophrenia treated with therapeutic doses of ziprasidone. *Am. J. Psychiatry*, **2004,** *161,* 818-825.

[59] Schotte, A.; Janssen, P.F.; Gommeren, W.; Luyten, W.H.; Van Gompel, P.; Lesage, A.S.; De Loore, K.; Leysen, J.E. Risperidone compared with new and reference antipsychotic drugs: in vitro and in vivo receptor binding. *Psychopharmacology (Berl.)*, **1996,** *124,* 57-73.

Table 1. Percentage of monoamine receptor occupancy of the different antipsychotic drugs at the doses used in the present study.

Antipsychotic Drug (dose)	**Dopamine D2**	**5-HT$_{2A}$**	**5-HT$_{1A}$**	**α_1-adrenergic**
Clozapine (5 mg/kg)	20-30% [40,55]	70% [55]	40% [55,56]	60% [47]
Olanzapine (5 mg/kg)	50-60% [40,41]	60-80% [41,57]	NA	NA
Ziprasidone (10 mg/kg)	50% [40]	80% [58]	Negligible [46]	Negligible [59]
Haloperidol (0.1 mg/kg)	50% [40,55]	<10% [55]	Negligible [55]	<10% [47,55]
Chlorpromazine (5 mg/kg)	40% [41]	40% [41]	NA	NA

Data taken or estimated from references given between parentheses.

NA, data not available.

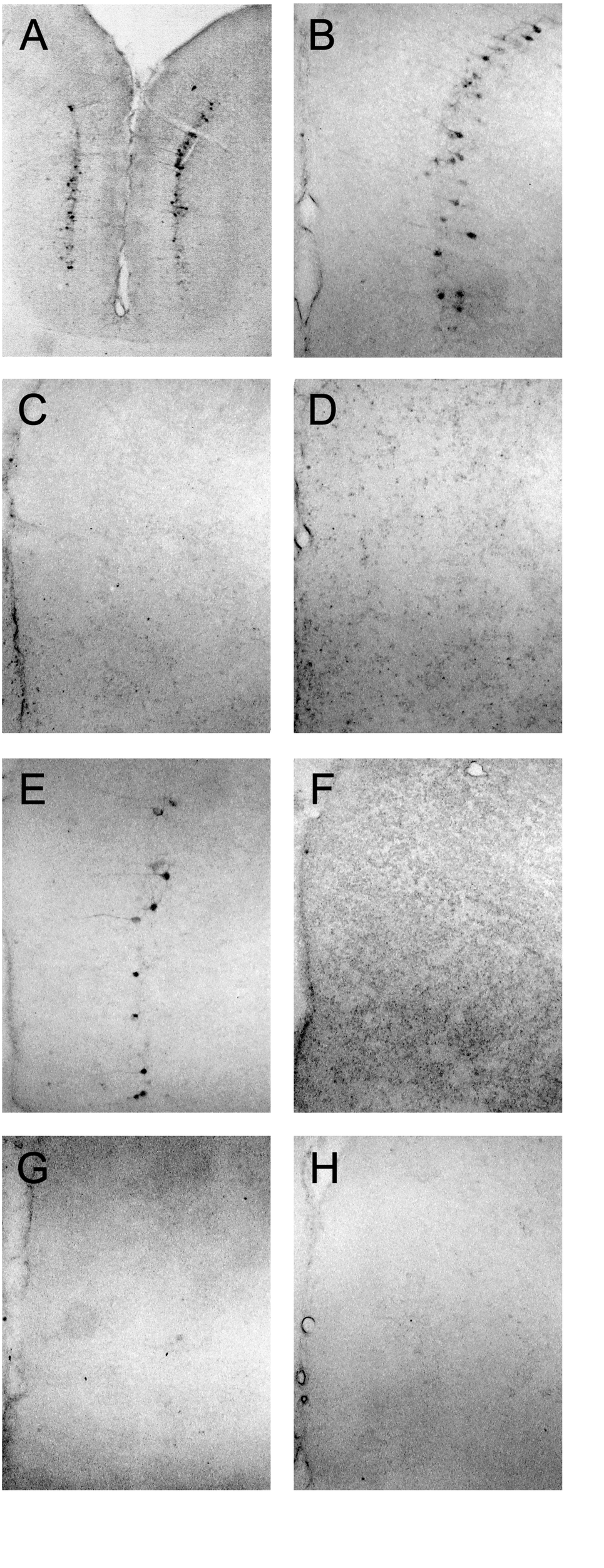
FIGURE LEGENDS

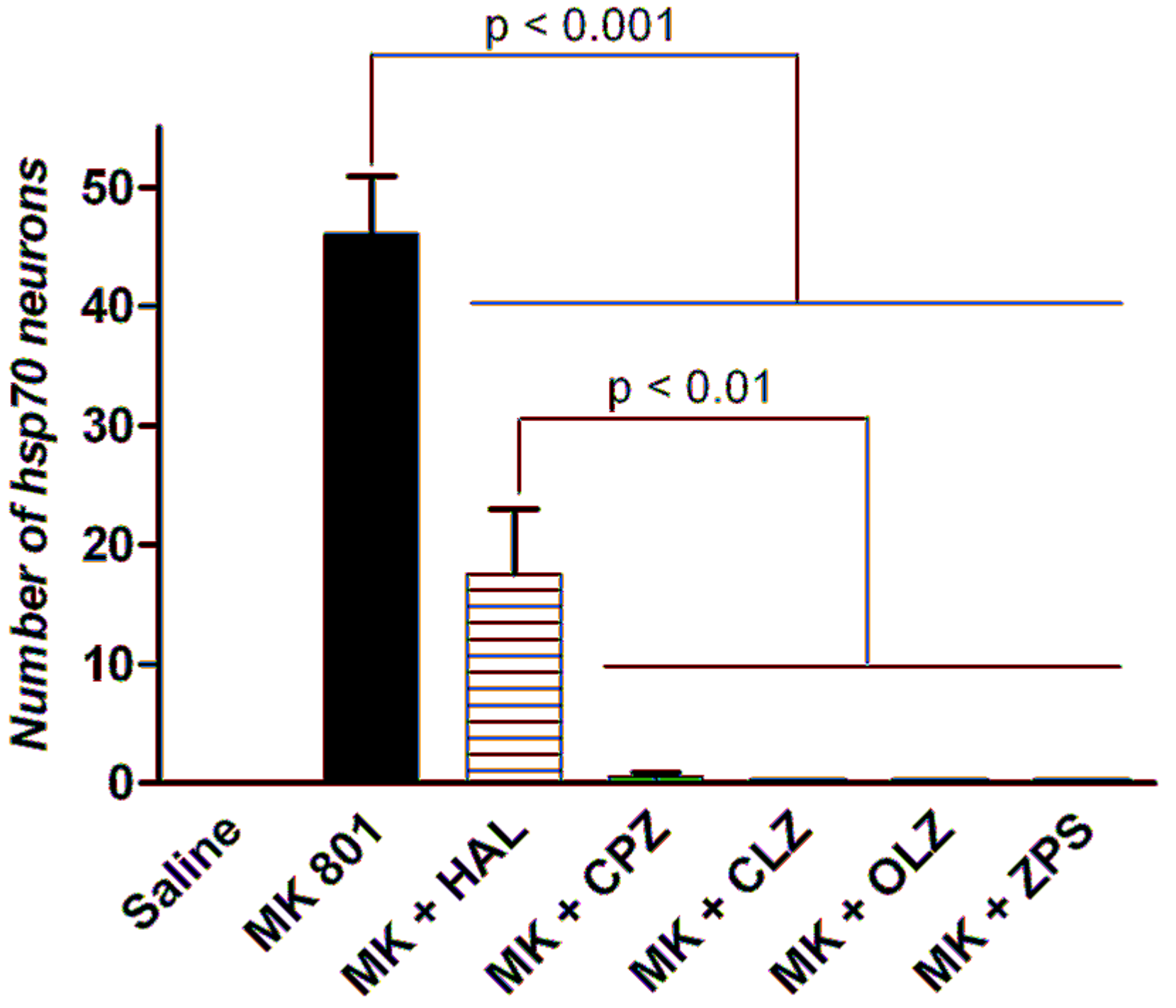
Figure 1: Representative photomicrographs of the immunolabeling of Hsp72/73 induced by MK-801 in the layer III of the retrosplenial cortex of a male rat (A, B) and its blockade by the systemic administration of clozapine (D), haloperidol (E), olanzapine (F), chlorpromazine (G) and ziprasidone (H). The injection of vehicle (C) did not cause induction of Hsp72. Scale bars, 100 µm.

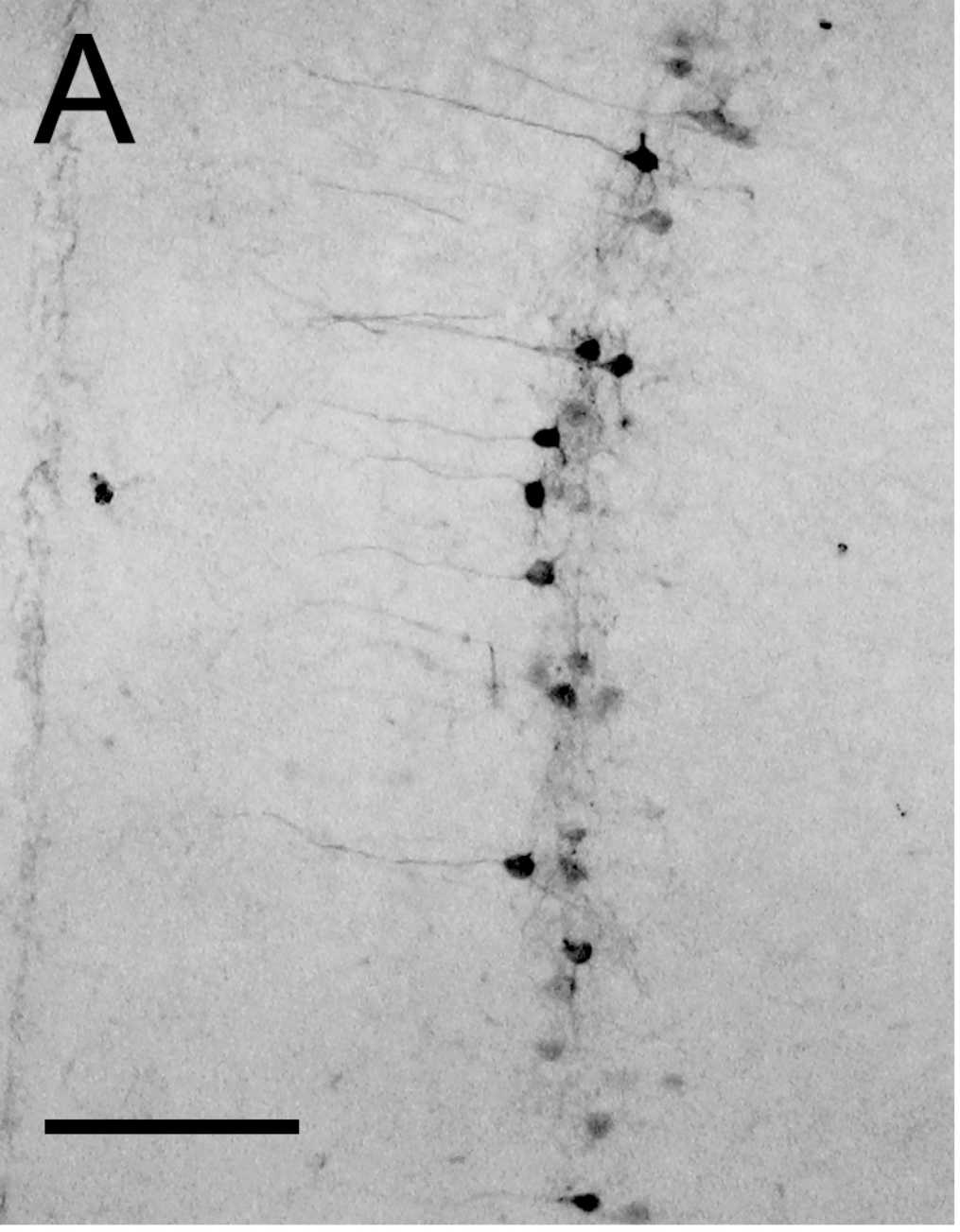
Figure 2: Blockade of the MK-801-induced number of Hsp72/73 cells by pretreatment with 0.1 mg/kg haloperidol (HAL), 5 mg/kg chlorpromazine (CPZ), 5 mg/kg clozapine (CLZ), 5 mg/kg olanzapine (OLZ), and 10 mg/kg ziprasidone (ZPS). Data expressed as mean ± SEM of four animals per group measured in triplicate. Statistical differences assessed by Newman-Keuls tests.

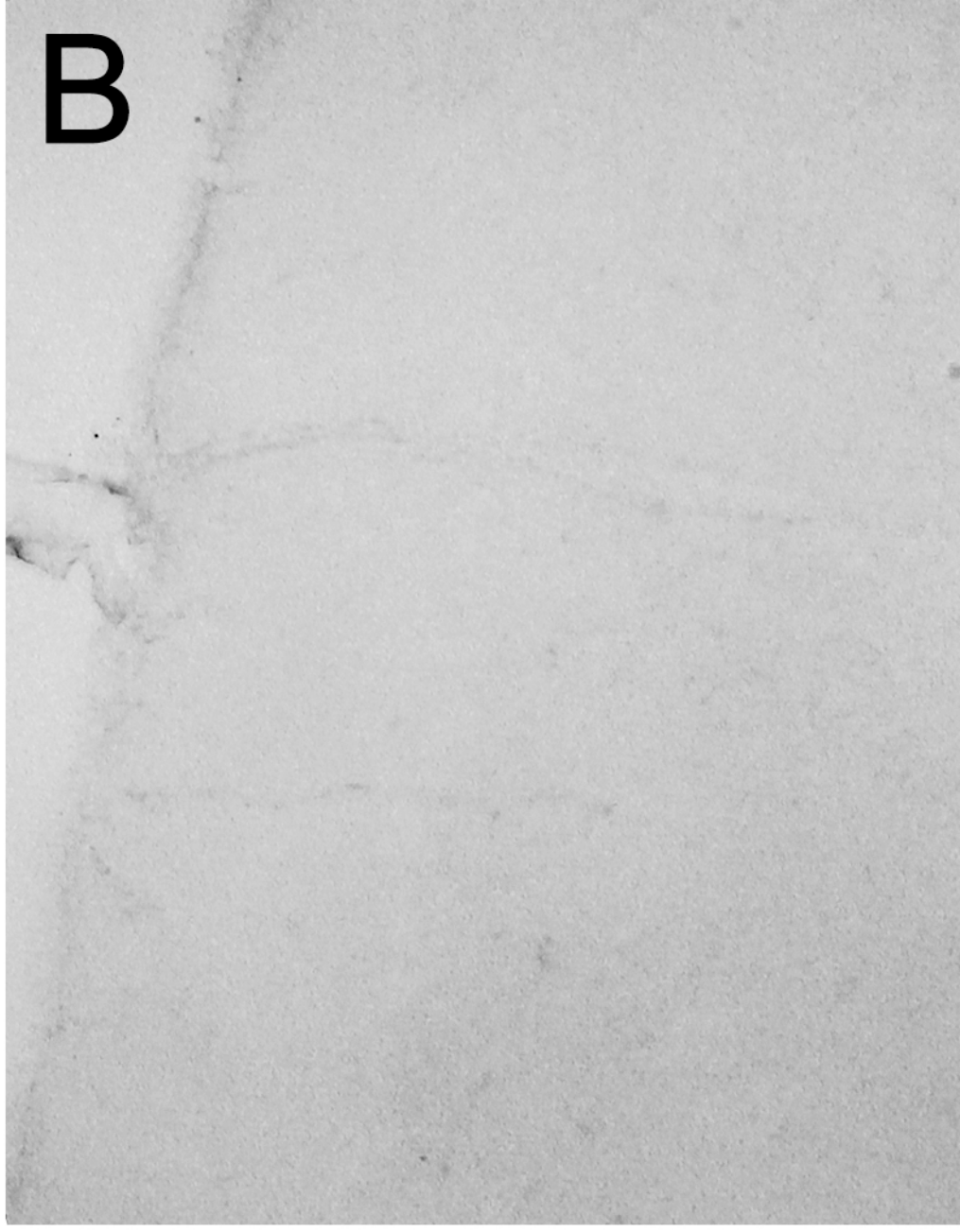
Figure 3: Representative photomicrographs of the immunolabeling of Hsp72/73 induced by MK-801 in the layer III of the retrosplenial cortex of a male rat (A) and its blockade by the systemic administration of raclopride (C), M100907 (D), BAY x 3702 (E), and prazosin (F). The injection of vehicle (B) did not cause induction of Hsp72. Scale bar, 100 µm.

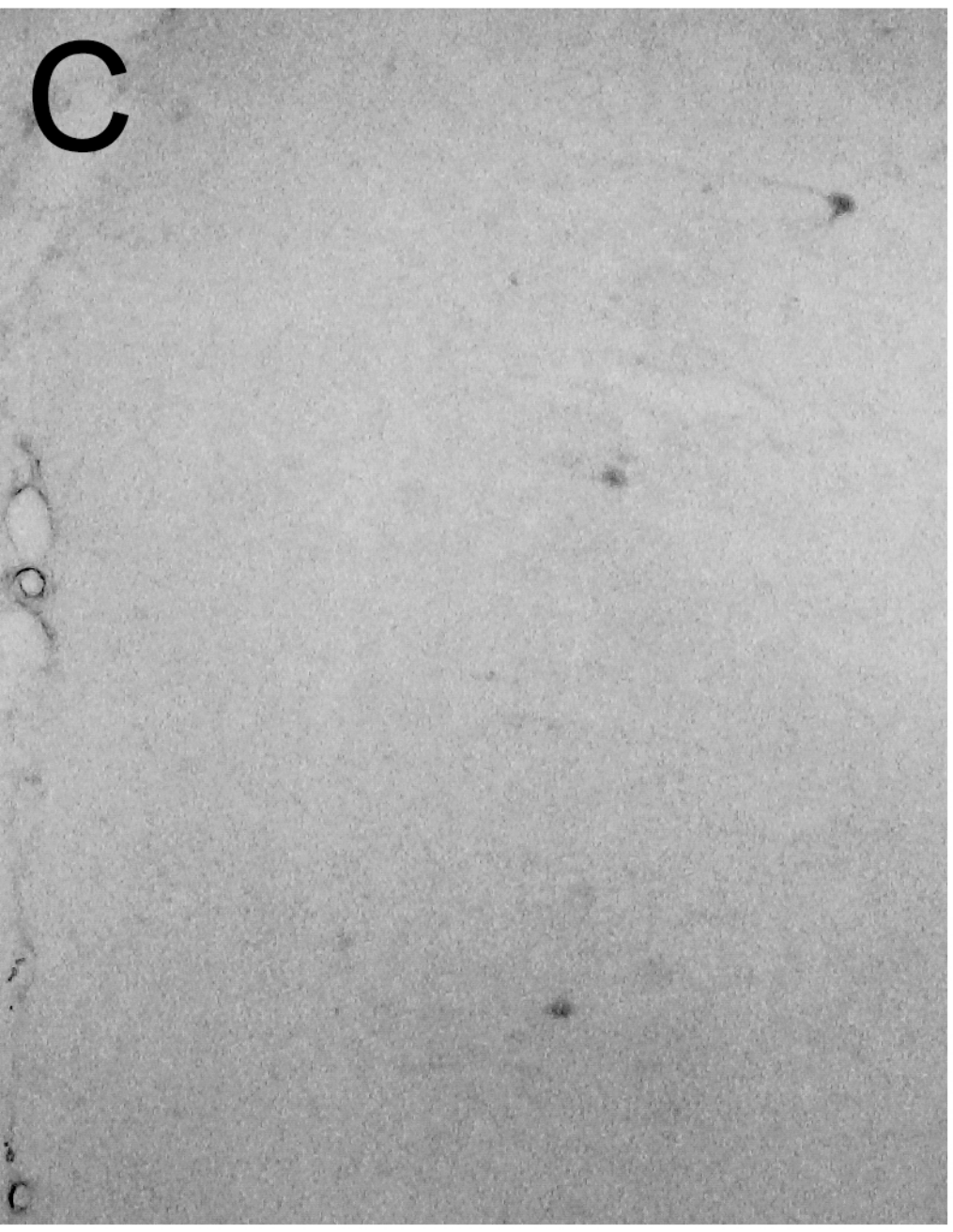
Figure 4: Blockade of the MK-801-induced number of Hsp72/73 cells by pretreatment with 1 mg/kg raclopride (RAC), 0.3 mg/kg M100907 (M100), 0.1 mg/kg BAY x 3702 (BAY), and 0.3 mg/kg prazosin (PRZ). Data expressed as mean ± SEM of four animals per group measured in triplicate. Statistical difference assessed by Newman-Keuls test.

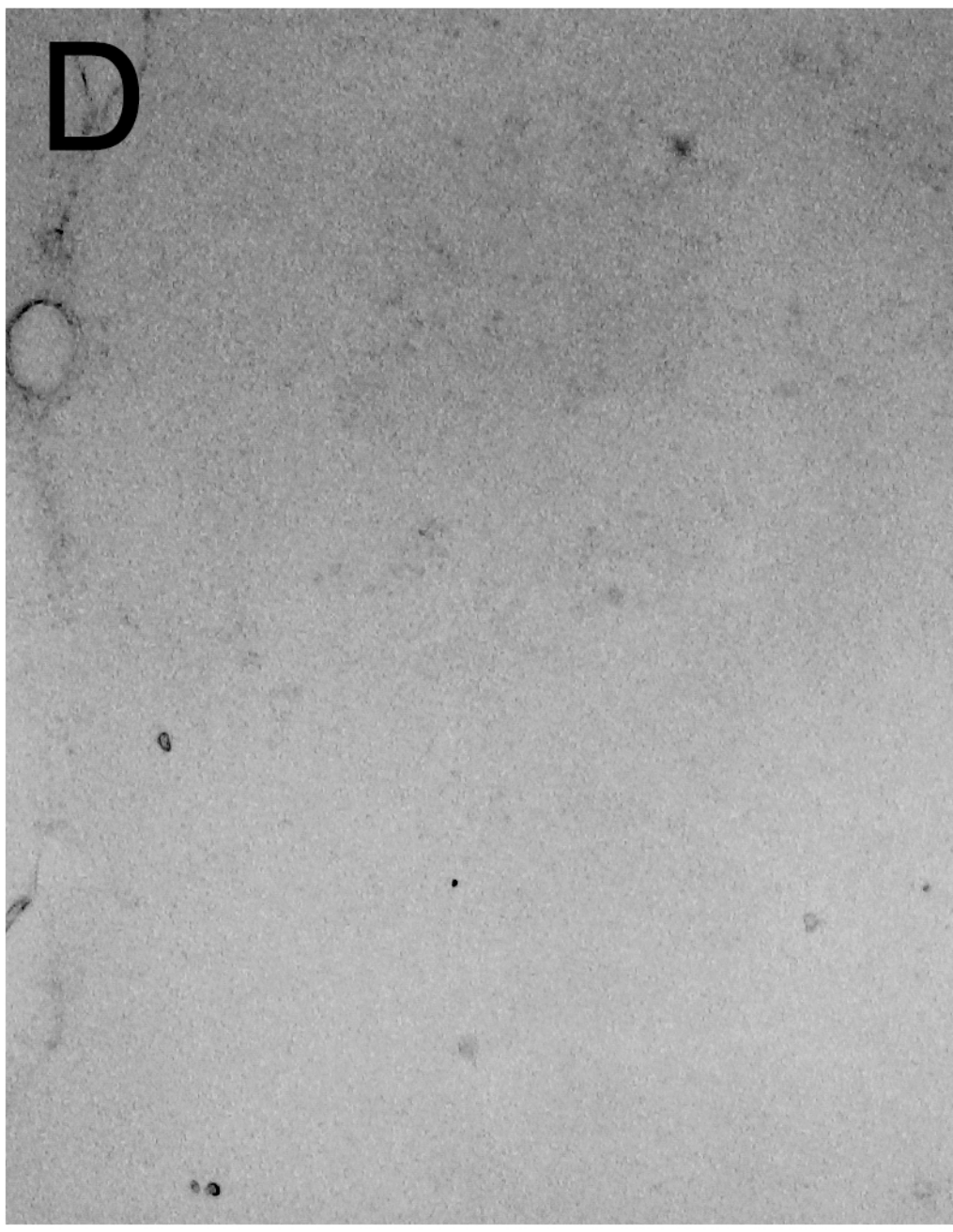

A
B
C
D
E
F
G
H

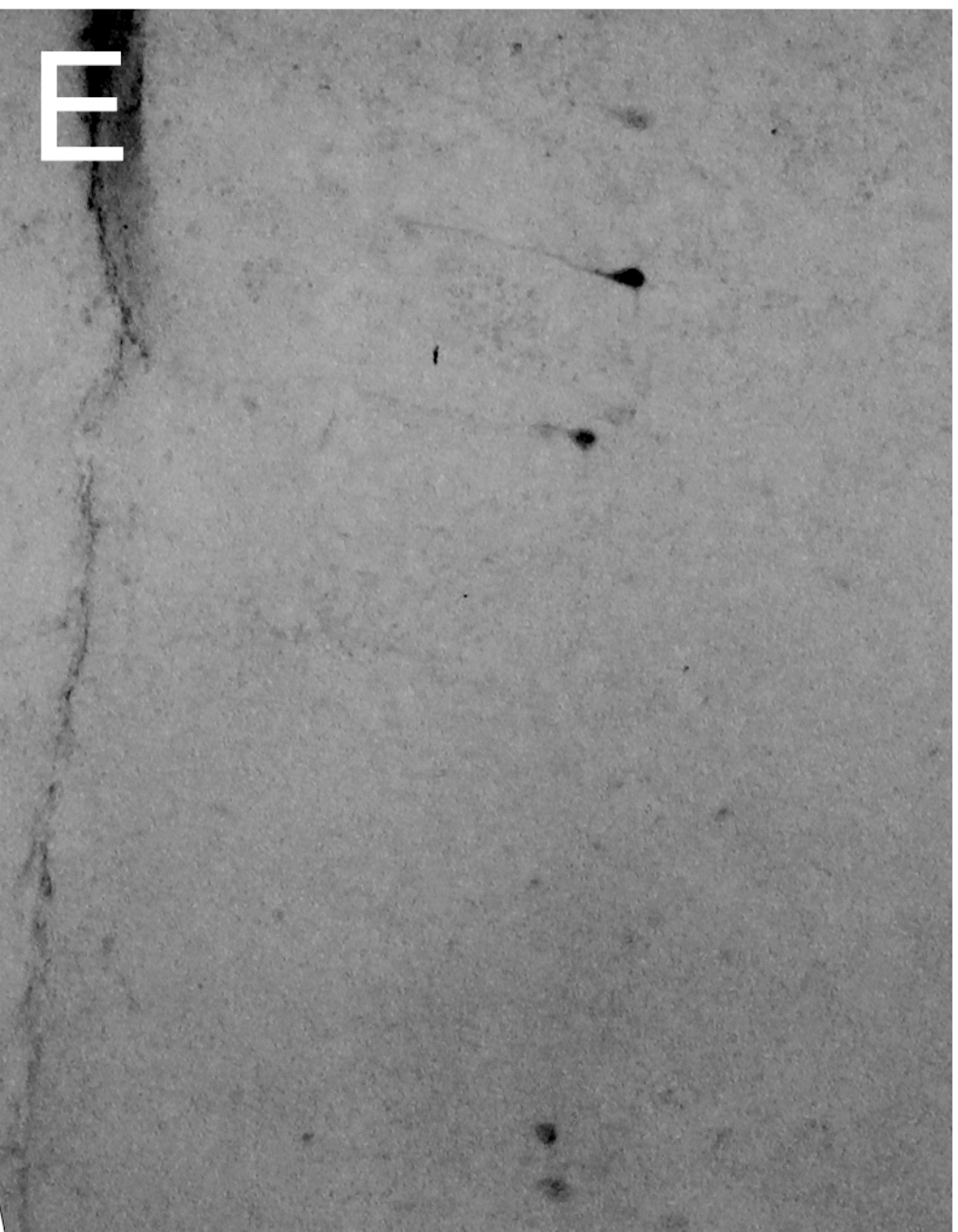

p < 0.001
p < 0.01
Number of hsp70 neurons
0
10
20
30
40
50
Saline
MK 801
MK + HAL
MK + CPZ
MK + CLZ
MK + OLZ
MK + ZPS

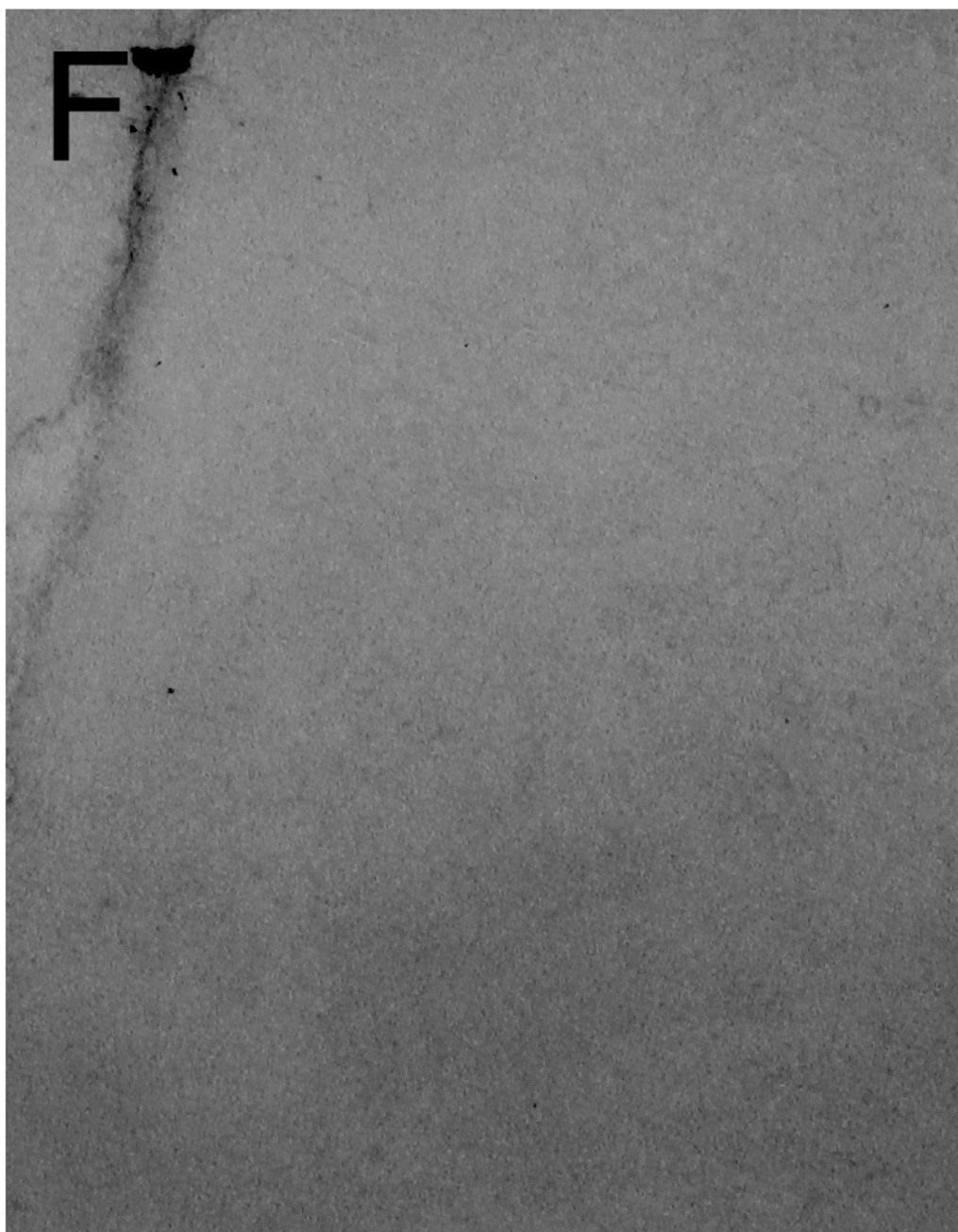

A

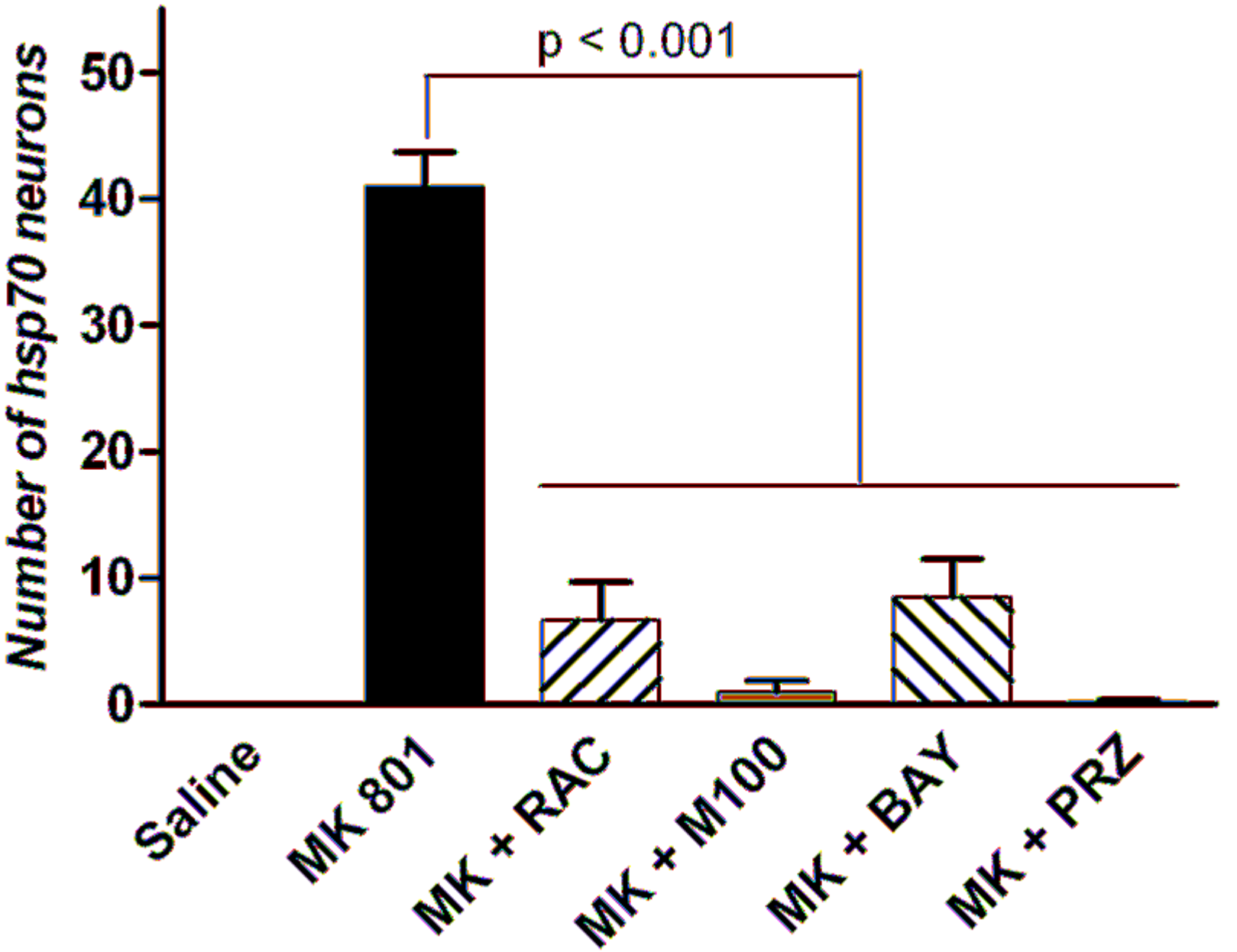

B


C


D


E


F


p < 0.001
Number of hsp70 neurons
0
10
20
30
40
50
Saline
MK 801
MK + RAC
MK + M100
MK + BAY
MK + PRZ