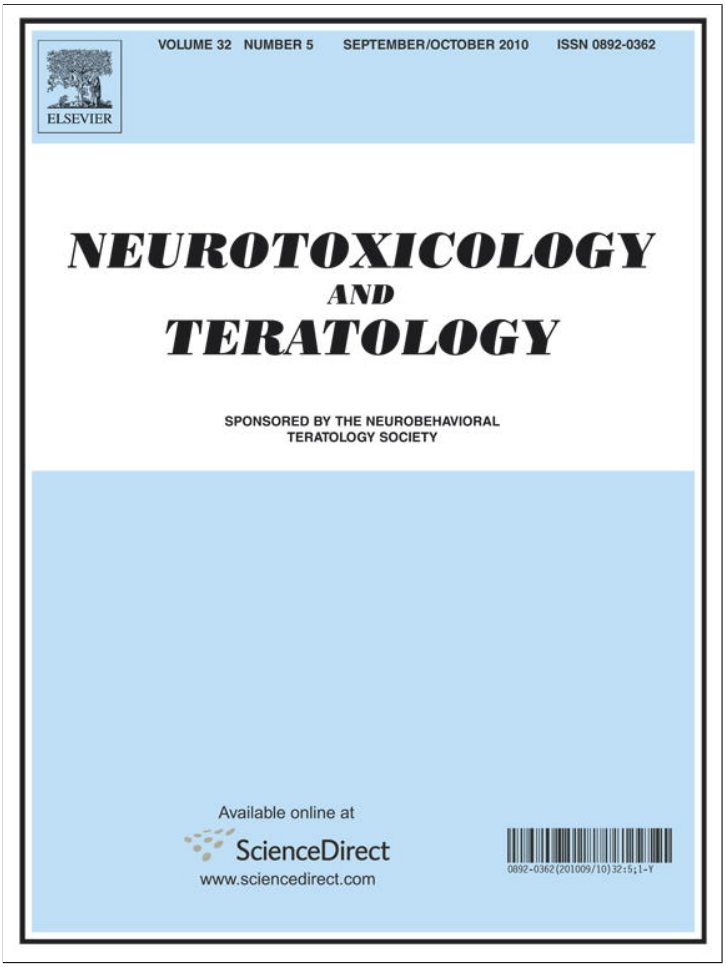

Neurotoxicology and Teratology 32 (2010) 542–550

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

journal homepage: www.elsevier.com/locate/neutera

Comparative analyses of the neurodegeneration induced by the non-competitive NMDA-receptor-antagonist drug MK801 in mice and rats

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ARTICLE INFO

Article history:
Received 2 March 2010
Received in revised form 3 May 2010
Accepted 5 May 2010
Available online 12 May 2010

Keywords:
MK801
Neurodegeneration
Mice
Non-competitive NMDA antagonist
Animal models

ABSTRACT

Non-competitive NMDA-receptor-antagonist drugs such as dizocilpine (MK801) induce behavioral changes and neurotoxicity that have made an impact in different fields of neuroscience. New approaches in research use transgenic mice to elucidate cellular mechanisms and circuits involved in the effects of these drugs. However, the neurodegeneration induced by these drugs has been extensively studied in rats, but the data in mice is limited. Therefore it is important to characterize if the neurotoxic pattern in mice corresponds to that of rats.

A comparative analysis of the neurodegeneration induced by MK801 (10 mg/kg) between Wistar rats, and CD-1, CF-1, and C57BL/6-129/Sv mice of both sexes, at different survival times (15, 24, 32, 48, 56 and 72 h) was analysed with the amino-cupric-silver and fluoro-jade B techniques. To compare different administration patterns, groups of mice received subchronic treatments with different doses (final doses of 20 and 40 mg/kg).

Results showed that mice treated with MK801 presented different neurotoxic profiles, such as excitotoxic-like cell death in the retrosplenial cortex, terminal degeneration in CA1 and apoptotic-like degeneration in the olfactory bulb. Unlike rats, mice subjected to the same treatment failed to show neurodegeneration in corticolimbic areas such as piriform cortex and dentate gyrus. The amount of degeneration was lower in mice, and the subchronic administration of MK801 did not change the neurotoxic pattern. Additionally, mice lacked the sexually dimorphic response to MK801 toxicity observed in rats. Altogether these results indicate important species dissimilarities. Neurotoxicological studies aimed to explore pathways and mechanisms of MK801 toxicity should consider these differences when using mice as rodent models.

1. Introduction

Dizocilpine maleate (MK801) is a non-competitive NMDA-receptor-antagonist drug that, like other NMDA antagonist drugs (NMDA-A), is neuroprotective in several animal models of pathologies that involve the hyperactivation of NMDA receptors, such as stroke, ischemia, epilepsy, neuropathic pain and alcohol abstinence [11,37,62]. However, the potential clinical application of these compounds is limited because, in addition to their protective properties, they cause neurodegeneration in some critical brain areas with a single application [8,22]. In humans NMDA-A drugs such as phencyclidine and ketamine induce cognitive deficits and psychotic symptoms [46,47], therefore the administration of these drugs is widely used as a pharmacological model of psychotic disorders such as schizophrenia [45–47,53,61,64].

The neurotoxic effect of NMDA-A has been extensively studied in rats. The systemic administration of MK801 with doses between 0.5 and 1 mg/kg induces vacuolisation and the expression of heat shock protein 70 in the retrosplenial cortex [52,60]. Both are signs of reversible neurotoxicity because they disappear after a time and no signs of neurodegeneration are observed. Higher doses (2–10 mg/kg) or subchronic treatments (2–5 days) with moderate doses, induce neuronal death (irreversible effects) not only in retrosplenial cortex but also in hippocampus, olfactory cortex, olfactory bulb, entorhinal cortex and other cortical-limbic nuclei [8,9,20,24,33,52].

Both neurotoxic and behavioral studies have shown that female rats are more sensitive than males to MK801 or PCP treatment [1,3,13,16,32,48,49,68,69]. Considerable neurodegeneration occurs in the female brain following treatment with a non-competitive NMDA antagonist while little occurs in the male rat brain. Based on these observations, most studies used the female rat for the identification of neuronal subpopulations affected by the neurotoxic effects of NMDA-A.

Abbreviations: RSC, retrosplenial cortex; RSG, granular retrosplenial cortex; RSD, dysgranular retrosplenial cortex; MOB, main olfactory bulb; ENT, entorhinal cortex; CA1, ammon's horn 1; PLCo, posterolateral cortical amygdaloid nuclei; DG, dentate gyrus; Pir, piriform cortex; A-Cu-Ag, amino-cupric-silver; FJ-B, fluoro-jade B.

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doi:10.1016/j.ntt.2010.05.002

Mice have gained importance in the study of the psychotropic mechanisms because of the possibility of generating genetic variations as tools for understanding cellular and molecular mechanisms involved in different experimental paradigms [56,67]. Also, this species is extensively used in order to analyse the behavioral changes and the potential use of antipsychotic drugs with NMDA-A [10,25,28,29,40]. However, reports about neuropathological effects induced by NMDA-A in mice are few and they have focalized on the reversible neurotoxic effects [6,7,51,65]. Systematic studies focusing on the irreversible neurotoxic effects (neurodegeneration) using specific histochemical techniques for detecting neuronal degeneration have not yet been performed. Moreover, a comparative analysis of MK801-induced neurodegeneration between rats and mice has not been executed. This is important since there has been an increase in the literature concerning neurobiological differences between rats and mice [2,30,66].

In this study we analyse if the neurotoxic effects evidenced in rats are extendable to mice. In order to compare the MK801-induced neurodegeneration between these two species, we use the amino-cupric-silver [14] and fluoro-jade B [59] techniques, which specifically stain degenerating neurons. The present study comparatively analyses the neurodegeneration induced by the same treatment of MK801 in rats and mice, along with a comprehensive and detailed description in mice of the neuroanatomical structures that were irreversibly damaged by MK801. In addition CD-1 (ICR) mice, that have previously shown to be sensitive to MK801-induced reversible toxicity [6], were compared with CF-1 and C57BL/6-129/Sv mice which have different genetic backgrounds and are currently used to generate knockout mice useful to study behavioral alterations related to psychiatric disorders [57,58].

This report shows that the sexually dimorphic response to MK801-induced toxicity observed in rats is absent in mice, and that mice are significantly less sensitive than rats to MK801-induced neurodegeneration in different brain structures. Concomitantly this data could be valuable for future studies aimed to explore MK801 toxicity in genetically modified mice and to applied neurotoxicology associated to schizophrenia and related disorders.

2. Methods

2.1. Animals

Young adult (70–80 days) Wistar rats from the Instituto de Investigaciones Médicas Mercedes y Martín Ferreyra vivarium of both sexes weighing 200–240 g ($N=10$) and, CD-1 ($N=10$), CF-1 ($N=28$), and C57BL/6-129/Sv ($N=25$) adult mice weighing 27–34 g were used. The animals were housed in a plastic home cage at a controlled temperature (20–22 °C) with food and water available ad libitum. CF-1 mice were donated by the Instituto de Ingeniería Genética y Biología Molecular (Buenos Aires, Argentina). All experiments conformed to named local and international guidelines on the ethical use of animals. Every effort was made to minimize discomfort of the animals and the overall number of animals used.

2.2. Treatments

Based on previous neurotoxic studies in rats [8,9,15] and mice [70], both species were administrated with one dose of 10 mg/kg of MK801 (dizocilpine maleate, (+)-5-methyl-10, 11-dihidro-5H-dibenzo (a, d) cyclohepten-5, 10-imine hydrogen maleate, SIGMA) and killed at 72 h. Control animals received an intraperitoneal (i.p.) injection of 0.9% NaCl (2 ml/kg). The earliest time point that the A-Cu-Ag technique detected consistent signs of neurodegeneration in mice was at 15 h post-treatment, therefore the time course of MK801 neurodegeneration was evaluated at 15, 24, 32, 48, 56 and 72 h post-injection. To compare different administration patterns, a subchronic treatment group of mice received 2 i.p. injections of MK801 (10 mg/kg) separated by 5 h and killed 24 h after the last injection. Another group received a subchronic administration of MK801, with the first dose of 20 mg/kg, followed by two injections of 10 mg/kg separated by 4 h. These animals were also killed 24 h after the last injection.

2.3. Tissue collection

Animals were anaesthetized i.p. with 30% chloral hydrate; perfused transcardially with washing solution (0.8% sucrose, 0.8% NaCl and 0.4% glucose) and fixed with 4% paraformaldehyde in 0.2 M borate buffer (pH 7.4). Brains were left overnight in the skull and afterwards removed and placed in 30% sucrose. Once the brains sank in the sucrose, coronal sections of 40 μm were obtained in a freezing microtome and stored at 4 °C until processing either in 0.01 M phosphate buffer (PBS) in order to immediately process for fluoro-jade B technique [59], or in 4% paraformaldehyde in order to assess neurotoxicity using the amino-cupric-silver technique [14]. The olfactory bulb and some brains were cut in sagittal sections in order to confirm the anatomical neurodegeneration observed in the coronal sections.

2.4. Histopathological techniques

The amino-cupric-silver stain (A-Cu-Ag) is a more recent version of the cupric-silver method and is suitable for staining degenerating perikarya, dendrites, stem axons, and their terminal ramifications (synaptic endings) in brain tissue subjected to different experimental and neuropathological conditions [14,17]. The procedure was carried out following the protocol previously described [14] except that before starting, the sections were pre-incubated in 40% ethanol in slight agitation for 30 min. This procedure eliminated the unspecific staining that was present in the mouse tissue. The pre-treatment also resulted beneficial to rat tissue, without affecting the specific stain. After that, sections were rinsed in double-distilled water and incubated in a pre-impregnating solution of silver nitrate at 50 °C. After cooling to room temperature, sections were rinsed with acetone and transferred to a concentrated impregnating silver nitrate solution for 40 min. Sections were then immersed in a reducing solution for 25 min and the reaction was stopped in 0.5% acetic acid. Bleaching was done in two steps to eliminate the non-specific deposits of silver on the tissue. After stabilization in a thiosulphate solution, the sections were mounted.

Fluoro-jade B technique (FJ-B) was performed as indicated by Schmued and Hopkins [59] except that the sodium hydroxide solution was substituted by xylene, in order to avoid losing sections. After 5 min in xylene, slides were transferred in ethanol absolute and then in 50% ethanol solution, 2 min for each one. This was followed by 2 min in distilled water and 10 min in 0.06% in potassium permanganate in slight agitation. The slides were then rinsed in distilled water for 2 min and after that submerged in FJ-B solution (0.004% in acetic acid 0.1%, Chemicon) for 20 min, rinsed in distilled water and dried on a slide warmer. Afterwards the slides were cleared in xylene and coverslipped with DPX.

2.5. Histological analyses

All brains were carefully analysed to identify different types of neurodegeneration (apoptotic, somatic and terminal degeneration). For anatomical references the atlas for rats and mice of Paxinos and Watson [54,55] was used. Quantitative assessment was accomplished with a light microscope at 40× objective lens, equipped with a Leica LC200 video camera, which acquired and saved images with a Photoshop 7.0 program. Afterwards, positive argyrophilic neurons were counted in selected brain areas using the SCION program from

the NIH. The counting was carried out by a treatment blind investigator and performed using a predefined area of an identical size for each brain region. The anterior–posterior distribution of neurotoxicity induced by NMDA-A has been previously described and indicates that in the rat retrosplenial cortex (RSC) the greatest amount of systematic degeneration can be found between planes −6.3 and 6.7 [3,16,24,69]. Therefore to get an average number of each case, 5 hemisections of these planes were considered an accurate representation of the sensitivity towards MK801-induced toxicity. Analyses of the neurodegeneration pattern induced in the RSC of mice corroborated the anterior–posterior distribution that was observed in rats and confirmed that in mice −3.1 and −3.5 would be equivalent to planes −6.3 and −6.7 of the rat. Sagittal sections were used to analyse the olfactory bulb at 1.44 mm lateral to the medial line in rats and 0.75 in mice. The mean number of positive argyrophilic cells per animal ($n=4$ per group) was used for statistical analysis.

2.6. Statistical analyses

The analysis of variance (ANOVA) was applied to the data using post hoc LSD Fischer test. The differences were considered statistically significant when $p<0.05$.

3. Results

3.1. Behavioral observations

In both rats and mice, behavioral disturbances started within 5–10 min after the administration of MK801 (10 mg/kg). Rats rapidly presented recumbency (animals lying down on the floor unable to rise or walk), while mice never became recumbent, instead they showed severe ataxia, specially marked for the hind limbs and displayed repetitive movements around the corner of the home cage. In mice of both sexes and male rats these behavioral disturbances induced by MK801 disappeared 24 h post-injection. By contrast, female rats treated with MK801 remained affected until the day of sacrifice (72 h post-injection).

3.2. Comparative analysis of the sexually dimorphic response to MK801-induced neurotoxicity in rats and mice

To analyse neurodegeneration induced by MK801, mice and rats of both sexes received a single i.p. injection of saline (control) or MK801 (10 mg/kg) and sacrificed 72 h post-treatment. Neurodegeneration was evaluated with the A-Cu-Ag and FJ-B techniques. Saline-treated animals stained with the A-Cu-Ag were free of signs of neurodegeneration, with the exception of the olfactory bulb of mice, which presented a basal amount of apoptotic-like somas. This has been described previously as a common feature in mice, in which TUNEL-positive cells were found [18], and will be described in detail down below.

MK801-induced neurotoxicity was sexually dimorphic in rats. Female rats displayed robust somatodendritic and terminal degeneration in several brain regions including the main olfactory bulb (MOB), anterior olfactory nucleus, primary olfactory cortex, olfactory tubercle, tenia tecta, posterolateral cortical amygdaloid nuclei (PLCo), entorhinal cortex (ENT), ammon's horn 1 (CA1), dentate gyrus (DG), subiculum, anterior cingulate cortex, retrosplenial cortex (RSC) (Fig. 1A), sensory cortex, visual cortex and motor cortex (Table 1). In contrast, male rats only presented signs of sparse neurodegeneration in RSC (Fig. 1B), PLCo, ENT, CA1, MOB and neocortical areas (Table 1).

Surprisingly, the pronounced sexual dimorphism seen in rats (Fig. 1A,B) was absent in mice, which presented practically indistinguishable degeneration profiles between sexes (Fig. 1C,D). All three strains of mice treated with MK801 showed degeneration that was mostly confined to RSC, MOB, and CA1. In mice of both sexes, somatodendritic degeneration was restricted to layer IV of the granular division of the retrosplenial cortex (RSG), while terminal degeneration was observed in layers I and IV, and extended to the dysgranular portion of the retrosplenial cortex (RSD) (Fig. 1C,D) (Table 1).

Similar to rats, MK801-treated mice also showed axonal-like terminal degeneration, restricted to the lacunosum molecular layer of the hippocampus (Fig. 2A,B). Mice also presented few sporadic and random degenerating neurons with excitotoxic-like appearance in layer III of the posterior part of ENT (Fig. 2C,D) (Table 1). However, in the piriform cortex, female (but not male) rats presented somatic and terminal degeneration that was only occasionally observed in the CD-1 mouse strain (data not shown). Other structures strongly sensitive to the neurotoxic effects of MK801 in female rats, such as the DG and PLCo, (Fig. 3A and C), were completely free of signs of neurodegeneration in mice (Fig. 3B and D) (Table 1).

Quantitative analysis of the argyrophilic somas in the RSC confirmed that rats were significantly more sensitive than mice and that sexually dimorphic neurotoxic effects of MK801 found in rats were absent in mice (Fig. 4). Degenerating argyrophilic somas in the rat RSC averaged 233 ± 38 (by hemisection) in females and 22 ± 5 in males, while in the mice strains, female CD-1, C57BL/6-129/Sv and CF-1, the average was 5 ± 2, 8 ± 1 and 3 ± 1, respectively (Fig. 4). The two way ANOVA indicated a significant effect of species ($F_{1,15}=19.61$, $p<0.01$), sex ($F_{1,15}=14.27$, $p<0.01$) and an interaction between both ($F_{1,15}=14.53$, $p<0.01$). Post hoc analysis indicated that female rats were significantly different from male rats, and from mice of both sexes ($p<0.0001$), however male and female mice did not differ from each other ($p<0.98$). Additionally, when comparing male rats and mice, the LSD Fisher post hoc test showed that male rats were significantly different from all mice groups ($p<0.001$) indicating that mice had significantly less positive neurons than rats. Altogether these results indicate that mice are less sensitive to the neurotoxic effect of MK801 than rats, and that the dimorphic effect observed in rats is absent in mice.

In FJ-B stained sections no signs of neurodegeneration were observed in control (saline-treated) animals of either species. In MK801-treated female rats, numerous FJ-B positive neurons were observed in the RSC, DG, PLCo, ENT and the primary olfactory cortex. However the robust terminal degeneration revealed by the A-Cu-Ag in the RSC and CA1 was not detected by the FJ-B technique (Fig. 5). In male rats we detected somas only in RSC and PLCo. When comparing genders in mice, similar quantities of FJ-B positive degenerative somas were detected exclusively in RSC, confirming the lack of sexual dimorphism and the less sensitivity towards MK801-induced neurotoxicity that was previously described with the A-Cu-Ag.

3.3. Time-course evaluation of the neurotoxic effect of MK801 in mice

To determine the temporal pattern of neurodegeneration induced by MK801 in mice, a time-course analysis (15, 24, 32, 48, 56 and 72 h) was performed using the A-Cu-Ag technique. In the RSC, evidence of golgi-like neuronal profiles with dendritic arborizations that were still preserved in layer IV and terminal degeneration in layer I were found at 15 h post-treatment, indicating an early stage of degeneration. The degenerating profile became more evident between 24 and 48 h post-treatment where most of the perikarya and dendritic processes displayed a typical pattern of excitotoxic death. Thereafter neuronal cell bodies, dendrites and axons started to show fragmentation, which is consistent with advanced stages of neuronal death. The number of degenerating somas was not significantly different at these survival times (ANOVA, $F_{4,23}=0.21$, $p=0.9$) suggesting that degeneration of a limited number of neurons began few hours after MK801 treatment, but no other neurons were recruited thereafter.

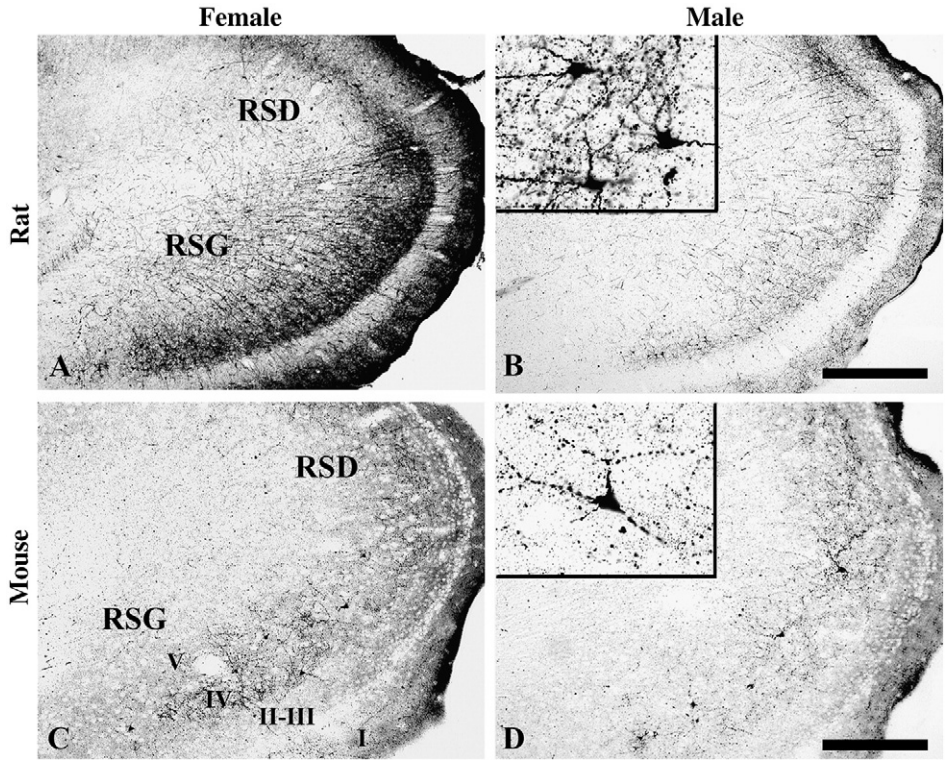

Fig. 1. Comparative analysis of MK801-induced neurotoxicity in rat and mouse in coronal sections of the retrosplenial cortex. MK801-treated (10 mg/kg) female and male rats (A and B) and mice (C and D) were evaluated with the A-Cu-Ag technique at 72 h post-treatment. Note that somatic degeneration is confined to layer IV of the granular retrosplenial cortex (RSG), while terminal degeneration was observed in layers I, IV and V, and extended to the dysgranular portion of the retrosplenial cortex (RSD). Images at the left upper corner of B and D are representative high power magnifications of the excitotoxic-like neuronal death in layer IV of the RSG. Increased intensity of neuronal degeneration is observed in the female rats (A), but not in the female mouse (C). Scale bar 400 μm for rat and 200 μm for mice.

Control mice presented scattered apoptotic-like neurons in the MOB (Fig. 6A). An increase of argyrophilic neurons was observed at 15 h post-MK801 treatment where most of these degenerating neurons depicted an apoptotic-like appearance (Fig. 6B and insert). The two way ANOVA indicated that there was a significant effect of treatment ($F_{1,24}=6.13$, $p<0.03$), survival time ($F_{5,24}=3.34$, $p<0.02$) and treatment × survival time ($F_{5,24}=3.52$, $p<0.02$). Post hoc analysis indicated that only the MK801-treated animals at 15 h were different from saline ($p<0.001$) (Fig. 6C) and all the other groups. The larger amount of apoptotic-like argyrophilic cells scored at earlier survival times in the MOB would be consistent with the fact that the apoptotic-like death occurs at a faster rate than the excitotoxicity induced by MK801 [50]. Finally, we observed that in mice terminal degeneration in CA1 was present at all survival times studied, while the somatodendritic degeneration in ENT was detected in some cases only at 24 h (data not shown).

Table 1
Distribution of somatodendritic (S), terminal (T) and apoptotic-like (*) degeneration detected by the A-Cu-Ag in brain structures of MK801 (10 mg/kg)-treated rats and mice of both sexes. The small letters (s, t) indicate sporadic appearance of degeneration.

Brain structure	Rat		Mouse	
	Female	Male	Female	Male
Olfactory bulb	*S	*S	*S	*S
Anterior olfactory nucleus	T	–	–	–
Primary olfactory cortex	ST	–	–	–
Olfactory tubercle	T	–	–	–
Tenia tecta	S	–	–	–
Olfactory amygdala	ST	ST	–	–
Entorhinal cortex	ST	ST	s	s
Ammon's horn 1	T	T	T	T
Dentate gyrus	ST	–	–	–
Subiculum	T	t	–	–
Anterior cingulate cortex	T	t	–	–
Retrosplenial cortex	ST	ST	ST	ST
Sensory cortex	ST	T	t	t
Motor cortex	T	t	–	–

3.4. Subchronic treatment of MK801 in mice

A previous report showed that MK801 toxicity is higher when administered subchronically [33]. To examine the possibility that in mice other brain areas could be affected by a different administration pattern of MK801, subchronic doses of 20 and 40 mg/kg of MK801 were applied and neurotoxicity was evaluated with A-Cu-Ag and FJ-B at 24 h after the last injection.

A significant dose-dependent increase (ANOVA $F_{2,13}=4{,}85$, $p<0.03$) in the number of degenerating neurons in the RSC of subchronic-treated animals was observed (Fig. 7), in which the post hoc LSD analyses indicated that only the 40 mg/kg group was statistically different ($p<0.01$). Despite the increase in the number of degenerating neurons in RSC, no additional areas were affected, confirming that RSC, MOB, EC and CA1 are the only sensitive areas to MK801-induced neurodegeneration in mice. Additionally, we confirmed that in the subchronic treatment in mice, MK801-induced neurodegeneration affected the same neuroanatomical structures in both, males and females (data not shown). These observations corroborate that, on the contrary to rats, the neurotoxic effect of MK801 is not sexually dimorphic in mice, and that subchronic treatments do not change the neurotoxic pattern.

4. Discussion

In this study we show dramatic differences in the neurotoxic damage induced by MK801 between rats and mice, being the later much less vulnerable. We formally compared the sensitivity of different rat strains to MK801-induced toxicity and found only subtle

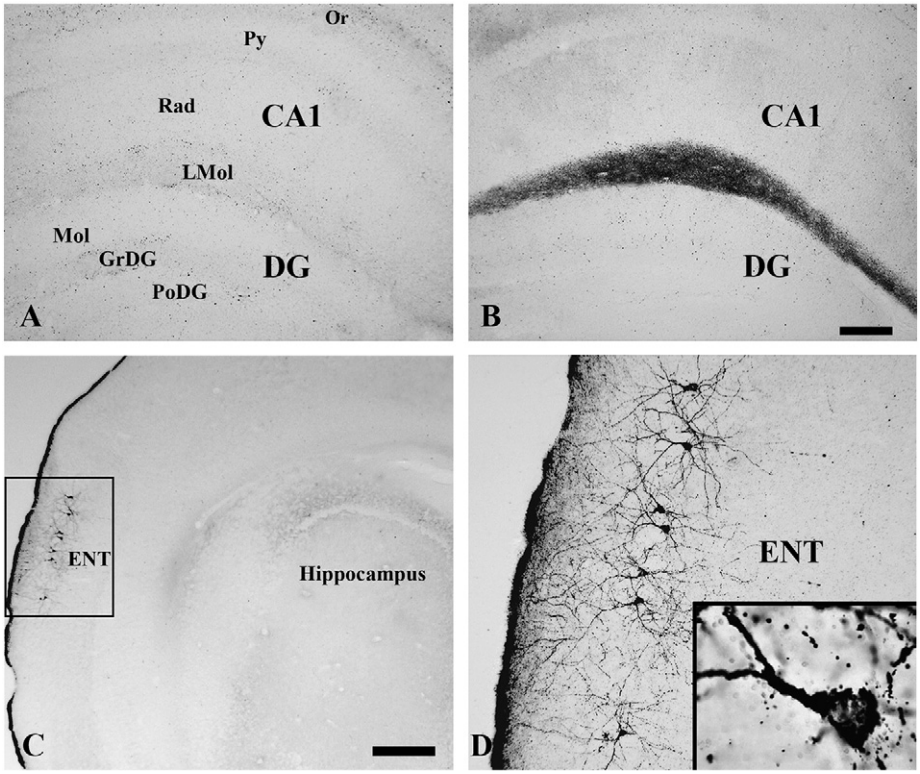

Fig. 2. Terminal and excitotoxic-like neurodegeneration induced by MK801 in hippocampal regions. (A) Mice treated with 0.9% NaCl (control) or (B–D) MK801 (10 mg/kg) at 24 h post-treatment were evaluated with the A-Cu-Ag technique. CA1 showed dense axonal-like terminal degeneration that was not observed in control animals (A), but was restricted to the lacunosum molecular layer of the hippocampus (LMol) in MK801-treated mice (B). Scale bar 100 μm. (C) MK-801 treated mice presented degenerating neurons with excitotoxic-like appearance in layer III of the posterior part of the entorhinal cortex (ENT). Scale bar 300 μm. (D and insert) Higher magnifications in the ENT show the typical argyrophilic cell bodies with corkscrew and fragmented dendrites. Scale bar 100 μm. CA1: CA1 field of the hippocampus; DG: dentate gyrus; GrDG: granule cell layer of DG; Mol: molecular layer of DG; PoDG: polymorph layer of DG; Py: pyramidal layer of the hippocampus; Rad: radiatum layer of the hippocampus; Or: orions layer of the hippocampus.

differences [9], and in this study, we observed no significant differences between mice strains. Similarly, it was reported that no differences were found between C3H, DBA2 and FVB inbred mouse strains to ketamine, another NMDA-A [42]. Altogether, these observations demonstrate that, regardless of the strain, sensitivity to NMDA-A is significantly different between rats and mice. The evaluation of the irreversible neurotoxic effects of MK801 showed that somatodendritic and terminal degeneration in mice of both sexes affects systematically the RSC, MOB, CA1, and in some cases ENT, in both acute and subchronic treatments. In mice, the quantitative analysis of argyrophilic cells confirmed the lack of sexually dimorphic response to MK801-induced toxicity, which was observed in rats. Another significant difference between these two species was that the quantity of dead cells in the RSC was significantly higher in rats than in mice. Interestingly, the behavioral response towards recumbency observed in rats, was totally absent in mice. Altogether these results indicate important species differences in both behavioral and neurotoxic changes induced by this NMDA-A drug.

In this report due to the high-sensitivity of the A-Cu-Ag technique, which not only detects somas of degenerating neurons, but also depicts their axons, dendrites and synaptic terminals [14,27,63], we were able to observe in mice neurodegeneration in areas that have not been previously reported, such as the MOB, CA1 and somatosensory cortex. The neuroanatomical detection of axonal and terminal degeneration could play an important role for the identification of neuronal circuits that are functionally affected after MK801 treatment. For example, in rats, we were able to correlate prolonged activation of different immediate early genes in cortical areas where MK801 promoted axonal and terminal degeneration, but no somatic death, suggesting that terminal degeneration was followed by long-lasting functional changes [15]. Interestingly, different research groups showed that in mice the administration of MK801 induces c-Fos activation in the RSC and CA1 [19,38,51]. With the A-Cu-Ag we detected intense terminal degeneration in both of these structures, which could be related to the deafferentation to which these areas undergo. Furthermore, degeneration of synaptic terminals in cortical and hippocampal structures could be related to the behavioral alterations reported after MK801 administration in mice [70].

Another interesting finding of our study was that MK801 induced the same pattern and intensity of neurodegeneration in both male and female mice, indicating that its neurotoxic effect is not sexually dimorphic, as it is in rats. In rats, females are much more sensitive than males to MK801-induced degeneration [3,16,34,44]. On the contrary, female mice did not show increased sensitivity to MK801, and consequently, when comparing the amount of argyrophylic cells in the RSC, no differences were observed between sexes. Also, when compared to female rats, mice were significantly less sensitive to the neurodegenerative effects of MK801. We observed that many areas, such as the PLCo or the DG, that were severely affected in female rats were free of damage in mice, even when administrated in a subchronic pattern that, in rats, has been associated to an enhancement of neurotoxicity [33,35].

When comparing the neurodegeneration after MK801 treatment in both species, the numbers of argyrophilic cells were dramatically higher in the female rat (more than 200) than in the mouse (less than 10). Stereological studies showed that, compared to mice, the number of cortical neurons in the rat is approximately 2.8 folds [30,31]. Consequently, this basic interspecies neuroanatomical characteristic could only explain a limited part of the difference in the number of degenerating neurons between mice and rats reported here. Several other factors could also determine the different sensitivity of rats and mice to MK801 toxicity. The efficiency to metabolise and/or eliminate

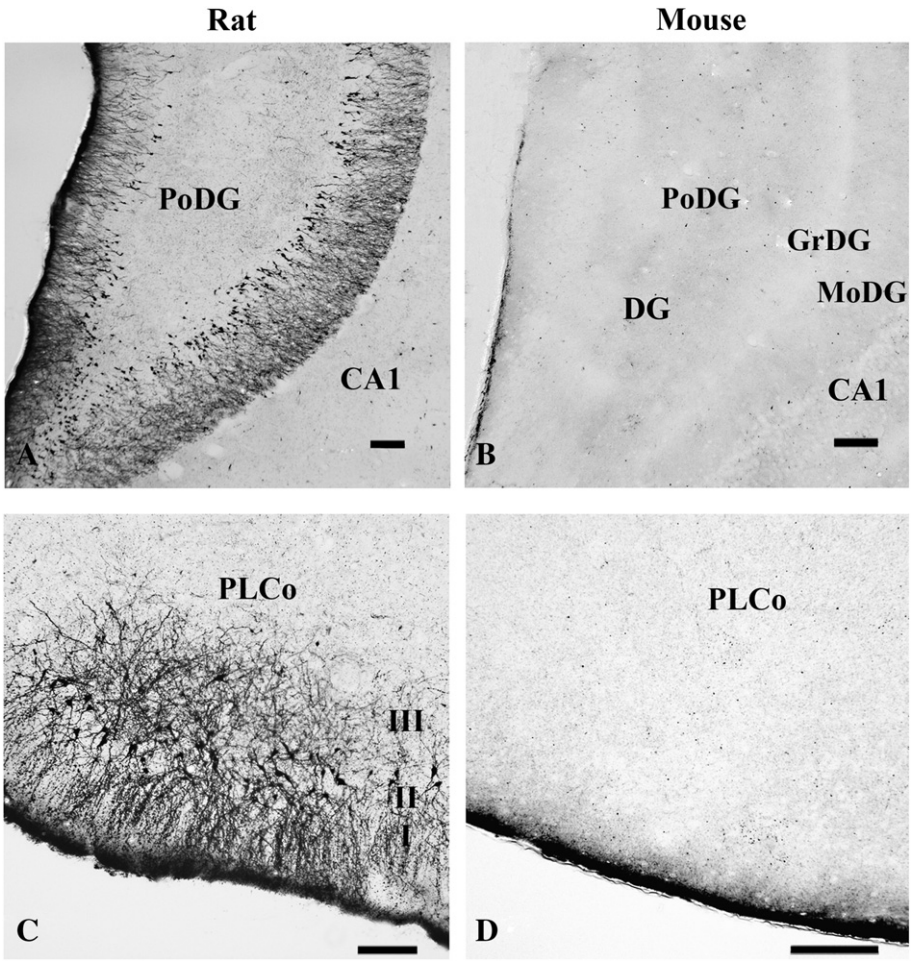

Fig. 3. Structures sensitive to the neurotoxic effects of MK801 in female rats were completely free of any signs of neurodegeneration in female mice. MK801-treated (10 mg/kg) female rats (A and C) and mice (B and D) were evaluated with the A-Cu-Ag technique at 72 h post-treatment. Note that the robust somatodendritic neurogeneration in the dentate gyrus of the rat (A) is totally absent in the mouse (B). Excitotoxic-like degeneration in layers II and III of the posterolateral cortical amygdaloid nucleus of the rat (C) were absent in mice (D). Scale bars = 100 μm. CA1: CA1 field of the hippocampus; DG: dentate gyrus; GrDG: granule cell layer of DG; Mol: molecular layer of DG; PoDG: polymorph layer of DG; PLCo: posterolateral cortical amygdaloid nucleus.

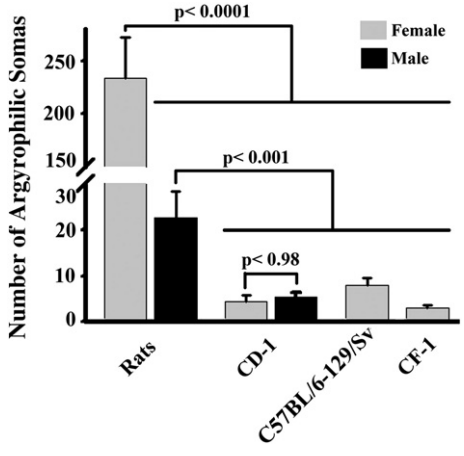

Fig. 4. Quantitative analysis of the argyrophilic somas in layer IV of the RSC of rats and mice. Degenerating argyrophilic somas were analysed in the male and female rat and mouse strain CD-1. Female mouse strains C57BL/6–129/Sv and CF-1 were also analysed. The two way ANOVA indicated a significant effect of species ($F_{1,15} = 19.61$, $p < 0.01$), sex ($F_{1,15} = 14.27$, $p < 0.01$) and an interaction between both ($F_{1,15} = 14.53$, $p < 0.01$). Post hoc analysis indicated that female rats were significantly different from male rats, and from mice of both sexes ($^* = p < 0.0001$), however male and female mice did not differ from each other ($p < 0.98$). Additionally, when comparing male rats and mice, the LSD Fisher post hoc test showed that male rats were significantly different from all mice groups ($\# = p < 0.001$). These results indicate that mice are less sensitive to the neurotoxic effect of MK-801 than rats, and that the dimorphic effect observed in rats is absent in mice.

a drug will determine the bioavailability of the psychotropic substance. For example, ethanol is eliminated faster in mice than in rats [41]. However, there are no studies regarding the pharmacokinetics of MK801 in mice, therefore it is not possible to ascertain if this variable plays any role in the interspecies differences to MK801-sensitivity. Another important difference between species is that at 72 h post-treatment the neurodegeneration in mice showed much more fragmentation than rats, indicating a more advanced stage of neurodegeneration. This suggests that the neurotoxic process induced by MK801 advanced more quickly in mice than in rats. Similar conclusions have been reported in mice treated with kainic acid, in which a time-dependent increase of argyrophilic neurons could be found between 12 h and 3 days post-treatment in some of the hippocampal structures analysed, which were later followed by neuronal fragmentation [5]. Both models present excitotoxic-like lesions that affect layer IV neurons of the RSC, however the degeneration induced by kainate acid exceeds that induced by MK801, promoting neuronal damage in extensive regions of the brain, including, among others, the neocortex, amygdala, thalamus, hippocampus, olfactory bulb and striatum [4]. The mechanism of MK801-induced toxicity has not been elucidated, but probably involves misregulation of several neurotransmitters, including glutamate, GABA, acetylcholine, serotonin, and noradrenaline. Therefore, another factor that may have relevance in MK801 toxicity is the chemo-structural organization of the brain. Some authors propose that MK801 toxicity depends on the imbalance of excitatory and

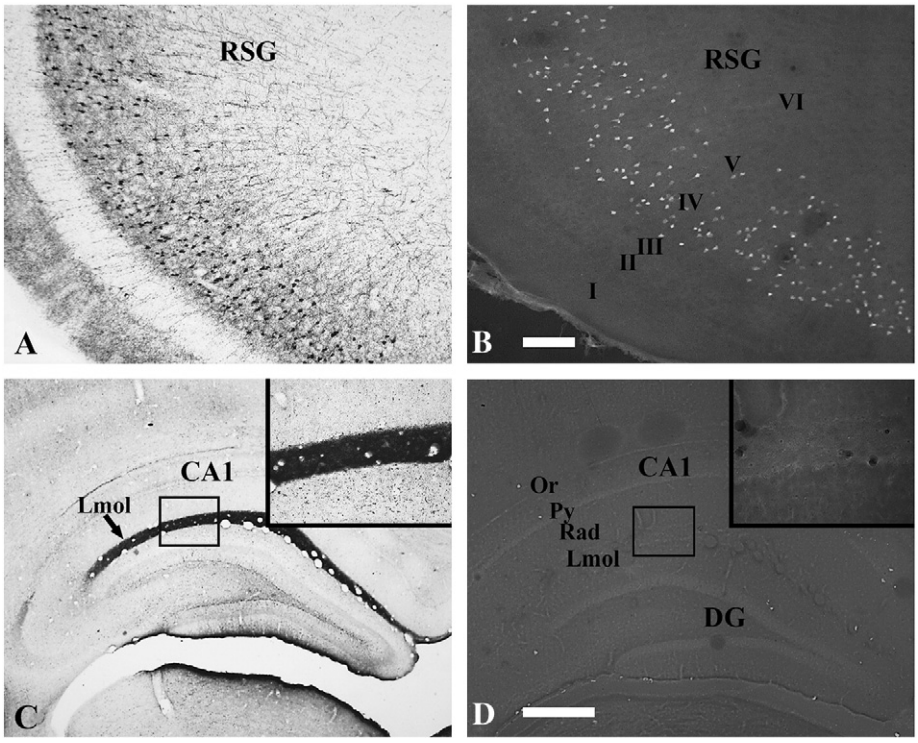

Fig. 5. Neurodegeneration in MK801-treated (10 mg/kg) female rat in the granular retrosplenial cortex (RSG) (A and B) and the hippocampus (C and D) evaluated with the A-Cu-Ag (left) and FJ-B (right) techniques at 72 h post-treatment. Similar patterns of degenerating somas in layer IV of the RSG were observed with both techniques. On the contrary, intense terminal degeneration in layer I of RSG and in the lacunosum molecular (Lmol) of CA1 was detected only with the A-Cu-Ag. Scale bar 100 μm in A–B and 500 μm in C–D.

inhibitory inputs, and suggest that cholinergic inputs to the RSC play a critical role in neuronal damage [12,22]. There are reports showing that on the contrary to rats, mice lack cortical cholinergic neurons, which could explain the reduced neurotoxic response [39].

In mice, we revealed two types of degenerating neurons after MK801 treatment, those in RSC and ENT with somatodendritic morphology compatible with excitotoxic cell death, and those in the MOB with apoptotic-like morphology. Similar observations were

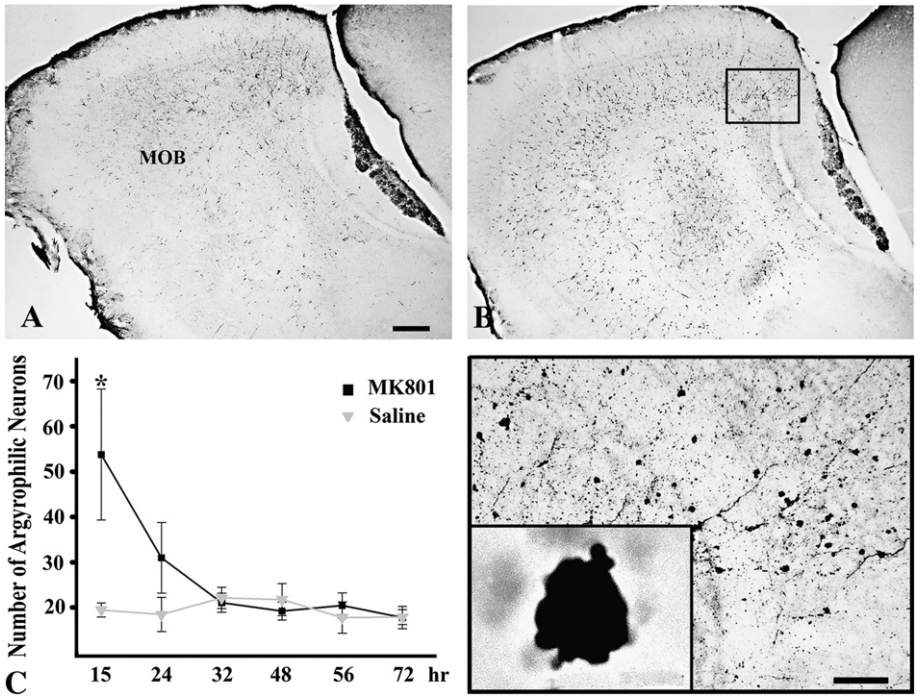

Fig. 6. Evaluation of MK801 toxicity in the mouse main olfactory bulb (MOB). (A) Saline-treated mice presented scattered apoptotic-like neurons in the MOB. Scale bar = 200 μm. (B) An increase in the amount of argyrophilic neurons was observed at 15 h post-MK801 treatment. Magnification and insert show how most of these degenerating neurons depicted an apoptotic-like appearance. Scale bar 50 μm. (C) A time-course evaluation at 15, 24, 32, 48, 56 and 72 h using the A-Cu-Ag technique determined the temporal pattern of expression of the neurodegeneration induced by MK801 (10 mg/kg) in mice. The two way ANOVA indicated that there was a significant effect of treatment ($F_{1,24} = 6.13$, $p < 0.03$), survival time ($F_{5,24} = 3.34$, $p < 0.02$) and treatment × survival time ($F_{5,24} = 3.52$, $p < 0.02$). Post hoc analysis indicated that only the MK801-treated animals at 15 h were different from saline ($p < 0.001$) and all the other groups.

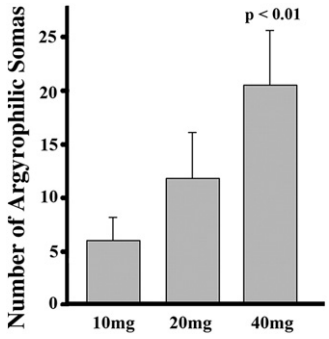

Fig. 7. Quantification of argyrophilic neurons in the RSC using subchronic treatments of different doses of MK801 in mice. Subchronic doses of 20 and 40 mg/kg of MK801 were applied and neurotoxicity was evaluated with A-Cu-Ag at 24 h after the last injection. A significant dose-dependent increase (ANOVA $F_{2,13} = 4{,}85$, $p < 0.03$) in the number of degenerating neurons in the RSC of subchronic-treated animals was observed (Fig. 6), in which the post hoc LSD analyses indicated that only the 40 mg/kg group was statistically different ($p < 0.01$).

previously reported in female rats treated with MK801 [8,9], indicating that different morphological patterns of neuronal death are preserved after MK801 treatment in these species. These observations suggest that the mechanism of neuronal cell death induced by MK801 is dependent on the intrinsic properties of a particular neuronal population. The olfactory bulb is the brain structure with the highest level of adult neurogenesis, mainly in the granular and periglomerular layers [26]. Interestingly it is in these layers where MK801-induced apoptotic cells are found. Recent studies have suggested that in developing stages (pre and early postnatal period) NMDA-receptor activation serves as a "trophic" survival signal in several brain regions [36] including the olfactory bulb [23]. The increase in apoptotic-like cells in the adult MOB after the treatment of NMDA-A could be due to the lack of the pro-survival-signaling of the NMDA receptors and thereby triggers an enhanced programmed cell death. Supporting this interpretation, there are reports where deprivation of olfactory sensorial pathways induced a dramatic increase of apoptosis of newly generated granule cells of the MOB [43,71].

Many studies [21] have analysed the excitotoxic cell death observed in the RSC, and propose that the neurodegeneration in this area is caused by the hypofunction of NMDA receptors, which abolish the inhibitory control over excitatory cholinergic and glutamatergic inputs to the RSC neuron. The disinhibition hyperactivates the RSC neuron causing multiple intracellular signals that induce hypermetabolism and, depending on how long the disruption lasts, cell death. A similar mechanism is suggested for other corticolimbic areas such as the ENT. However, the comprehension of the specificity of the layer affected (IV in RSC and III in ENT) and the neuronal circuits involved have still not been elucidated.

5. Conclusion

The fact that NMDA antagonists have the potential to induce significant damage to neuronal subpopulations in the rat brain has limited their application to palliate neuropathologic mechanisms in which they might have a potential therapeutic value (i.e., epilepsy, cerebral ischemia, ethanol abstinence, neuropathic pain, etc). This work shows that doses of MK801 that induce important neuronal damage in the rat brain promote only modest neurodegeneration in mice and that specific areas that presented neuronal death in rats were absent in mice. Also, significant sexually dimorphic effects of MK801-induced toxicity observed in rats were absent in mice, indicating that there exists an important disparity in the neurotoxic effects of NMDA-A drugs between these species. Thus, precaution must be taken when inferring results from rodent species to human beings, and further research is required to elucidate which species better models the effect of NMDA-A in humans.

Conflict of interest

No conflict of interest is declared by the authors.

Acknowledgements

This work has been supported by grants of Antorchas and Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) PICT# 05-12360 to JdeO and PICT# 06-01941 to AL. SdeO, and AL are career members of CONICET, and CB is a fellow from CONICET. The authors would like to thank Dr. Marcelo Rubinstein for generously providing the CF-1 mice strain. This work is dedicated to the memory of Dr. Jose S. de Olmos.

References

[1] P. Andiné, R. Widermark, R. Axelson, G. Nyberg, G. Olofsson, E. Martenson, M. Sandberg, Characterization of MK-801-induced behavior as a putative rat model of psychosis, J. Pharmacol. Exp. Ther. 290 (1999) 1393–1408.
[2] E. Asan, D.M. Yilmazer-Hanke, M. Eliava, M. Hantsch, K.P. Lesch, A. Schmitt, The corticotropin-releasing factor (CRF)-system and monoaminergic afferents in the central amygdala: investigations in different mouse strains and comparison with the rat, Neuroscience 131 (2005) 953–967.
[3] R.N. Auer, Effect of age and sex on N-Methyl-D-Aspartate antagonist-induced neuronal necrosis in rats, Stroke 27 (1996) 743–746.
[4] S.A. Benkovic, J.P. O'Callaghan, D.B. Miller, Regional neuropathology following kainic acid intoxication in adult and aged C57BL/6J mice, Brain Res. 1070 (2006) 215–231.
[5] S.A. Benkovic, J.P. O'Callaghan, D.B. Miller, Sensitive indicators of injury reveal hippocampal damage in C57BL/6J mice treated with kainic acid in the absence of tonic–clonic seizures, Brain Res. 1024 (2004) 59–76.
[6] G. Brosnan-Watters, T. Ogimi, D. Ford, L. Tatekawa, D. Gilliam, E.J. Bilsky, D. Nash, Differential effects of MK-801 on cerebrocortical neuronal injury in C57BL/6J, NSA, and ICR mice, Neuropsychopharmacol. Biol. Psychiatry 24 (2000) 925–938.
[7] G. Brosnan-Watters, D.F. Wozniak, A. Nardi, J.W. Olney, Parallel recovery of MK-801-induced spatial learning impairment and neuronal injury in male mice, Pharmacol. Biochem. Behav. 62 (1999) 111–122.
[8] A. Bueno, S. de Olmos, L. Heimer, J.S. de Olmos, NMDA-antagonist MK-801 induced neuronal degeneration in Wistar rat brain detected by the amino-cupric silver method, Exp. Toxicol. Pathol. 54 (2003) 319–334.
[9] A. Bueno, S. de Olmos, M. Manzini, N. Desmond, J.S. de Olmos, Strain differences in the neurotoxic sequelae of MK801 visualized with the amino-cupric silver method, Exp. Toxicol. Pathol. 55 (2003) 287–294.
[10] C. Chavez, A. Gogos, M.E. Jones, M. van den Buuse, Psychotropic drug-induced locomotor hyperactivity and prepulse inhibition regulation in male and female aromatase knockout (ArKO) mice: role of dopamine D1 and D2 receptors and dopamine transporters, Psychopharmacology 206 (2009) 267–279.
[11] S.R. Chen, G. Samoriski, H.L. Pan, Antinociceptive effects of chronic administration of uncompetitive NMDA receptor antagonists in a rat model of diabetic neuropathic pain, Neuropharmacology 57 (2009) 121–126.
[12] F. Colbourne, D. Rakić, R.N. Auer, The effects of temperature and scopolamine on N-methyl-D-aspartate antagonist-induced neuronal necrosis in the rat, Neuroscience 90 (1999) 87–94.
[13] D.N. D'Souza, R.E. Harlan, M.M. Garcia, Sexual dimorphic effect of morphine and MK-801: sex steroid-dependent and -independent mechanism, J. Appl. Physiol. 92 (2002) 493–503.
[14] J.S. de Olmos, C.A. Beltramino, S. de Olmos, Use of an amino-cupric-silver technique for the detection of early and semiacute neuronal degeneration caused by neurotoxicants, hypoxia, and physical trauma, Neurotoxicol. Teratol. 16 (1994) 545–561.
[15] S. de Olmos, C. Bender, J.S. de Olmos, A. Lorenzo, Neurodegeneration and prolonged immediate early gene expression throughout cortical areas of the rat brain following acute administration of dizocilpine, Neuroscience 164 (2009) 1347–1359.
[16] S. de Olmos, A. Bueno, C. Bender, A. Lorenzo, J. de Olmos, Sex differences and influence of gonadal hormones on MK801-induced neuronal degeneration in the granular retrosplenial cortex of the rat, Brain Struct. Funct. 213 (2008) 229–238.
[17] J.S. de Olmos, S.O.E. Ebbesson, L. Heimer, Silver methods for the impregnation of degenerating axoplasm, in: L. Heimer, M.J. RoBards (Eds.), Neuroanatomical Tract-tracing Methods, Plenum Press, 1981, pp. 117–170.
[18] X. Deng, B. Ladenheim, S. Jayanthi, J.L. Cadet, Methamphetamine administration causes death of dopaminergic neurons in the mouse olfactory bulb, Biol. Psychiatry 61 (2007) 1235–1243.
[19] G. Duncan, S. Miyamoto, H. Gu, J. Lieberman, B. Koller, J. Snouwaert, Alterations in regional brain metabolism in genetic and pharmacological models of reduced NMDA receptor function, Brain Res. 951 (2002) 166–176.
[20] G. Ellison, Competitive and non-competitive NMDA antagonists induce similar limbic degeneration, NeuroReport 5 (1994) 2688–2692.

[21] N.B. Farber, The NMDA receptor hypofunction model of psychosis, Ann. N.Y. Acad. Sci. 1003 (2003) 119–130.
[22] N.B. Farber, S.H. Kim, K. Dikranian, X.P. Jiang, C. Heinkel, Receptor mechanisms and circuitry underlying NMDA antagonist neurotoxicity, Mol. Psychiatry 7 (2002) 32–43.
[23] B.K. Fiske, P.C. Brunjes, NMDA receptor regulation of cell death in the rat olfactory bulb, J. Neurobiol. 47 (2001) 223–232.
[24] A.S. Fix, J. Ross, S.R. Stitzel, R.C. Switzer, Integrated evaluation of central nervous system lesions: stains for neurons, astrocytes, and microglia reveal the spatial and temporal features of MK-801-induced neuronal necrosis in the rat cerebral cortex, Toxicol. Pathol. 24 (1996) 291–304.
[25] X.M. Gao, G.I. Elmer, B. Adams-Huet, C.A. Tamminga, Social memory in mice: disruption with an NMDA antagonist and attenuation with antipsychotic drugs, Pharmacol. Biochem. Behav. 2 (2009) 236–242.
[26] E. Gould, How widespread is adult neurogenesis in mammals? Nat. Rev. Neurosci. 8 (2007) 481–488.
[27] G. Grant, H. Holländer, H. Aldskogius, Suppressive silver methods—a tool for identifying axotomy-induced neuron degeneration, Brain Res. Bull. 62 (2004) 261–269.
[28] S.M. Grauer, V.L. Pulito, R.L. Navarra, M.P. Kelly, C. Kelley, R. Graf, B. Langen, S. Logue, J. Brennan, L. Jiang, et al., Phosphodiesterase 10A inhibitor activity in preclinical models of the positive, cognitive, and negative symptoms of schizophrenia, J. Pharmacol. Exp. Ther. 331 (2009) 574–590.
[29] K. Hashimoto, Y. Fujita, M. Horio, S. Kunitachi, M. Iyo, D. Ferraris, T. Tsukamoto, Co-administration of a D-amino acid oxidase inhibitor potentiates the efficacy of D-serine in attenuating prepulse inhibition deficits after administration of dizocilpine, Biol. Psychiatry 65 (2009) 1103–1106.
[30] S. Herculano-Houzel, C.E. Collins, P. Wong, J.H. Kaas, Cellular scaling rules for primate brains, Proc. Natl. Acad. Sci. USA 104 (2007) 3562–3567.
[31] S. Herculano-Houzel, B. Mota, R. Lent, Cellular scaling rules for rodent brains, Proc. Natl. Acad. Sci. USA 103 (2006) 12138–12143.
[32] D. Hönack, W. Löscher, Sex differences in NMDA receptor mediated responses in rats, Brain Res. 620 (1993) 167–170.
[33] Z.C. Horvath, MK-801-induced neuronal damage in rats, Brain Res. 753 (1997) 181–195.
[34] G.H. Hur, W.C. Son, S. Shin, J.K. Kang, Y.B. Kim, Sex differences in dizocilpine (MK801) neurotoxicity in rats, Environ. Toxicol. Pharmacol. 7 (1999) 143–146.
[35] V. Jevtovic-Todorovic, J. Beals, N. Benshoff, J.W. Olney, Prolonged exposure to inhalational anesthetic nitrous oxide kills neurons in adult rat brain, Neuroscience 122 (2003) 609–616.
[36] A.M. Kaindl, C. Ikonomidou, Glutamate antagonists are neurotoxins for the developing brain, Neurotox. Res. 11 (2007) 203–218.
[37] L.V. Kalia, S.K. Kalia, M.W. Salter, NMDA receptors in clinical neurology: excitatory times ahead, Lancet Neurol. 7 (2008) 742–755.
[38] S. Kelly, A. Bieneman, J.B. Uney, J. McCulloch, Cerebral glucose utilization in transgenic mice overexpressing heat shock protein 70 is altered by dizocilpine, Eur. J. Neurosci. 15 (2002) 945–952.
[39] C.A. Kitt, C. Höhmann, J.T. Coyle, D.L. Price, Cholinergic innervation of mouse forebrain structures, J. Comp. Neurol. 341 (1994) 117–129.
[40] J. Kocerha, M.A. Faghihi, M.A. Lopez-Toledano, J. Huang, A.J. Ramsey, M.G. Caron, N. Sales, D. Willoughby, J. Elmen, H.F. Hansen, et al., MicroRNA-219 modulates NMDA receptor-mediated neurobehavioral dysfunction, Proc. Natl. Acad. Sci. USA 106 (2009) 3507–3512.
[41] D.J. Livy, S.E. Parnell, J.R. West, Blood ethanol concentration profiles: a comparison between rats and mice, Alcohol 29 (2003) 165–171.
[42] C.R. Majewski-Tiedeken, C.R. Rabin, S.J. Siegel, Ketamine exposure in adult mice leads to increased cell death in C3H, DBA2 and FVB inbred mouse strains, Drug Alcohol Depend. 92 (2008) 217–227.
[43] N. Mandairon, F. Jourdan, A. Didier, Deprivation of sensory inputs to the olfactory bulb up-regulates cell death and proliferation in the subventricular zone of adult mice, Neuroscience 119 (2003) 507–516.
[44] H. Matsuki, Y. Shirayama, K. Hashimoto, A. Tanaka, Y. Minabe, Effects of age and gender on the expression of brain-derived neurotrophic factor mRNA in rat retrosplenial cortex following administration of dizocilpine, Neuropsychopharmacology 25 (2001) 258–266.
[45] B. Moghaddam, M.E. Jackson, Glutamatergic animal models of schizophrenia, Ann. N.Y. Acad. Sci. 1003 (2003) 131–137.
[46] C.J. Morgan, L. Muetzelfeldt, H.V. Curran, Consequences of chronic ketamine self-administration upon neurocognitive function and psychological wellbeing: a 1-year longitudinal study, Addiction 105 (2009) 121–133.
[47] C.J. Morgan, L. Muetzelfeldt, H.V. Curran, Ketamine use, cognition and psychological wellbeing: a comparison of frequent, infrequent and ex-users with polydrug and non-using controls, Addiction 104 (2009) 77–87.
[48] T. Nabeshima, K. Kitaichi, Y. Noda, Functional changes in neuronal systems induced by phencyclidine administration, Ann. N.Y. Acad. Sci. 801 (1996) 29–38.
[49] T. Nabeshima, K. Yamaguchi, H. Furukawa, T. Kameyama, Role of sex hormones in sex-dependent differences in phencyclidine-induced stereotyped behaviors in rats, Eur. J. Pharmacol. 105 (1984) 197–206.
[50] K.K. Noguchi, B. Nemmers, N.B. Farber, Age has a similar influence on the susceptibility to NMDA antagonist-induced neurodegeneration in most brain regions, Brain Res. Dev. Brain Res. 158 (2005) 82–91.
[51] M.F. O'Neill, C.A. Hicks, G. Shaw, T. Parameswaran, G.P. Cardwell, M.J. O'Neill, Effects of 5-hydroxytryptamine2 receptor antagonism on the behavioral activation and immediate early gene expression induced by dizocilpine, J. Pharmacol. Exp. Ther. 287 (1998) 839–846.
[52] J. Olney, J. Labruyere, M. Price, Pathological changes induced in cerebrocortical neurons by phencyclidine and related drugs, Science 244 (1989) 1360–1362.
[53] J.W. Olney, J.W. Newcomer, N.B. Farber, NMDA receptor hypofunction model of schizophrenia, J. Psychiatr. Res. 33 (1999) 523–533.
[54] G. Paxinos, K.B. Franklin, The Mouse Brain in Stereotaxic Coordinates, EditionAcademic Press, San Diego, 2001.
[55] G. Paxinos, C. Watson, The Rat Brain Stereotaxic Coordinates, EditionAcademic Press, San Diego, 2007.
[56] M.R. Picciotto, K. Wickman, Using knockout and transgenic mice to study neurophysiology and behavior, Physiol. Rev. 78 (1998) 1131–1163.
[57] M. Rubinstein, C. Cepeda, R.S. Hurst, J. Flores-Hernandez, M.A. Ariano, T. Falzone, L.B. Kozell, C.K. Meshul, J.R. Bunzow, M.J. Low, et al., Dopamine D_4 receptor-deficient mice display cortical hyperexcitability, J. Neurosci. 21 (2001) 3756–3763.
[58] M. Rubinstein, J.S. Mogil, M. Japón, E.C. Chan, R.G. Allen, M.J. Low, Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis, PNAS 93 (1996) 3995–4000.
[59] L.C. Schmued, K.J. Hopkins, Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration, Brain Res. 874 (2000) 123–130.
[60] F.R. Sharp, P. Jasper, J. Hall, L. Noble, S.M. Sagar, MK801 and ketamine induce heat shock protein hsp72 in injured neurons in posterior cingulate and retroesplenial cortex, Ann. Neurol. 30 (1991) 801–809.
[61] F.R. Sharp, M. Tomitaka, M. Bernaudin, S. Tomitaka, Psychosis: pathological activation of limbic thalamocortical circuits by psychomimetics and schizophrenia? Trends Neurosci. 24 (2001) 330–334.
[62] T.W. Stone, J.I. Addae, The pharmacological manipulation of glutamate receptors and neuroprotection, Eur. J. Pharmacol. 447 (2002) 285–296.
[63] R.C. Switzer, Application of silver degeneration stains for neurotoxicity testing, Toxicol. Pathol. 28 (2000) 70–83.
[64] C.A. Tamminga, A.C. Lahti, D.R. Medoff, X.M. Gao, H.H. Holcomb, Evaluating glutamatergic transmission in schizophrenia, Ann. N.Y. Acad. Sci. 1003 (2003) 113–118.
[65] J. Väisänen, T. Saarelainen, E. Koponen, E. Castrén, Altered trkB neurotrophin receptor activation does not influence the N-methyl-D-aspartate receptor antagonist-mediated neurotoxicity in mouse posterior cingulate cortex, Neurosci. Lett. 350 (2003) 1–4.
[66] T. van Groen, I. Kadish, J.M. Wyss, Species differences in the projections from the entorhinal cortex to the hippocampus, Brain Res. Bull. 57 (2002) 553–556.
[67] L.N. Wei, Transgenic animals as new approaches in pharmacological studies, Annu. Rev. Pharmacol. Toxicol. 37 (1997) 119–141.
[68] W.D. Wessinger, Sexual dimorphic effects of chronic phencyclidine in rats, Eur. J. Pharmacol. 277 (1995) 107–112.
[69] D. Wozniak, K. Dikranian, M. Ishimaru, A. Nardi, T. Corso, T. Tenkova, J. Olney, A. Fix, Disseminated corticolimbic neuronal degeneration induced in rat brain by MK-801: potential relevance to Alzheimer disease, Neurobiol. Dis. 5 (1998) 305–322.
[70] D.F. Wozniak, G. Brosnan-Watters, A. Nardi, M. McEwen, T.D. Corso, J.W. Olney, A.S. Fix, MK-801 neurotoxicity in male mice: histologic effects and chronic impairment in spatial learning, Brain Res. 707 (1996) 165–179.
[71] M. Yamaguchi, K. Mori, Critical period for sensory experience-dependent survival of newly generated granule cells in the adult mouse olfactory bulb, Proc. Natl. Acad. Sci. USA 102 (2005) 9697–9702.