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Neurohistopathology of postnatal IP dose of MK-801 in juvenile rats

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Abstract

Characterization of low occurrence of isolated minimal or mild degeneration (DEG) and apoptosis (APO) in control brains is imperative to determine the difference between relationship of normal changes and the effects of a test article. The effects of MK-801 (a non-competitive NMDA antagonist with known neurotoxic properties) on the development of juvenile Sprague-Dawley rat brains were characterized. The MK-801 group received a single dose (3 mg/kg, IP) on PNDs 7, 8, 9, 11 13, 16, 23, 39, 69 or 111). The control article (distilled water, 5 mL/Kg, oral gavage) was dosed daily from PND 7 until the day prior to termination. Thirty or twenty rats per sex per time-point had brains perfused/ harvested on PNDs 8, 9, 10 and 12, or on PNDs 14, 17, 24, 40, 71 and 113, respectively. The brains were embedded, coronally sectioned at 40µ (through the entire brain length), and stained with amino cupric silver (DEG changes) and caspase 9 stain (APO). Neurohistopathological evaluation of the entire brain for DEG and APO was performed.

APO and DEG was prevalent in numerous brain regions of younger control animals (primarily from PND 8 through PND 24). Therefore DEG and APO treatment effects are changes present in a given brain area, specific for each PND, that were greater in incidence or severity than control values. Increased DEG and APO were present in MK-801 animals at all time-points. Increased DEG and APO in MK-801 rats were present in a large number of brain sites in the earlier PNDs with severities ranging from minimal to marked while these changes at later PNDs were substantially diminished. Females generally had more brain sites involved than did males especially at the later PNDs. By PND 40, males typically had only 3 or 4 sites at which DEG was observed. APO that was present in MK-801 rats was generally distinct and unequivocal when compared with the control animals. Based on this data, isolated minimal or mild occurrences of DEG and APO should not be considered treatment related events in juvenile Sprague-Dawley rats.

Introduction

MK-801 (dizocilpine maleate) is a potent non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist known to induce neurotoxicity (neuronal degeneration and apoptosis) in the rat while it has also been shown to be neuroprotective in several animal models of pathologies that involve hyperactivation of the MDMA receptors, such as stroke, ischemia, epilepsy, and neuropathic pain (Bender, 2010).

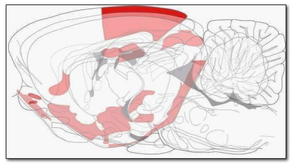
Olney *et al.* (1989) observed transient intracytoplasmic vacuoles in rat brains following MK-801 administration. Olney *et al.* (1990, 1993) further observed that MK-801 caused neuronal degeneration that was co-located at vacuole sites. Importantly, neurodegeneration was also found in regions of the brain distant from the vacuole sites. Jevtovic-Todorovic, V., *et al.* (2000, 2003a, 2003b) demonstrated that exposure of the developing brain to anesthetic agents that block NMDA receptors during the period of synaptogenesis can trigger widespread apoptotic neurodegeneration. During development in the rat, especially during postnatal days (PND) 7–14, the central nervous system exhibits enhanced susceptibility to the toxic effects of modulation of the NMDA receptor system. This enhanced susceptibility has been suggested to be derived from the increased expression of specific NMDA receptor subunits (Miyamoto *et al.,* 2001). Neonatal rats administered the MK-801 during the first two weeks of life have been shown to develop abnormal axonal arborization in the retinal connections to the superior colliculus, interfering with normal visual responses (Haberny, 2002).

The current data describes the neurodegeneration (DEG) and apoptosis (APO) in the juvenile rat brain caused by acute MK-801 treatment at different intervals. Figure 1 details the brain regions in the adult rat where acute MK-801 treatment was associated with neurodegeneration and the timing of those findings. The peak observable time for neurodegeneration in adult rat lasts ~3 days as shown in Figure 2. The window of opportunity for measurable neurodegeneration in the developing brain shrinks from days to hours for the younger ages (Figure 3). The sites and incidence at which DEG and APO was observed in MK-801 treated male and female rats are shown in Tables 1 through 4. Examples of AmCuAg-stained DEG in the Thalamic Nuclei and Retrosplenial Cortex on PNDs 8 and 14 are shown in Figures 4 through 7 and examples of Caspase 9-stained APO in the Thalamic Nuclei on PNDs 8 and 14 are shown in Figures 8 and 9. Some control animals exhibited DEG and APO in several brain sites that were not involved in remaining animals of that sex/age group. This occurred at the earlier PNDs, most prominently from PND 8 through PND 24. The location and severity of the lesions are presented (Table 5).

Methods

The MK-801 group received a single dose (3 mg/kg, IP) on PNDs 7, 8, 9, 11, 13, 16, 23, 39, 69 or 111). The control group (distilled water, 5 mL/Kg, oral gavage) was dosed daily via gavage from PND 7 until the day prior to termination. The age at initiation of dosing and the duration is the period of rat development which corresponds with the ages of a pediatric population (from newborn through adult). Thirty or 20 rats per sex per interval had brains perfused/ harvested on PNDs 8, 9, 10 and 12, or on PNDs 14, 17, 24, 40, 71 and 113, respectively. The brains were embedded, coronally sectioned at 40µ (through the entire cerebrum length) using MultiBrain® Technology (NSALabs®), and stained with amino cupric silver and caspase 9 stain. The disintegrative degeneration stain (DEG) was chosen for this study because it has been demonstrated to be effective in assessing for the neurodegeneration associated with Olney lesions. Brains (PNDs 8 to 40 only) were evaluated for apoptosis using a caspase-9 antibody stain because increased apoptosis is believed to be the main adverse effect for this drug class (N-methyl-D-aspartate [NMDA] receptor antagonist neuropathology) at this age in rats of both genders. Degeneration and apoptosis were graded using a 4 point scale i.e. 1 (minimal), 2 (mild), 3 (moderate), and 4 (marked). Location of degeneration and apoptosis (in excess of vehicle control levels) within the brain was recorded. Neurohistopathological evaluation for DEG and APO of the entire cerebrum was performed.

Figure 1: Acute neurodegeneration profile for MK801 in adult rats



- Location: retrosplenial cortex; dentate gyrus; pyriform cortex; tenia tecta; amygdala; entorhinal cortex
- Timing
  - 1 day post-administration: scattered degeneration, mainly in retrosplenial cortex
  - 2 days post-administration: darkly stained neurons observed in all regions listed above
  - 3 days post-administration: peak observability of neurodegeneration
  - 4 days post-administration: degeneration diminished in many brain regions, but still high in retrosplenial cortex
  - 7 days post-administration: degeneration barely detectable

Figure 2: The peak observable time of degeneration following administration of MK-801 to adult rat lasts ~ 3 days

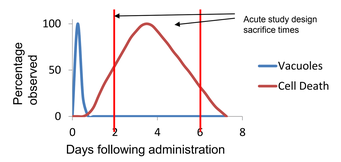


Figure 3: In the developing brain the window of opportunity for measurable neurodegeneration is shrunk from days to hours

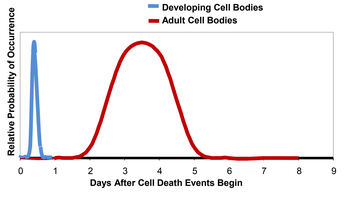


Table 1: Sites at which degeneration was observed in MK-801 male rats using the AmCuAg degeneration stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	16	16	16	16	16	16	13	13	21
Number Examined	16	16	16	16	16	16	17	16	16	21
Entorhinal cortex	12	12	15	9	8	0	5	0	0	1
Cingulate cortex	16	16	13	16	11	0	0	0	7	15
Lateral cortex anterior	16	16	16	13	0	0	0	0	0	0
Prelimbic cortex	8	13	4	11	1	0	0	0	0	0
Infralimbic/prelimbic cortex	0	0	0	1	1	0	0	0	0	0
Piriform cortex	3	2	0	9	9	6	0	7	1	5
Globus pallidus	16	11	12	11	2	0	0	0	0	0
Thalamic nuclei	16	16	16	14	12	0	0	0	0	0
Presubiculum	9	14	15	9	2	0	0	0	0	0
Postsubiculum	13	16	16	15	1	0	0	0	0	0
Hippocampus	16	15	15	16	9	0	0	0	0	0
Somatosensory cortex	0	0	0	1	2	0	0	0	0	0
Retrosplenial cortex	16	16	16	16	16	5	0	6	13	21
Mammillary nuclei	14	11	14	15	15	7	0	0	0	0
Lateral cortex middle/ posterior	15	16	16	16	15	0	0	0	0	0
Frontal association cortex	14	13	11	12	1	0	0	0	0	0
Septal nuclei	7	4	6	5	5	0	0	0	0	0
Accumbens	16	16	16	13	1	0	0	0	0	0
Pontine nuclei	15	16	10	11	12	2	0	0	0	0
Motor cortex	16	15	15	16	1	0	0	0	0	0
Caudate putamen	16	16	15	12	0	0	0	0	0	0
Dentate	1	4	0	4	3	7	0	2	0	0
Hypothalamic nuclei	15	12	7	9	4	0	0	0	0	0
Amygdaloid nuclei	16	15	14	10	0	0	0	0	0	0
Dorsal peduncular nucleus	6	9	2	9	0	0	0	0	0	0
Dorsal peduncular cortex	0	0	2	0	0	0	0	0	0	0
Bed nuclei stria terminalis	2	1	0	2	2	0	0	0	0	0
Dorsal tenia tecta	9	7	5	3	0	0	0	0	0	0
Orbital cortex	1	5	5	1	0	0	0	0	0	0
Ventral pallidum	1	0	1	0	1	0	0	0	0	0
Claustrum	1	1	0	0	0	0	0	0	0	0
Navicular nuclei	0	1	1	0	0	0	0	0	0	0

Table 2: Sites at which degeneration was observed in MK-801 female rats using the amcuag degeneration stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	16	16	16	16	16	18	16	16	21
Number Examined	16	16	16	16	16	16	18	16	16	21
Olfactory lobe	6	8	6	15	7	5	8	8	13	8
Olfactory bulb	0	0	0	0	8	5	9	8	0	0
Olfactory nucleus	0	1	0	0	0	0	1	0	0	0
Olfactory tubercle	0	0	0	2	0	0	0	0	0	0
Entorhinal cortex	9	11	13	14	13	3	5	9	15	20
Cingulate cortex	15	14	14	16	14	0	0	1	10	19
Lateral cortex anterior	15	15	16	12	0	0	0	0	0	0
Prelimbic cortex	9	8	7	12	5	0	0	0	4	1
Piriform cortex	1	0	2	5	12	12	2	16	16	21
Globus pallidus	14	8	13	7	0	0	0	0	0	0
Thalamic nuclei	16	15	15	16	13	5	0	0	0	0
Presubiculum	11	15	12	9	4	0	0	0	0	0
Postsubiculum	13	15	15	16	2	0	0	0	0	0
Hippocampus	16	16	15	16	9	0	0	1	8	4
Somatosensory cortex	0	0	0	0	0	0	0	0	0	1
Retrosplenial cortex	16	16	16	16	16	4	0	16	16	21
Mammillary nuclei	13	13	13	13	13	12	0	0	0	0
Lateral cortex middle/ posterior	14	16	16	16	15	0	0	0	1	0
Frontal association cortex	12	13	12	11	2	0	0	0	0	0
Septal nuclei	6	4	4	1	3	0	0	0	1	4
Accumbens	14	15	15	13	0	0	0	0	0	0
Pontine nuclei	10	14	10	14	12	0	0	0	0	0
Motor cortex	16	15	16	13	0	0	0	0	0	0
Caudate putamen	15	15	14	10	0	0	0	0	0	0
Dentate	1	2	0	4	7	8	2	1	7	12
Hypothalamic nuclei	12	9	8	9	0	0	0	0	0	0
Amygdaloid nuclei	16	15	12	6	0	0	0	0	4	0
Dorsal peduncular nucleus	0	3	4	1	0	0	0	0	5	0
Dorsal peduncular cortex	7	2	1	6	1	0	0	0	0	4
Bed nuclei stria terminalis	0	0	0	2	1	0	0	0	0	0
Dorsal tenia tecta	6	9	1	0	0	0	0	0	3	0
Orbital cortex	1	0	2	2	0	0	0	0	0	0
Ventral pallidum	1	0	0	0	0	0	0	0	0	0
Claustrum	2	1	0	0	0	0	0	0	0	0
Cortex layers 1-4	0	0	0	0	0	0	0	0	4	0

Table 3: Sites at which degeneration was observed in MK-801 male rats using the caspase apoptosis stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	16	16	16	17	12	3	1	0	0
Number Examined	16	16	16	16	17	16	17	16	16	21
Olfactory lobe	9	7	10	13	8	2	1	0	0	0
Olfactory nucleus	0	0	0	1	1	0	1	0	0	0
Olfactory bulb	0	0	0	0	7	1	1	0	0	0
Entorhinal cortex	13	13	12	10	10	0	0	0	0	0
Cingulate cortex	15	15	16	9	7	0	0	0	0	0
Lateral cortex anterior	8	14	13	5	4	0	0	0	0	0
Prelimbic cortex	3	6	3	10	1	0	0	0	0	0
Piriform cortex	0	0	1	4	9	8	0	0	0	0
Globus pallidus	3	0	1	2	0	0	0	0	0	0
Thalamic nuclei	15	14	15	14	12	0	0	1	0	0
Presubiculum	2	7	8	6	1	0	0	0	0	0
Postsubiculum	14	15	15	14	0	0	0	0	0	0
Hippocampus	16	16	16	15	3	0	0	0	0	0
Retrosplenial cortex	15	16	16	16	17	4	0	0	0	0
Mammillary nuclei	2	0	3	7	16	10	0	0	0	0
Lateral cortex middle/ posterior	8	16	16	14	0	0	0	0	0	0
Frontal association cortex	6	8	2	3	0	0	0	0	0	0
Septal nuclei	5	3	3	1	2	0	0	0	0	0
Accumbens	12	8	6	4	1	0	0	0	0	0
Pontine nuclei	1	6	6	10	10	5	0	1	0	0
Motor cortex	10	13	12	5	0	0	0	0	0	0
Caudate putamen	10	7	6	3	0	0	0	0	0	0
Hypothalamic nuclei	1	3	3	8	0	0	0	0	0	0
Amygdaloid nuclei	7	8	9	5	0	0	0	0	0	0
Dorsal peduncular nucleus	3	3	0	0	0	0	0	0	0	0
Dorsal peduncular cortex	0	0	0	2	0	0	0	0	0	0
Bed nuclei stria terminalis	0	0	0	1	0	0	0	0	0	0
Dorsal tenia tecta	2	4	1	0	0	0	0	0	0	0
Orbital cortex	0	0	0	1	1	0	0	0	0	0
Visual cortex	0	0	0	0	1	0	0	0	0	0
Corpus callosum	1	0	1	0	0	0	0	0	0	0
Hypothalamus	0	0	0	0	2	0	0	0	0	0

Table 4: Sites at which degeneration was observed in MK-801 female rats using the caspase apoptosis stain

Age (Postnatal days)	8	9	10	12	14	17	24	40	71	113
No.Animals with Lesions	16	15	16	16	16	15	11	11	16	21
Number Examined	16	16	16	16	16	16	18	16	16	21
Olfactory lobe	7	6	9	11	8	3	0	6	0	0
Olfactory bulb	0	0	0	0	7	2	10	4	0	0
Entorhinal cortex	10	10	12	9	13	0	0	0	0	0
Cingulate cortex	14	13	15	11	9	0	0	0	0	0
Lateral cortex anterior	8	14	14	2	0	0	0	0	0	0
Prelimbic cortex	2	2	5	6	3	0	0	0	0	0
Infralimbic/prelimbic cortex	0	0	0	0	1	0	0	0	0	0
Piriform cortex	0	0	2	2	10	9	4	7	0	0
Globus pallidus	0	0	5	1	0	0	0	0	0	0
Thalamic nuclei	15	11	16	12	14	0	0	0	0	0
Presubiculum	5	7	11	3	0	0	0	0	0	0
Postsubiculum	14	15	16	10	3	0	0	0	0	0
Hippocampus	15	15	16	13	7	0	0	0	0	0
Retrosplenial cortex	15	11	16	15	15	3	0	0	0	0
Mammillary nuclei	3	2	5	8	13	12	0	0	0	0
Lateral cortex middle/ posterior	12	15	16	13	2	0	0	0	0	0
Frontal association cortex	4	4	4	3	0	0	0	0	0	0
Septal nuclei	1	2	2	0	1	0	0	0	0	0
Accumbens	4	9	7	2	1	0	0	0	0	0
Pontine nuclei	2	6	3	10	10	3	0	0	0	0
Motor cortex	10	11	14	5	0	0	0	0	0	0
Caudate putamen	4	3	6	3	0	0	0	0	0	0
Dentate	0	0	0	0	0	1	0	0	0	0
Hypothalamic nuclei	0	0	1	4	4	0	0	0	0	0
Amygdaloid nuclei	7	5	12	3	0	0	0	0	0	0
Dorsal peduncular nucleus	0	0	0	0	0	0	0	0	0	0
Dorsal peduncular cortex	1	2	0	0	0	0	0	0	0	0
Bed nuclei stria terminalis	0	0	0	0	0	0	0	0	0	0
Dorsal tenia tecta	1	5	0	0	0	0	0	0	0	0
Orbital cortex	0	1	0	0	1	0	0	0	0	0
Visual cortex	0	0	0	0	1	0	0	0	0	0
Corpus callosum	2	0	1	0	0	0	0	0	0	0
Subiculum	0	0	0	0	2	0	0	0	0	0
Anterior commissure	0	0	1	0	0	0	0	0	0	0
Fimbria hippocampus	1	0	0	0	0	0	0	0	0	0

Table 5: Sites at which degeneration was observed in vehicle control rats

Degeneration and to a greater extent, apoptosis, were present in numerous brain sites of males and females from vehicle control groups at earlier PNDs, most prominently from PND 8 through PND 24.

Males Site	PND	Incidence	Severity Minimal	Severity Mild
Accumbens	9	2/10	1/10	1/10
Pontine nuclei	9	2/10	0/10	1/10
Piriform cortex	14	1/10	1/10	0/10
Dentate	17	6/10	6/10	0/10
Piriform cortex	24	10/10	5/10	5/10

Females Site	PND	Incidence	Severity Minimal	Severity Mild
Accumbens	9	8/10	8/10	0/10
Entorhinal cortex	9	1/10	1/10	0/10
Lateral cortex middle/posterior	9	1/10	1/10	0/10
Caudate Putamen	9	1/10	1/10	0/10
Caudate Putamen	10	1/10	0/10	1/10
Thalamic nuclei	10	1/10	0/10	1/10
Corpus callosum	10	1/10	0/10	1/10
Dentate	17	8/10	7/10	1/10
Piriform cortex	24	9/9	3/9	6/9

Figure 4a and b: Thalamic nuclei, PND 8, AmCuAg degeneration stain

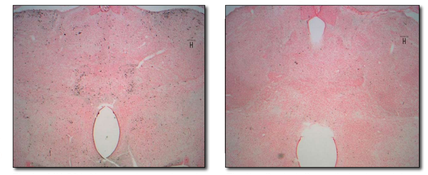


Fig 4a. MK-801; Note greater number of Thalamic Nuclei involved as compared to PND 14. Calibration mark = 50µ

Fig 4b. Control; Same thalamic nuclei as shown in Fig 4a. Black dots are RBCs in capillaries. Calibration mark = 50µ

Figure 5a and b: Retrosplenial cortex, PND 8, AmCuAg degeneration stain

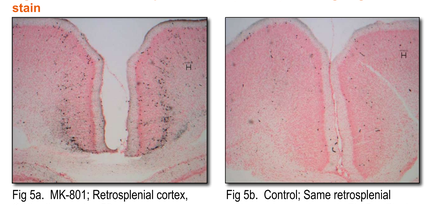


Fig 5a. MK-801; Retrosplenial cortex, Calibration mark = 50µ

Fig 5b. Control; Same retrosplenial cortex region as shown in Fig 5a. Black dots are RBCs in capillaries. Calibration mark = 50µ

Figure 6a and b: Thalamic nuclei, PND 14, AmCuAg degeneration stain

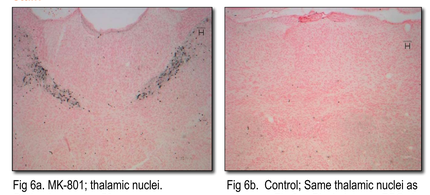


Fig 6a. MK-801; thalamic nuclei. Calibration mark = 50µ

Fig 6b. Control; Same thalamic nuclei as shown on Fig 6a. Black dots are RBCs in capillaries

Figure 7a and b: Retrosplenial cortex, PND 14, AmCuAg degeneration stain

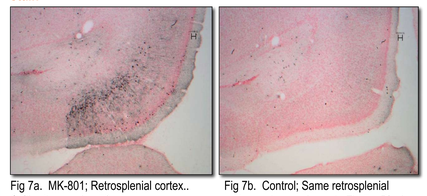


Fig 7a. MK-801; Retrosplenial cortex.. Calibration mark = 50µ

Fig 7b. Control; Same retrosplenial cortex region as shown in Fig 7a. Black dots are RBCs in capillaries. Calibration mark = 50µ

Figure 8a and b: Thalamic nuclei, PND 8, caspase stain

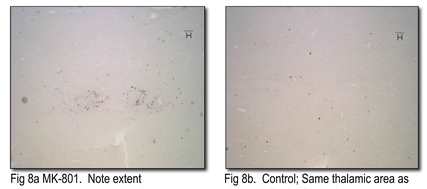


Fig 8a MK-801. Note extent of involvement as compared to degeneration stain (Fig 5a). Calibration mark = 50µ

Fig 8b. Control; Same thalamic area as shown in Fig 8a Black dots represent background staining. Calibration mark = 50µ

Figure 9a and b: Thalamic nuclei, PND 14, caspase stain

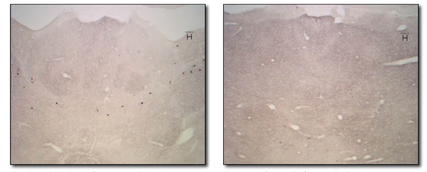


Fig 9a. MK-801; Same nuclei as in Fig 6a, Calibration mark = 50µ

Fig 9b. Control; Same thalamic area as shown in Fig 9a. Calibration mark = 50µ

Conclusions

DEG and APO were present in numerous brain sites in MK-801 treated rats in the earlier PNDs (PNDs 8 through 14), intermediate at PND 17, and few sites were observed at PNDs 24, 40, 71 and 113.

Females generally had more brain sites involved than did males especially at the later PNDs but were generally equivalent in the early PNDs. By PND 40, males typically had only 3 or 4 sites at which DEG was observed.

DEG and APO in early neonatal rats changes rapidly (within a day); evaluation of changes resulting from administration of a test chemical must account for DEG and APO as seen in age matched controls.

DEG (as shown by the CuAg degeneration stain) was generally more sensitive in detecting changes as compared to APO (as shown in the caspase 9 stain) in both numbers of involved sites and intensity of changes within involved sites.

APO that was present in MK-801 treated rats was generally distinct and unequivocal when compared with the control rats.

Based on this data, isolated minimal or mild occurrences of DEG and APO should not be considered treatment related events in juvenile Sprague-Dawley rats.

[1]Huntingdon Life Sciences, [2]MW ToxPath Sciences LLC, [3]NeuroScience Associates, Inc.