Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 475 (2003) 55–60
www.elsevier.com/locate/ejphar

Role of the mesotelencephalic dopamine system in learning and memory processes in the rat

Vasile Hefco[a,*], Kiyofumi Yamada[b], Andreea Hefco[a], Lucian Hritcu[a], Adrian Tiron[a], Toshitaka Nabeshima[b]

[a] *Department of Physiology, Al.I. Cuza University, Blv. Carol I, nr. 11, 6600 Iasi, Romania*
[b] *Department Neuropsychopharmachology and Hospital Pharmacy, Nagoya University Graduate School of Medicine, Nagoya 466-8560, Japan*

Received 17 April 2003; received in revised form 8 July 2003; accepted 11 July 2003

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) or 6-hydroxydopamine (8 μg/4 μl) 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.

Keywords: Dopamine; Kainic acid; 6-OHDA (6-hydroxydopamine); Memory; Substantia nigra; Ventral tegmental area

1. Introduction

The mesotelencephalic dopamine system is generally divided into three components: mesostriatal (also commonly called nigrostriatal), mesolimbic and mesocortical (White, 1996). The mesostriatal dopamine fibres arise mostly from the substantia nigra pars compacta projecting predominantly to the caudate–putamen (Fallon, 1988). The mesolimbic dopamine fibres arise predominantly from the ventral tegmental area with a minor component originating in various parts of the substantia nigra. These mesolimbic dopamine fibres project mostly to the nucleus accumbens, amygdala, nucleus of stria terminalis, lateral septal area, etc. The mesocortical dopamine fibres predominantly arise from the ventral tegmental area, although some originate from different parts of the substantia nigra. These fibres primarily innervate the medial prefrontal cortex, the anterior cingulate cortex and the suprarhinal cortex (Gardner and Ashby, 2000).

In the present study, we investigated the role of the dopaminergic neuronal system and other neurons from the substantia nigra pars reticulata and ventral tegmental area in short- and long-term memory by injecting 6-hydroxydopamine, a specific neurotoxin for dopamine neurons, or kainic acid (which destroys only the soma of different type of neurons without affecting the passing fibres). Our data suggest that mainly dopaminergic neurons in both substantia nigra and ventral tegmental area have a role in short- and long-term memory retention, the role of ventral tegmental area being more prominent than that of substantia nigra. In contrast, the acquisition of memory was not impaired by lesions of ventral tegmental area and substantia nigra.

2. Materials and methods

Male Wistar rats weighing 250–300 g at the start of the experiment were used. The rats were treated in accordance with the Guidelines for Animal Experiments of the "Al.I.-Cuza" University and of the U.S. National Institute of the Health Guide for the Care and Use of Laboratory Animals. The rats were housed three per cage with free access to food and water under controlled laboratory conditions (a 12-h light/dark cycle with lights on at 8:00 a.m., 22 ± 0.5 °C)

* Corresponding author. Tel.: +40-232-201502; fax: +40-232-201201.
E-mail addresses: vhefco@uaic.ro (V. Hefco), tnabeshi@tsuru.med.nagoya-u.ac.jp (T. Nabeshima).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0014-2999(03)02115-0

2.1. Neurosurgery

The rats were anesthetized with sodium pentobarbital (45 mg/kg b.w. i.p.).

The substantia nigra was lesioned bilaterally by stereotaxic microinjections of two doses of kainic acid (50 ng in 250 nl and 100 ng in 500 nl) at a rate of 100 nl/min. After each infusion, the Hamilton syringe was left in place for 1 min to allow for adequate diffusion of the solution. The sham-operated groups were injected with saline. Specific lesions of the dopaminergic neurons located in substantia nigra pars reticulata were produced with 6-hydroxydopamine (Sigma). Eight migrograms (free base) 6-hydroxydopamine, dissolved in 4 μl physiological saline containing 0.1% ascorbic acid were administered through the Hamilton syringe over 4.50 min, and the syringe was left in place for 5 min after injection before being slowly removed. The rats were pretreated 30 min before the 6-hydroxydopamine infusion with 25 mg/kg i.p. desipramine (Sigma) to protect noradrenergic projections. Sham-operated rats received an injection of desipramine, followed by vehicle only in the substantia nigra. The following coordinates were used: 5.5 mm posterior to bregma; 2.0 mm lateral to the midline; 7.4 mm ventral to the surface of the cortex (Paxinos and Watson, 1986). For lesioning the ventral tegmental area, the same quantity of kainic acid or 6-hydroxydopamine was injected bilaterally according to the following coordinates: 5.6 mm posterior to bregma; 0.5 mm lateral to the midline; 7.6 mm ventral to the surface of the cortex. The sham-operated groups were treated as described for substantia nigra sham-operated groups. Learning and memory tests began 2 weeks after the operation.

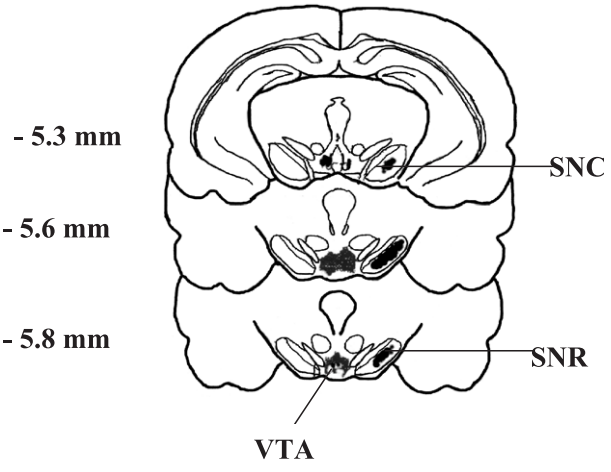

Fig. 1. Bilateral chemical lesion with 6-hydroxydopamine (8 μg) or kainic acid (100 ng) of the substantia nigra pars reticulata (SNR) and ventral tegmental area (VTA). Mean sites of the lesion are drawn on coronal brain sections running from −5.3 to −5.8 mm posterior to the bregma. The lesioned sites of SNR are indicated only on right sites. SNC means SN pars compacta. The figure represents the histological verification of the lesion sites from substantia nigra or ventral tegmental area lesioned rats. Plates were modified from Paxinos and Watson (1986).

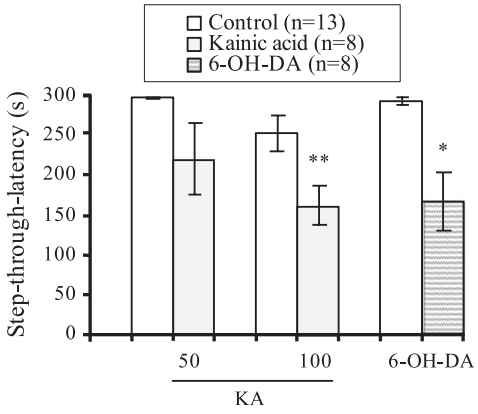

Fig. 2. Alterations of step-through latency induced by substantia nigra lesion with kainic acid (KA: 50 ng in 250 nl and 100 ng in 500 nl) or 6-hydroxydopamine (8 μg in 4 μl in group 6-OHDA) at 24 h after acquisition training in multi-trial passive avoidance test. Values are means ± S.E.M. The number of rats is given in brackets. *$P<0.05$, **$P<0.02$ vs. control group.

2.2. Learning and memory tasks

2.2.1. The multi-trial passive avoidance test

In brief, a step-through-type passive avoidance apparatus consisting of two compartments (25 × 15 × 15 cm high), one light and one dark, both equipped with a grid floor was used. The two compartments were separated by a guillotine door. In the acquisition trial, each rat was placed in the light compartment and a foot shock (0.3 mA/5 s) was delivered via the grid floor when the rat stepped in the dark compartment. 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 at least 120-s periods. Twenty-four hours later, each rat was placed in the light compartment and the step-through latency was recorded until 300 s had elapsed (retention trial). The number of training trials in the first day was used as the index of memory acquisition. The step-through latency in the retention trial was used as the index

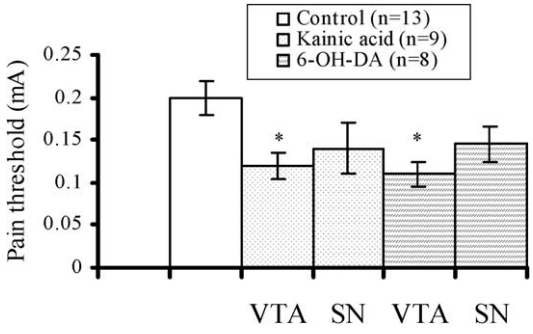

Fig. 3. Alterations of pain threshold (mA DC) induced by kainic acid (100 ng) or 6-hydroxydopamine (8 μg) lesion of substantia nigra (SN) and ventral tegmental area (VTA). Values are means ± S.E.M. The number of rats is given in brackets. *$P<0.05$ vs. control group.

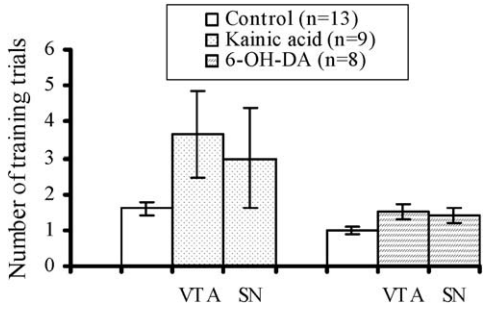

Fig. 4. Alterations of number of training trials induced by kainic acid (100 ng in 500 nl) or 6-hydroxydopamine (8 μg in 4 μl) lesion of substantia nigra (SN) and ventral tegmental area (VTA) in the acquisition stage in multi-trial passive avoidance test. Values are means ± S.E.M. The number of rats is given in brackets.

of retention of training experience (Yamada et al., 1996). Longer retention latencies were interpreted as indicating better retention of the training experience.

2.2.2. Y-maze task

Spatial working memory and short-term memory were assessed by testing spontaneous alternation behavior in a 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 an 8-min session. An arm entry was counted when the hind paws of the rat were completely within the arm.

Spontaneous alternation behavior was defined as successive entries into the three arms, in overlapping triplet sets. The percent spontaneous alternation behavior was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries—2) × 100. Spontaneous alternation is considered to involve spatial memory of the "working" type (Yamada et al., 1996). This experiment was performed 24 h before the shock passive avoidance test.

2.3. Pain threshold test

Because the multi-trial passive avoidance test uses a pain stimulus, the reaction of the rats could be modified by pain sensitivity. The nociceptive electric threshold of vocalization and jumping was recorded as mA of a DC electric foot shock delivered via the grid floor of a passive avoidance apparatus.

2.4. Histological control

The rats were killed with an overdose of sodium pentobarbital (100 mg/kg i.p.) followed by a transcardial infusion of 0.9% saline and a 10% formalin solution. The brains were removed and placed in a 30% sucrose/formalin solution. The brains were frozen and cut into coronal sections (50 μm) using a freezing microtome and stained with cresyl violet for verification of the point of the syringe needle. Only experimental data from lesions correctly located in the ventral tegmental area and substantia nigra were used for statistical analysis.

2.5. Statistical analysis

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

3. Results

3.1. Histological verifications

After chemical lesions the rats recovered quickly and gained weight by 1 week. Fig. 1 illustrates the rostrocaudal extension of the mean lesion sites of the substantia nigra and ventral tegmental area.

In the majority of substantia nigra-lesioned rats (9/12), the point of the syringe needle was positioned symmetrically in the central part of the substantia nigra pars reticulata

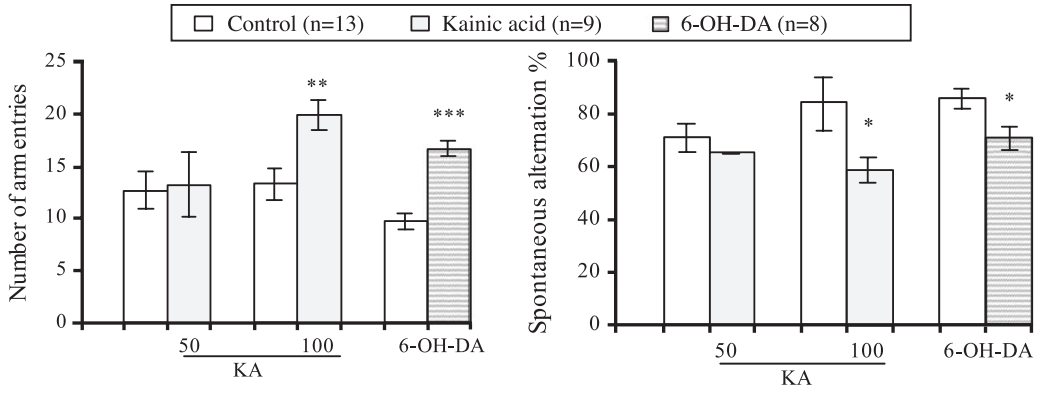

Fig. 5. Alterations of number of arm entries and spontaneous alternation % induced by substantia nigra lesion with kainic acid (KA: 50 ng in 250 nl and 100 ng in 500 nl) or 6-hydroxydopamine (8 μg in 4 μl in group 6-OHDA). Values are means ± S.E.M. The number of rats is given in brackets. *$P < 0.05$, **$P < 0.02$, ***$P < 0.001$ vs. control group.

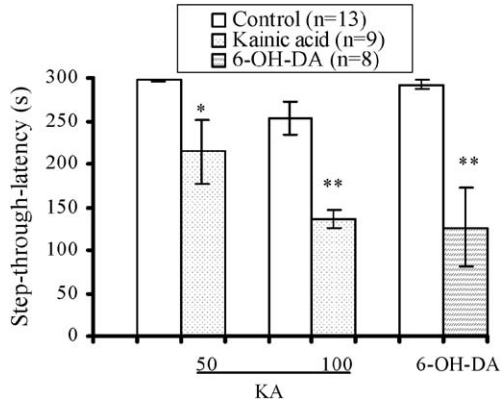

Fig. 6. Alterations of step-through latency induced by ventral tegmental area lesion with kainic acid (KA: 50 ng in 250 nl and 100 ng in 500 nl) or 6-hydroxydopamine (8 μg in 4 μl in group 6-OHDA) at 24 h after acquisition training in multi-trial passive avoidance test. Values are means ± S.E.M. The number of rats is given in brackets. *$P<0.05$, **$P<0.01$ vs. control group.

and the lesions extended from −5.3 to −5.8 mm posterior to bregma, without any significant damage to adjacent structures including substantia nigra pars compacta and ventral tegmental area.

In the case of ventral tegmental area-lesioned rats, the point of the needle was positioned in the middle of the brain under the red nucleus (Fig. 1), in three rats, however, being in the very posterior part of the ventral tegmental area. In all rats whose data were retained for statistical analysis, there was no noticeable variation in the lesion extent among the nine ventral tegmental area-lesioned rats. The extent of lesioning with the high dose of kainic acid was about the same as with 6-hydroxydopamine.

3.2. The effects of substantia nigra pars reticulata

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. 2, group KA 50 ng). High doses of kainic acid (100 ng) and 6-hydroxydopamine (8 μg), a specific dopaminergic neurotoxin, induced a significant decrease in step-through latency 24 h after the acquisition (Fig. 2, group KA 100 ng and 6-OHDA), suggesting that especially lesioning dopaminergic neurons impaired long-term memory retention. This cannot be attributed to the changes in pain sensitivity because the pain threshold did not change significantly after the lesion of substantia nigra (Fig. 3).

Neither type of chemical substantia nigra lesion significantly affected the number of training trials in the acquisition training, suggesting that substantia nigra has no effect on acquisition (Fig. 4).

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. 5, group KA 50 ng).

Lesioning of substantia nigra with high doses of kainic acid (100 ng/500 nl) or 6-hydroxydopamine significantly increased the number of arm entries and decreased spontaneous alternation % (Fig. 5, group KA 100 ng and 6-OHDA), suggesting that the lesion impaired short-term memory. As can be deduced from the number of arm entries (Fig. 5, group KA 100 ng and 6-OHDA), locomotor activity increased significantly after substantia nigra lesion.

3.3. The effects of ventral tegmental area

The lesions of ventral tegmental area with both doses of kainic acid, or 6-hydroxydopamine, increased the number of training trials in the acquisition training, but due to the individual variation, the increase was not statistically significant (Fig. 4), suggesting that the lesion does not affect memory acquisition. Conversely, lesion of the ventral tegmental area significantly decreased the step-through latency 24 h after acquisition (Fig. 6), suggesting that the lesion impaired long-term memory retention. These changes cannot be attributed to changes in pain sensitivity which increased significantly after lesioning with high doses of kainic acid or 6-hydroxydopamine (Fig. 3).

With regard to short-term memory, ventral tegmental area lesions with low doses of kainic acid had no significant effect on spontaneous alternation %, although the lesion

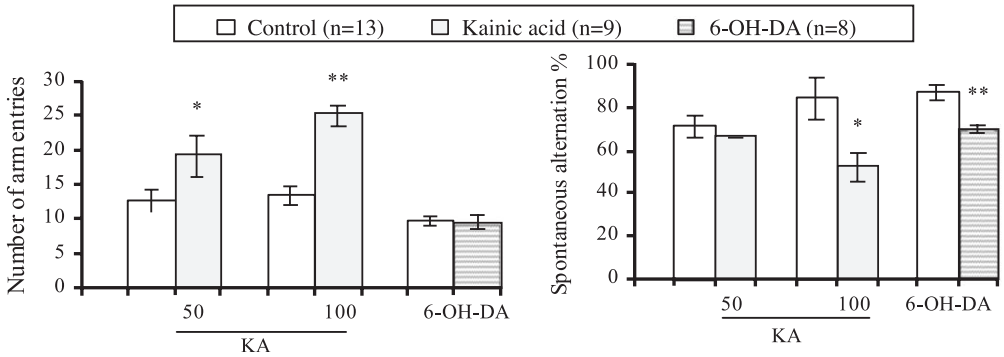

Fig. 7. Alterations of number of arm entries and spontaneous alternation % in Y-maze task induced by ventral tegmental area lesion with kainic acid (KA: 50 ng and 100 ng) or 6-hydroxydopamine (8 μg in group 6-OHDA) Values are means ± S.E.M. *$P<0.05$, **$P<0.02$ vs. control group.

significantly increased the number of arm entries (Fig. 7, group KA 50 ng). High doses of kainic acid as well as 6-hydroxydopamine induced a significant decrease in spontaneous alternation % (Fig. 7, group KA 100 ng and 6-OHDA), suggesting that the lesion of ventral tegmental area neurons impaired short-term memory too.

4. Discussion

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. Because testing of long-term memory supposes avoidance of a pain stimulus delivered in the acquisition period in the dark compartment of the passive avoidance apparatus, the retaining of information could depend on the extent of pain sensitivity. Our results showed that ventral tegmental area lesions enhanced pain sensitivity, because the pain threshold for electric foot shock and hot-plate test (data not shown) was significantly decreased. From these data, we can conclude that decreasing of the long-term memory cannot be attributed to changes in pain sensitivity. The ventral tegmental area may be involved in the processing of nociceptive information. Activation of mesolimbic dopamine neurons of the ventral tegmental area, naturally triggered by exposure to stress, facilitates the analgesic process through the endogenous release of opioids and substance P in the midbrain (Altier and Stewart, 1999; Sotres-Bayan et al., 2001). Our data show that chemical lesions of ventral tegmental area by means of kainic acid or 6-hydroxydopamine also decrease the analgesic effect of dopaminergic neurons.

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). Moreover, it was shown that injections of kainic acid in the rostral pontine tegmentum caused not only lesions to local cell bodies, but also destruction of γ-aminobutyric acid (GABA)ergic and dopaminergic cells in the substantia nigra (McGeer and McGeer, 1984). The ventral tegmental area contains a considerable number of nondopaminergic neurons that project largely to the same terminal regions as the dopaminergic cells. There is good evidence that the nondopaminergic part of the projection from ventral tegmental area to the medial prefrontal cortex contains GABA as its neurotransmitter (Tzschentke, 2001). Furthermore, a high density of neurotensin-containing perikarya and enkephalin-containing neuronal fibers and perikarya are present in the ventral tegmental area dopamine region (Kalivas and Abhold, 1997; Kalivas and Miller, 1984). Most neurons in the ventral tegmental area are also immunopositive for serotoninergic and dopaminergic markers (Wirtschafter and Sheppard, 2001). All these types of neuronal perikarya can be damaged by kainic acid treatment.

The effects on memory processes, recorded after kainic acid lesions of the ventral tegmental area and substantia nigra, were similar to those observed in our experiments with 6-hydroxydopamine, a specific dopaminergic neurotoxin, suggesting that effects observed after kainic acid may be attributed mainly to the lesions of dopaminergic neurons. The dose of 6-hydroxydopamine used in the present experiments was high enough to damage dopamine neurons. We reported previously that 6-hydroxydopamine injection into the nigra reduced the dopamine content in the striatum to 30% of the control level 1 day after lesion and that this reduced persisted until 28 days after lesioning (Nitta et al. 1992), indicating that dopaminergic neurons were damaged.

The mesotelencephalic dopamine system could also be involved in the appearance of the stimulatory nicotinic effects on learning and memory as we observed in our previous experiments (Hefco et al., 2000; Nitta et al., 1994). Nicotinic acetylcholine receptors on dopaminergic neurons in the ventral tegmental area are thought to be a prime target for nicotine's stimulatory effects. We have reported that i.c.v. administration of nicotine increased extracellular dopamine concentration in the striatum and acetylcholine in the region extending from frontal cortex to hippocampus (Itoh et al., 1996). Nicotine stimulates the firing rate of ventral tegmental area dopamine neurons (White et al. 1995; Yin and French, 2000). Stimulation of ventral tegmental area at a frequency known to evoke dopamine overflow in the prefrontal cortex produces a long-lasting enhancement of the magnitude of the hippocampal-prefrontal cortex long-term potentiation (Gurden et al., 1999), a putative cellular mechanism underlying plasticity (Bao et al., 2001). Depletion of more than 50% of the cortical dopamine level which could be also achieved through chemical lesioning of the ventral tegmental area, as in our experiments, induces a dramatic decrease in hippocampal–prefrontal cortex long-term potentiation (Gurden et al., 1999).

As seen from our data, obtained following two doses of kainic acid and 6-hydroxydopamine lesioning, 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). The existence of a direct monosynaptic pathway from the ventral CA_1 region of the hippocampus and subiculum to specific areas of the prefrontal cortex provides useful model for conceptualizing the operations of hippocampal–prefrontal cortex communication in learning

and memory. Dopamine by means of D1 but not D2 dopamine receptors is crucial for the control of NMDA receptors mediated by synaptic response to a specific excitatory input to the prefrontal cortex. The interactions of dopamine D1 receptors and NMDA receptors may play a crucial role in the storage and transfer of hippocampal information in the prefrontal cortex (Gurden et al., 2000).

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 long-term memory is more sensitive to the decreased dopamine level in the nervous structures involved in processing and storage of information. The retention of memory can be attributed to some extent to interaction of mesotelencephalic dopamine receptors with other receptors such as muscarinic and nicotinic acetylcholine receptors and NMDA receptors. The integrity of the mesotelencephalic dopamine system may be necessary for processing and storage of information.

References

Altier, N., Stewart, J., 1999. The role of dopamine in the nucleus accumbens in analgesia. Life Sci. 65, 2269–2287.

Bao, S., Chan, V., Merzenich, M.M., 2001. Cortical remodeling induced by activity of ventral tegmental area9 dopamine neurons. Nature 412, 79–83.

Fallon, J.H., 1988. Topographic organization of the ascending dopaminergic projections. Ann. N.Y. Acad. Sci. 537, 1–9.

Gardner, E.L., Ashby, C.R., 2000. Heterogeneity of the mesotelencephalic dopamine fibres: physiology and pharmacology. Neurosci. Behav. Rev. 24, 115–118.

Guevara, B.H., Hoffmann, I.S., Cubeddu, L.X., 1997. Ventral and dorsal cholinergic neurons have different sensitivities to kainic acid. Neurochem. Int. 31, 723–730.

Gurden, H., Tassin, J.P., Jay, T.M., 1999. Integrity of the mesocortical dopaminergic system is necessary for complete expression of in vivo hippocampal–prefrontal cortex long-term potentation. Neuroscience 94 (4), 1019–1027.

Gurden, H., Takita, M., Jay, T.M., 2000. Essential role of D1 but not D2 receptors in the NMDA receptor-dependent long-term potentation of hippocampal–prefrontal cortex synapses in vivo. J. Neurosci. 20, 1–5.

Hefco, V., Olariu, A., Gavrilovici, C., Hefco, A., 2000. Nicotinic and muscarinic receptors interaction on learning and memory process. Ann. Univ. Iasi 46, 177–182.

Itoh, A., Nitta, A., Nadai, M., Nishimura, K., Hirose, M., Hasegawa, T., Nabeshima, T., 1996. Dysfunction of cholinergic and dopaminergic neuronal systems in β-amyloid protein-infused rats. J. Neurochem. 66, 1113–1117.

Kalivas, P.W., Abhold, R., 1997. Enkephalin release into the VTA in response to stress: modulation of mesocorticolimbic dopamine. Brain Res. 419, 339–348.

Kalivas, P.W., Miller, J.S., 1984. Neurotensin neurons in the ventral tegmental area project to the medial nucleus accumbens. Brain Res. 300, 157–160.

McGeer, E.G., McGeer, P.L., 1984. Substantia nigra cell death from kainic acid or folic acid injections into the pontine tegmentum. Brain Res. 298, 339–342.

Nitta, A., Furukawa, Y., Hayashi, K., Hiramatsu, M., Kameyama, T., Hasegawa, T., Nabeshima, T., 1992. Denervation of dopaminergic neurons with 6-hydroxydopamine increases nerve growth factor content in rat brain. Neurosci. Lett. 144, 152–156.

Nitta, A., Katano, Y., Itoh, A., Hasegawa, T., Nabeshima, T., 1994. Nicotine reverses scopolamine-induced impairment of performance in passive avoidance task in rats through its action on the dopaminergic neuronal system. Pharmacol. Biochem. Behav. 49 (4), 807–812.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates. Academic Press, New York.

Sotres-Bayan, F., Torres-Lopez, E., Lopez-Avila, A., del Angel, R., Pellicer, F., 2001. Lesion and electrical stimulation of the ventral tegmental area modify persistent nociceptive behavior in the rat. Brain Res. 898, 342–349.

Tzschentke, T.M., 2001. Pharmacology and behavioral pharmacology of the mesocortical dopamine system. Prog. Neurobiol. 63, 241–320.

White, F.J., 1996. Synaptic regulation of mesocorticolimbic dopamine neurons. Annu. Rev. Neurosci. 19, 405–436.

White, F.J., Hu, X.-T., Zhang, X.-F., Wolf, M.E., 1995. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. J. Pharmacol. Exp. Ther. 273, 445–454.

Wirtschafter, D., Sheppard, A.C., 2001. Localization of GABA (B) receptor in midbrain monoamine containing neurons in the rat. Brain Res. Bull. 1 (56), 1–5.

Yamada, K., Noda, Y., Hasegawa, T., Komori, Y., Nikai, T., Sugihara, H., Nabeshima, T., 1996. The role of nitric oxide in dizocilpine-induced impairment of spontaneous alternation behavior in mice. J. Pharmacol. Exp. Ther. 276, 460–666.

Yin, R., French, E.D., 2000. A comparison of the effects of nicotine on dopamine and non-dopamine neurons in the rat ventral tegmental area: an in vitro electrophysiological study. Brain Res. Bull. 51 (6), 507–514.