

KAINIC ACID LESION-INDUCED DEFICITS ON COGNITIVE PERFORMANCE IN WISTAR RATS

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Keywords: dopamine, kainic acid, memory, substantia nigra, ventral tegmental area

Abstract: The effects of lesioning the ventral tegmental area or substantia nigra pars reticulata by means of bilateral microinjections of two doses of kainic acid (50 ng/250 nl and 100 ng/500 nl) were investigated to clarify the role of the mesotelencephalic dopamine system in learning and memory processes. Our findings suggest that ventral tegmental area and substantia nigra dopaminergic neurons play an important role in retention of both short-term memory, tested in the Y-maze task and long-term memory evaluated with the multi-trial passive avoidance test, without affecting memory acquisition. As compared to short-term memory, long-term memory is more susceptible to the decreased dopamine level in nervous structures involved in processing and storage of information.

INTRODUCTION

Previous studies have suggested that Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by abnormal motor signs, akinesia, rigidity, tremor, and learning and memory deficits (Hefco et al., 2003; Piallat et al., 1996; Takada et al., 2000). It is the consensus that the pathogenesis of PD is the consequences of the loss on the dopaminergic neurons in the substantia nigra and dopamine depletion in the striatum (Piallat et al., 1996). Current neurosurgical strategies target overactive brains regions including the subthalamic nucleus, globus pallidus and thalamus to control various symptoms of PD. Substantia nigra pars reticulata (SNr) also becomes overactive in PD and there are few surgical studies in parkinsonian primates (Henderson et al., 2005). The mesocortical dopamine fibers predominantly arise from the ventral tegmental area (VTA), although some originate in the dorsal and medial aspects of the substantia nigra. These mesocortical dopamine fibers primarily innervate the medial prefrontal cortex, the anterior cingulate cortex, and the suprarhinal cortex. Thus, it can be appreciated that there is a significant degree of heterogeneity in the anatomic origins and terminal projection loci of the mesotelencephalic dopamine system (Gardner et al., 2000). This heterogeneity is far too often ignored by behavioral and cognitive neuroscientist developing models of mesotelencephalic dopamine function to explain human neuropsychiatric diseases such as schizophrenia, mania, drug addiction, and attention deficit/hyperactivity disorder. The dopaminergic system is strong candidate for mediating novelty acquisition and synaptic plasticity. The dopaminergic center in the brain is the ventral tegmental area (VTA). The VTA and hippocampus have been proposed to form a functional loop designed to detect novelty. This novelty signal would then gate behaviorally relevant information into long-term memory (Lemon et al., 2006; Lisman et al., 2005). There are also studies which have shown that kainic acid lesions enhanced locomotor responses to novelty (Bardgett et al., 1997), induced long-term loss of hippocampal plasticity manifesting as reduce capacity for long term potentiation and impaired spatial learning (Lynch et al., 2000). However, there are also studies which have shown that local microinjections of kainic acid clear induced impairment in measures of learning and memory, particularly so in bilaterally lesioned animals (Marien et al., 2004). In the present study, we investigated the role of neuronal system from substantia nigra pars reticulata (SNr) and ventral tegmental area (VTA) on working memory (which is a form of short-term memory) and reference memory (which is a form of long-term memory) performance in radial 8 arm-maze task by cell bodies lesions in these brain areas with kainic acid which destroys only the soma of neurons without affecting the passing fibers. Our data suggests that neurons in both SNr and VTA have a facilitatory role in short-term memory and long-term memory retention.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250 ± 25 g at the start of the experiment were used. The animals were housed in a temperature- and light-controlled room (22 °C, a 12-h cycle starting at 08:00 h) and were fed and allowed to drink water ad libitum. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Romania and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC).

Neurosurgery

All surgical procedures were conducted under aseptic conditions, under sodium pentobarbital (45mg/kg b.w., i.p., SIGMA) anesthesia. Rats were mounted in the stereotaxic apparatus with the nose oriented 110 below horizontal zero plane. Substantia nigra (SNr) was lesioned bilaterally by stereotaxic microinjection of kainic acid (25ng/250nl, SIGMA) at a rate of 100nl/min. After each infusion the syringe was left in place for 1 min before being removed to allow the adequate diffusion of the solution. The following coordinates were used: 5.5 mm posterior to bregma; 1.8 mm to the midline; 7.4 mm ventral to the surface of the cortex. For lesion of ventral tegmental area, the same dose of kainic acid

was used: 25ng/250nl (SIGMA). The following coordinates were used: 5.3-5.6 mm posterior to bregma; 0.5 mm lateral to the midline; 7.3 mm ventral to the surface of the cortex (Paxinos and Watson, 2005). The sham-operated groups were injected with the same volume of saline. Learning and memory tests were began 2 weeks after the neurosurgery.

Y-maze task

Short-term memory was assessed by spontaneous alternation behavior in the Y-maze task. The Y-maze used in the present study consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. The rat was placed at the end of one arm and allowed to move freely through the maze for 8 min. The time limit in Y-maze test was 8 min., and every session was stopped after 8 min. An arm entry was counted when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2 and percent spontaneous alternation was calculated as (actual alternations/maximum alternations) X 100 (Hefco et al., 2003; Hritcu et al., 2007). Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

Step-through passive avoidance task

In brief, a step-through type passive avoidance apparatus consisting of two compartments (25×15×15 cm high), one illuminated and one dark, both equipped with a grid floor was used. The two compartments were separated by a guillotine door. In the acquisition trials, each rat was placed in the illuminated compartment; when the animal entered the dark compartment, the door was closed and an inescapable footshock (0.3 mA, 5 s) was delivered through the grid floor. The rat was removed after receiving the foot shock and was placed back into the light compartment. The door was again opened 30 s later to start the next trial. The training continued until the rat stayed in the light compartment for a 120-s period on a single trial. The rats were given 3-5 trials and trained to avoid punishment (remain on shock-free zone) for at least 60 s (acquisition trial). After 24 h, each rat was placed in the light compartment and the step-through latency was recorded until 300 s had elapsed (retention trial). The step-through latency in the retention trial was used as the index of retention of the training experience (Hefco et al., 2003; Hritcu et al., 2007). Longer retention latencies were interpreted as indicating better retention of the training experience.

Statistical analysis

The results were analyzed statistically by means of Student's t-test. Values of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSIONS

1. Effects of kainic acid-induced SNr and VTA neuron lesion on learning and memory

Lesioning of the substantia nigra neurons with a low dose of kainic acid (50 ng) induced no significant changes in step-through latency 24 h after the acquisition (Fig. 1). High doses of kainic acid (100 ng) induced a significant decrease in step-through latency 24 h after the acquisition (Fig. 1) suggesting that especially lesioning dopaminergic neurons impaired long-term memory retention. On the short-term memory (tested by means of Y-maze task) substantia nigra lesions with low doses of kainic acid produced a nonsignificant decrease in spontaneous alternation % (Fig. 2). Lesioning of substantia nigra with high doses of kainic acid (100 ng) significantly increased the number of arm entries and decreased spontaneous alternation % (Fig. 2), suggesting that the lesion impaired short-term memory. As can be deduced from the number of arm entries (Fig. 2), locomotor activity increased significantly after substantia nigra lesion. Lesion of the ventral tegmental area significantly decreased the step-through latency 24 h after acquisition (Fig. 3), suggesting that the lesion impaired long-term memory retention. With regard short-term memory, ventral tegmental area lesions with low doses of kainic acid had no significant effect on spontaneous alternation %, although the lesion significantly increased the number of arm entries (Fig. 4). High doses of kainic acid induced a significant decrease in spontaneous alternation % (Fig. 4), suggesting that the lesion of ventral tegmental area neurons impaired short-term memory too.

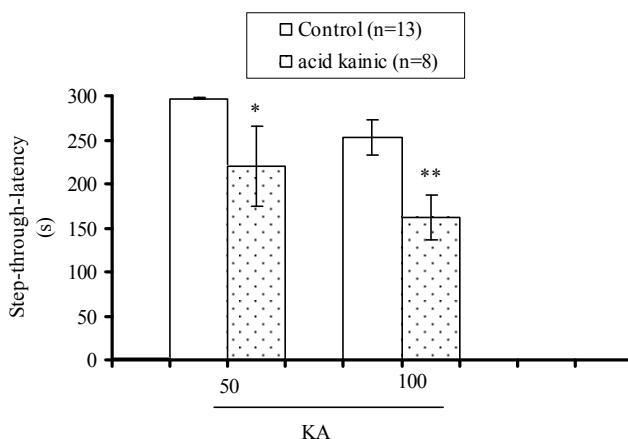


Fig. 1. Alterations of step-through latency induced by substantia nigra lesion with kainic acid (KA: 50 ng/250 nl and 100 ng/500 nl) at 24 h after acquisition training in multi-trial passive avoidance test. Values are means \pm S.E.M. The number of rats is given in brackets. *P < 0.05, **P < 0.02 vs. control group.

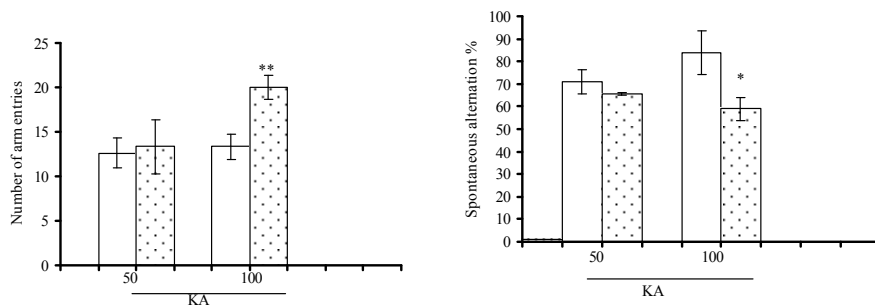


Fig. 2. Alterations of number of arm entries and spontaneous alternation % induced by substantia nigra lesion with kainic acid (KA: 50 ng/250 nl and 100 ng/500 nl). Values are means \pm S.E.M. The number of rats is given in brackets. *P < 0.05, **P < 0.02 vs. control group. Legend is described in Fig. 1.

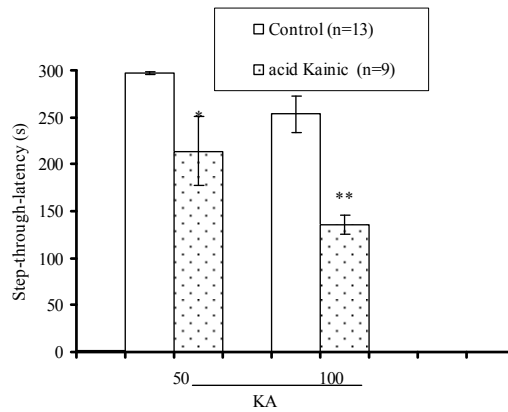


Fig. 3. Alterations of step-through latency induced by ventral tegmental area lesion with kainic acid (KA: 50 ng/250 nl and 100 ng/500 nl) at 24 h after acquisition training in multi-trial passive avoidance test. Values are means \pm S.E.M. The number of rats is given in brackets. * $P < 0.05$, ** $P < 0.01$ vs. control group.

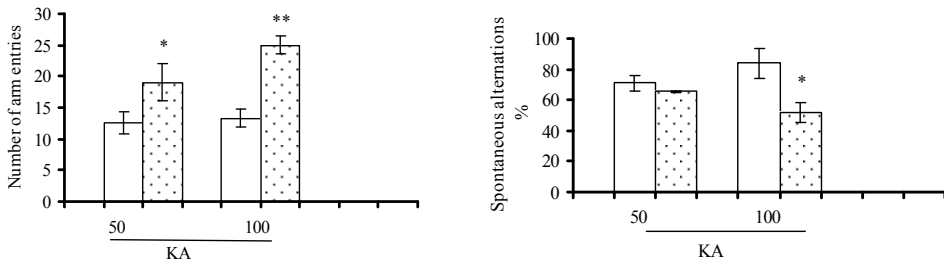


Fig. 4. Alterations of number of arm entries and spontaneous alternation % in Y-maze task induced by ventral tegmental area lesion with kainic acid (KA: 50 ng and 100 ng). Values are means \pm S.E.M. * $P < 0.05$, ** $P < 0.02$ vs. control group. Legend is described in Fig. 3.

Our experimental data suggest that ventral tegmental area dopaminergic neurons have a crucial role in short- and long-term memory retention without affecting memory acquisition. Substantia nigra dopaminergic neurons have a similar role, the effect of ventral tegmental area being more prominent. Kainic acid damages neuronal cell bodies, dendrites and terminals intrinsic to the structures injected, but spares axons passing through or terminating in the region (Guevara et al., 1997). As seen from our data, obtained following two doses of kainic acid, the normal concentration of dopamine in prefrontal cortex is more important for long-term than for short-term memory storage, because when the decrease of dopamine is less, as expected after ventral tegmental area lesion with a low dose of kainic acid, short-term memory is not affected while long-term memory is significantly impaired. Moreover, it seems that the role of ventral tegmental area dopaminergic neurons in processing and storage of information is more prominent than that of substantia nigra, as mesocortical dopaminergic fibres predominantly arise from the

ventral tegmental area, although some originate from the substantia nigra (Gardner and Ashby, 2000).

CONCLUSIONS

On the basis of our results obtained by chemical lesioning of the substantia nigra and the ventral tegmental area, we could suggest that ventral tegmental area and substantia nigra facilitate the retention of short- and long-term memory without affecting memory acquisition. It seems that longterm memory is more sensitive to the decreased dopamine level in the nervous structures involved in processing and storage of information.

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Acknowledgements. This research was supported by the National Council of Scientific Research and University Education (CNCSIS).

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