Neuroscience Letters 573 (2014) 19–23

Contents lists available at ScienceDirect

Neuroscience Letters

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

Intrastriatal grafts of fetal ventral mesencephalon improve allodynia-like withdrawal response to mechanical stimulation in a rat model of Parkinson's disease

Ryuichiro Takeda[a,b], Yasushi Ishida[b,*], Kosuke Ebihara[b], Hiroshi Abe[b], Hisae Matsuo[b], Tetsuya Ikeda[c], Go Koganemaru[b], Aki Kuramashi[b], Hideki Funahashi[b], Yasuhiro Magata[d], Keiichi Kawai[e], Toshikazu Nishimori[b]

[a] Healthcare and Security center, University of Miyazaki, 1-1 Gakuen Kibanadai-nishi, Miyazaki 889-1692, Japan
[b] Department of Psychiatry, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
[c] Division of Neurobiology, Faculty of Medicine, University of Miyazaki, 5200 Kihara, Kiyotake, Miyazaki 889-1692, Japan
[d] Laboratory of Genome Bio-Photonics, Photon Medical Research Center, Hamamatsu University School of Medicine, 1-20-1 Handayama, Hamamatsu 431-3192, Japan
[e] Department of Quantum Medical Technology, Division of Health Sciences, Graduate School of Medical Sciences, Kanazawa University, 5-11-80 Kodatsuno, Kanazawa 920-0942, Japan

HIGHLIGHTS

- A 6-OHDA rat model of Parkinson's disease showed allodynia-like withdrawal response.
- Fetal ventral mesencephalon (VM) cells were transplanted into the denervated striatum.
- The withdrawal threshold was increased in 6-OHDA rats with VM grafts.
- Transplantation of VM cells may ameliorate pain sensation in 6-OHDA rats.

ARTICLE INFO

Article history:
Received 17 March 2014
Received in revised form 2 May 2014
Accepted 3 May 2014
Available online 14 May 2014

Keywords:
Pain
6-Hydroxydopamine
Basal ganglia
Transplantation
Immunohistochemistry

ABSTRACT

We previously reported that a unilateral 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease showed allodynia-like withdrawal response to mechanical stimulation of the ipsilateral side of the rat hindpaw. The goal of this study was to investigate the effect of intrastriatal grafts of fetal ventral mesencephalon (VM) on the withdrawal response in 6-OHDA rats. The withdrawal threshold in response to the mechanical stimulation of the rat hindpaw was measured using von Frey filaments. In the ipsilateral side of the 6-OHDA lesions, the withdrawal threshold in response to mechanical stimulation significantly increased in 6-OHDA rats with VM grafts compared with those with sham grafts, but did not change in the contralateral side at 5 weeks after transplantation. The present results suggest that the intrastriatal grafts of fetal VM may relieve pain sensation induced by mechanical stimulation in 6-OHDA rats.

1. Introduction

Parkinson's disease (PD) is well characterized by dominating bradykinesia, rigidity, tremors, postural instability, and dementia as clinical features [20]. Apart from parkinsonian motor symptoms, PD is less widely considered a disease that causes a large variety of pain syndromes with a prevalence of ~40% [7]. Another recent clinical examination involving 176 home-living PD patients showed that 146 (83%) of the patients had pain-related symptoms [3]. However, little is known about the clinical predictors of pain in PD, and it remains unclear whether these painful symptoms are inherent in this disease or are caused by medication [24].

Rats with unilateral 6-hydoroxydopamine (6-OHDA) lesions of the mesostriatal dopamine pathway have been widely used as a rodent model for PD [26]. The 6-OHDA rat is valuable for clarifying the mechanisms of painful symptoms in patients with PD. The response of pain-related behaviors in this model animal is different from that in a naïve control animal. Unilateral

* Corresponding author. Tel.: +81 985852969; fax: +81 985855475.
E-mail address: ishiday@med.miyazaki-u.ac.jp (Y. Ishida).

http://dx.doi.org/10.1016/j.neulet.2014.05.007
0304-3940/

injections of 6-OHDA into the rat substantia nigra (SN) decreased the flinch-jump threshold of electrical footshock [5] and the latency of withdrawal responses in hot plate, paw pressure, and paw immersion tests [23]. We previously reported that a unilateral 6-OHDA-lesioned rat showed allodynia-like withdrawal response to mechanical stimulation of the ipsilateral side of the rat hindpaw [29]. These previous reports suggested that unilateral dopamine depletion might cause hypersensitivity to mechanical or noxious stimulus.

Many reports have been made in which intrastriatal grafts of fetal ventral mesencephalon (VM) could ameliorate motor function and neurochemical milieu in the brain of unilateral 6-OHDA-lesioned rats [12,13,16,20,21]; however, there is no evidence that neural transplantation affects pain-related behavior in PD model animals. Thus, the purpose of this study was to evaluate whether the intrastriatal transplants of fetal VM affect the allodynia-like withdrawal response to mechanical stimulation in 6-OHDA rats.

2. Materials and methods

Male Wistar rats (Charles River, Japan), weighting 120–130 g at the beginning of this study, were used. They were housed in a group of three or four per cage under a 12 h light/dark cycle with free access to food and water. The experimental procedures in this study were performed in strict adherence of the Guidelines of the University of Miyazaki for the care and use of experimental animals and under the approval of the ethical committee of animal experimentation at the University of Miyazaki. A unilateral lesion of the left medial forebrain bundle (MFB) was made by microinjection of 10 μg 6-OHDA hydrobromide (Sigma, MA, USA) in 5 μl of sterile saline containing 0.01% ascorbic acid per animal, as previously described [12,13,15]. Stereotaxic coordinates for the lesions were 3.3 mm rostral to the interaural line, 1.3 mm left of the midline, and 6.8 and 6.4 mm (injected 2.5 μl each) ventral to the dural surface [17]. Motor disturbance was assessed by counting full-rotations per min in a cylindrical container (30 cm diameter) at 10-min intervals for the first 60 min after methamphetamine (3 mg/kg, i.p.) administration [21]. Behavioral screening was carried out after 2 weeks of recovery, and animals that turned no less than 5 turns/min on the methamphetamine challenge were used as the 6-OHDA rats in the study. There was an immediate and almost complete destruction of dopamine neurons of the SN and of the ventral tegmental area (VTA), resulting in the near total depletion (2% of normal) of dopamine in the striatum ipsilateral to the 6-OHDA injections [12]. Seven and eight animals out of the 6-OHDA rats were allocated as the lesion plus VM graft group and lesion plus sham graft group, respectively. Neural transplantation was performed from fetal VM tissue according to the cell suspension method [4,8]. Six μl of the suspension containing 9.6×10^5 cells from 15-day-old rat embryos was injected into two sites (3 μl each site) of the denervated striatum: (1) AP = 1.0 mm rostral to the bregma, L = 2.5 mm left of midline, V = 5.0 mm ventral to the dural surface and (2) AP = 0.0 mm to the bregma, L = 3.2 mm left of midline, V = 5.0 mm ventral to the dural surface [22]. For animals receiving sham grafts, 3 μl aliquots of saline alone were injected at the same sites. All of the VM and sham grafted rats were tested again for rotational asymmetry at 4 weeks after transplantation.

One week before and 5 weeks after transplantation, the withdrawal threshold in response to mechanical stimulation of the rat hindpaw was measured using von Frey filaments in normal (n = 6), lesion plus sham grafted (n = 8), and lesion plus VM grafted animals (n = 7). The maximum and minimum cut-offs of the withdrawal thresholds were set at 100 and 1 g, respectively. The withdrawal responses using the von Frey filaments, at least two times out of five applications, were defined as the threshold against mechanical stimulation.

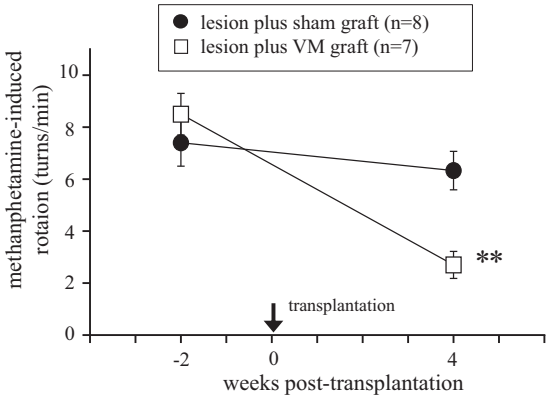

Fig. 1. Mean numbers of rotations (±SEM) ipsilateral to 6-OHDA lesions per min over 60 min test period in response to methamphetamine (3 mg/kg, i.p.) are shown for lesion plus sham graft group and lesion plus VM graft group. Ipsilateral rotation found in lesion plus VM graft group significantly decreased at 4 weeks after transplantation as compared to lesion plus sham graft group (**P < 0.01, two way ANOVA with repeated measures followed by Bonferroni post hoc tests).

After the behavioral test with mechanical stimulation, these animals were deeply anesthetized with an overdose of sodium pentobarbital (i.p.) and intracardially perfused with saline followed by 4% paraformaldehyde in a 0.1 M phosphate buffer (PB) for 30 min.

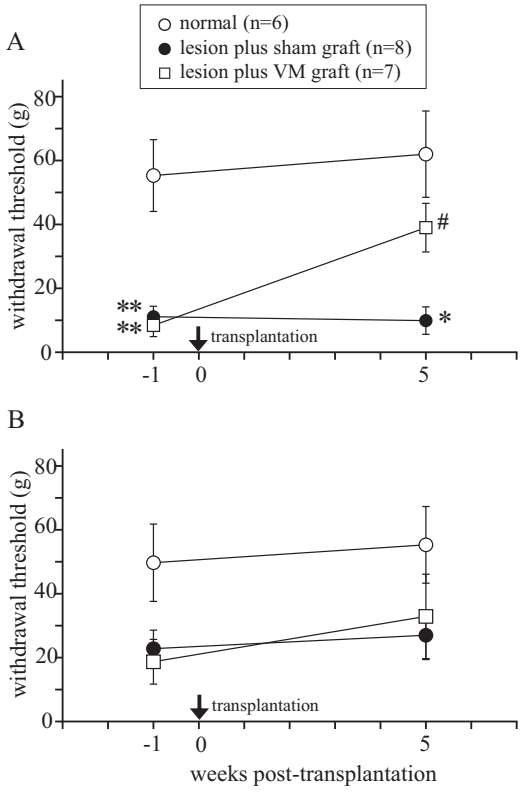

Fig. 2. Withdrawal threshold in response to mechanical stimulation of rat hindpaw by using von Frey filaments, (A) ipsilateral and (B) contralateral to 6-OHDA lesions and transplantation. Withdrawal threshold was examined in hindpaw bilaterally 1 week before and 5 weeks after transplantation for normal control rats, lesion plus sham grafted rats, and lesion plus VM grafted rats. Results are shown as means ± SEM; *P < 0.05, **P < 0.01 compared to each corresponding value in normal group (two way ANOVA with repeated measures followed by Bonferroni post hoc tests); #P < 0.05 compared to each corresponding value of lesion plus sham graft group (two way ANOVA with repeated measures followed by Bonferroni post hoc tests).

The brain was removed, postfixed for 1 h in the same fixative and cryoprotected in 10% sucrose for 1 h and 30% sucrose in PB overnight. Coronal serial sections at the SN and striatum, 50 μm thick, were frozen, cut, and collected in phosphate-buffered saline (pH 7.4) and processed for immunohistochemical staining for tyrosine hydroxylase (TH) as free-floating sections [15,16,29].

The data were analyzed using two way analysis of variance (ANOVA) with repeated measures (groups × time) followed by a Bonferroni post hoc test. P values <0.05 were regarded as being statistically significant.

3. Results

Before transplantation, methamphetamine (3 mg/kg, i.p.) induced strong ipsilateral rotation in the 6-OHDA-lesioned rats. In the lesion plus sham grafted animals ($n = 8$), methamphetamine-induced rotation continued to be observed 8 weeks following lesion generation. This behavior was significantly suppressed at 4 weeks after transplantation of the intrastriatal VM grafts ($n = 7$) [main effect of groups, $F_{1,13} = 2.09$, $P = 0.17$; main effect of time, $F_{1,13} = 29.38$, $P < 0.01$; groups × time interaction, $F_{1,13} = 13.87$, $P < 0.01$] (Fig. 1).

Before transplantation, the 6-OHDA rats (in both lesion plus sham graft and lesion plus VM graft groups) showed significantly lower withdrawal thresholds in response to mechanical stimulation of the hindpaw ipsilateral to the lesion compared with the normal control rats (Fig. 2A) but did not change in the contralateral side (Fig. 2B). In the ipsilateral side of the 6-OHDA lesions, the withdrawal thresholds in response to mechanical stimulation was significantly increased in the 6-OHDA rats with VM grafts compared with those with sham grafts [main effect of groups, $F_{2,18} = 22.92$, $P < 0.01$; main effect of time, $F_{1,18} = 4.87$, $P = 0.04$; groups × time interaction, $F_{2,18} = 3.26$, $P = 0.06$] (Fig. 2A) but did not change in the contralateral side at 5 weeks after transplantation [main effect of groups, $F_{2,18} = 3.38$, $P = 0.06$; main effect of time, $F_{1,18} = 2.10$, $P = 0.16$; groups × time interaction, $F_{2,18} = 0.32$, $P = 0.72$] (Fig. 2B).

Consistent with previous studies [15,16], injection of 6-OHDA into the left MFB produced a marked reduction in TH immunoreactivity in the left SN and VTA, while there was abundant TH immunoreactivity in these two regions contralateral to the lesion (Fig. 3B). Similarly, TH immunoreactivity in the left striatum was remarkably reduced, whereas immunoreactivity in the contralateral side was localized in abundance for each 6-OHDA rat in the sham graft group (Fig. 3A). The VM grafts were all located almost in the middle part of the striatum lateromedially and surrounded by a rich TH-positive fiber network. However, it appeared that graft-derived fiber outgrowth was restricted to the area close to the graft (Fig. 3C and D).

4. Discussion

The present results of the von Frey test seem to be similar to those from our previous study in which a 6-OHDA rat elicited the reduction in withdrawal latency in response to mechanical stimulation of its hindpaw [29]. As we found for the first time in the present study, the allodynia-like withdrawal response to mechanical stimulation significantly improved in the rat hindpaw on the ipsilateral side 5 weeks after transplantation in the lesion plus VM graft group, whereas there was no significant change in the lesion

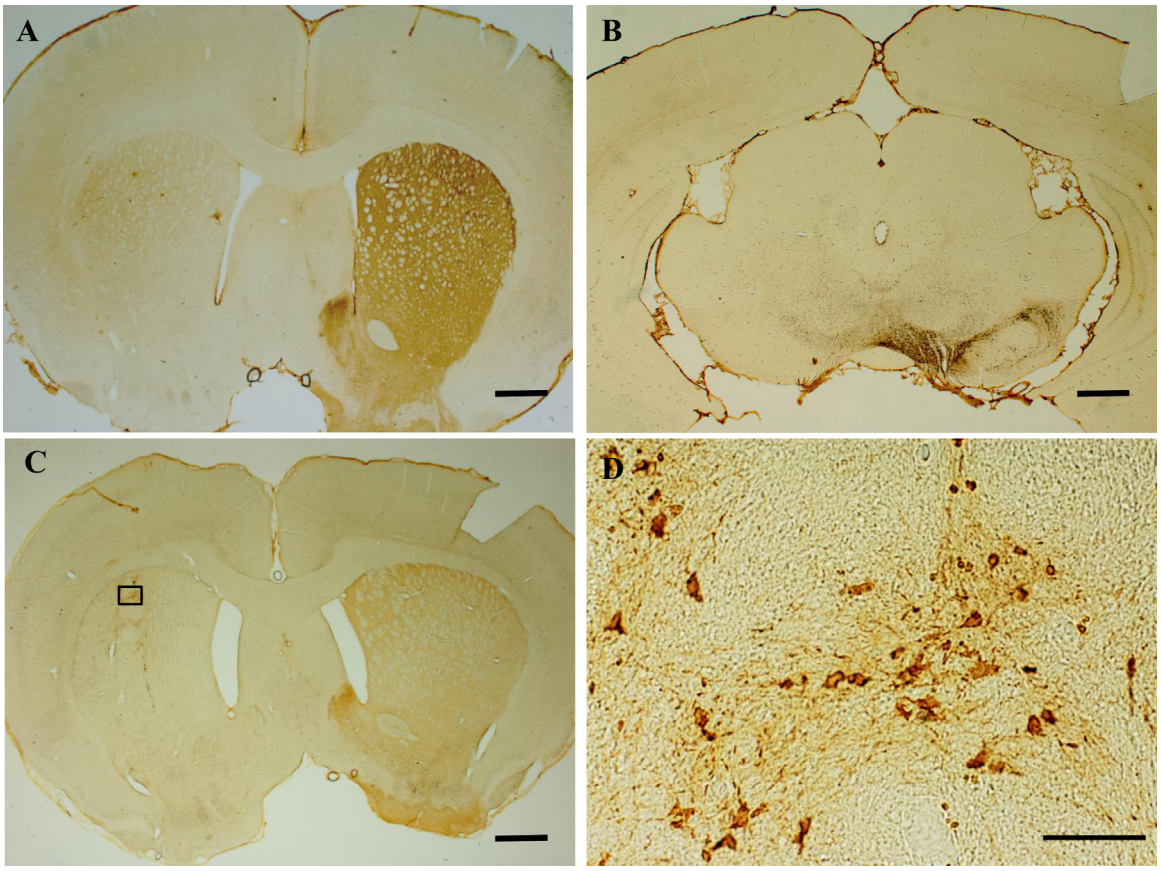

Fig. 3. Immunohistochemical visualization of tyrosine hydroxylase (TH) of rats with unilateral 6-OHDA lesions plus sham grafts (A and B) or VM grafts (C and D). (A) striatal region of sham grafted rat; (B) mesencephalon of sham grafted rat; (C) striatal region of VM grafted rat; (D) TH positive cells at higher magnification in VM graft. Lesioned/grafted side is on left in each section of (A–C). Boxed area in panel C is shown at high magnification in panel D. Scale bars = 1 mm (A–C), 100 μm (D).

plus sham graft group. These findings suggest that the intrastriatal transplants of fetal VM in unilateral 6-OHDA-lesioned rats could ameliorate not only motor but also sensory dysfunction in nociceptive processing.

As reviewed by Hagelberg et al. [10], striatal dopamine D2 receptors are involved in the regulation of pain in humans. Positron emission tomography indicated that low D2 receptor availability in the striatum of healthy subjects (indicating either a low density of D2 receptors or a high synaptic concentration of dopamine) is associated with a high cold pain threshold and a low capacity to recruit central pain inhibition by conditioned stimulation [10]. They also indicated that patients with chronic orofacial pain have higher D2 receptor availability than their age-matched controls. Unilateral 6-OHDA lesions of the mesostriatal dopamine pathway cause striatal upregulation of D2 receptors in rats, which is evident even at 3 days after 6-OHDA lesions [14]. The upregulation of D2 receptors caused by 6-OHDA lesions can be reversed by intrastriatal dopamine-rich grafts of fetal VM [25]. In VM grafted striatum, the extracellular concentration of dopamine was restored to 67% from 2% of normal in our previous study [12], which was carried out with similar methods as those in the present study. The restoration of dopamine levels might contribute to the reversal of D2 receptor upregulation, at least in part, in VM grafted striatum. Taken together, the present results are consistent with the previous findings, in which dopamine depletion and its restoration in the mesostriatal system affect the withdrawal response to mechanical stimulus.

However, the mechanisms of allodynia-like behavior on the side ipsilateral to the 6-OHDA lesions remain unclear. We are currently focusing on another possibility, unilateral impairment of the descending cathecholaminergic system involved in the A11 area. Because A11 descending fibers predominantly uncross [11,27] and the A11 cell group may provide a major source of dopaminergic innervation of the spinal cord that affects somatosensory processing [9], it can be speculated that the unilateral 6-OHDA lesions affect the impairment of the descending inhibition system involved in the A11 area, eliciting allodynia-like behavior on the side ipsilateral to the 6-OHDA lesions.

Both dopaminergic and nondopaminergic neurotransmissions may be involved in these mechanisms. The basal ganglia, specifically the striatum, are among the neuronal structures with highest concentrations of endogenous opiates and their receptors, which may be relevant to the mechanisms of endogenous analgesia [2]. The 6-OHDA lesioning decreases striatal opioid (μ, δ, κ) binding site density, increases proenkephalin mRNA, and decreases prodynorphin mRNA [28]. Tissue levels of substance P and neurokinin A have been reported to decrease in the SN and/or striatum in 6-OHDA rats, and simultaneous increases in both tissue and extracellular concentrations of GABA have been reported in the denervated striatum [18]. Therefore, alteration in these peptides (enkephalin, dynorphin, substance P, neurokinin A) and GABA activities in the mesostriatal system may have some effect on the alteration in the withdrawal response to mechanical stimulation in 6-OHDA rats. Transplantation of fetal nigral cells was reported to reverse the 6-OHDA lesion-induced alteration of preproenkephalin, prodynorphin, and preprotachykinin mRNA levels [1,6] and Met-enkephalin levels [19]. From the present study, the nondopaminergic neuromodulation due to intrastriatal transplantation might be related to the reversal of the allodynia-like withdrawal response in 6-OHDA rats.

Although further studies are needed to elucidate the role of the dopaminergic and nondopaminergic systems involved in sensory and/or motor processing in mesostriatum, we demonstrated that intrastriatal dopaminergic transplants might have, at least in part, a therapeutic potential to mitigate pain-related symptoms in PD.

References

[1] A. Bal, M. Savasta, M. Chritin, F. Mennicken, D.N. Abrous, M. Le Moal, C. Feuerstein, J.P. Herman, Transplantation of fetal nigral cells reverses the increase of preproenkephalin mRNA levels in the rat striatum caused by 6-OHDA lesion of the dopaminergic nigrostriatal pathway: a quantitative in situ hybridization study, Mol. Brain Res. 18 (1993) 221–227.

[2] A.C. Barceló, B. Filippini, J.H. Pazo, The striatum and pain modulation, Cell Mol. Neurobiol. 32 (2012) 1–12.

[3] A.G. Beiske, J.H. Loge, A. Rønningen, E. Svensson, Pain in Parkinson's disease: prevalence and characteristics, Pain 141 (2009) 173–177.

[4] A. Björklund, S.B. Dunnett, Neural transplantation in adult rats, in: S.B. Dunnett, A. Björklund (Eds.), Neural Transplantation. A Practical Approach, Oxford University Press, New York, 1992, pp. 57–78.

[5] R.J. Carey, Acute ipsilateral hyperalgesia and chronic contralateral hypoalgesia after unilateral 6-hydroxydopamine lesions of the substantia nigra, Exp. Neurol. 91 (1986) 277–284.

[6] M.A. Cenci, K. Campbell, A. Björklund, Neuropeptide messenger RNA expression in the 6-hydroxydopamine-lesioned rat striatum reinnervated by fetal dopaminergic transplants: differential effects of the grafts on preproenkephalin, preprotachykinin and prodynorphin messenger RNA levels, Neuroscience 57 (1993) 275–296.

[7] K.R. Chaudhuri, A.H. Schapira, Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment, Lancet Neurol. 8 (2009) 464–474.

[8] S.B. Dunnett, A. Björklund, Staging and dissection of rat embryos, in: S.B. Dunnett, A. Björklund (Eds.), Neural Transplantation. A Practical Approach, Oxford University Press, New York, 1992, pp. 1–19.

[9] S.M. Fleetwood-Walker, P.J. Hope, R. Mitchell, Antinociceptive actions of descending dopaminergic tracts on cat and rat dorsal horn somatosensory neurons, J. Physiol. 399 (1988) 335–348.

[10] N. Hagelberg, S.K. Jääskeläinen, I.K. Martikainen, H. Mansikka, H. Forssell, H. Scheinin, J. Hietala, A. Pertovaara, Striatal dopamine D2 receptors in modulation of pain in humans: a review, Eur. J. Pharmacol. 500 (2004) 187–192.

[11] T. Hökfelt, O. Phillipson, M. Goldstein, Evidence for a dopaminergic pathway in the rat descending from the A11 cell group to the spinal cord, Acta Physiol. Scand. 107 (1979) 393–395.

[12] Y. Ishida, H. Hashiguchi, K. Todaka, I. Kuwahara, Y. Ishizuka, H. Nakane, D. Uchimura, T. Nishimori, Y. Mitsuyama, Serotonergic activity in the rat striatum after intrastriatal transplantation of fetal nigra as measured by microdialysis, Brain Res. 788 (1998) 207–214.

[13] Y. Ishida, K. Kawai, Y. Magata, K. Ebihara, R. Takeda, H. Abe, M. Yoshimoto, H. Hashiguchi, K. Odagiri, H. Matsuo, T. Nishimori, Differential expression of Fos and Zif268 in the nigrostriatal system after methamphetamine administration in a rat model of Parkinson's disease, Synapse 62 (2008) 920–926.

[14] Y. Ishida, K. Kawai, Y. Magata, H. Abe, M. Yoshimoto, R. Takeda, H. Hashiguchi, T. Mukai, H. Saji, Alteration of striatal [^{11}C]raclopride and 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine uptake precedes development of methamphetamine-induced rotation following unilateral 6-hydroxydopamine lesions of medial forebrain bundle in rats, Neurosci. Lett. 389 (2005) 30–34.

[15] Y. Ishida, I. Kuwahara, K. Todaka, H. Hashiguchi, T. Nishimori, Y. Mitsuyama, Dopaminergic transplants suppress L-DOPA-induced Fos expression in the dopamine-depleted striatum in a rat model of Parkinson's disease, Brain Res. 727 (1996) 205–211.

[16] Y. Ishida, K. Todaka, H. Hashiguchi, R. Takeda, Y. Mitsuyama, T. Nishimori, Morphological changes in immunopositive cells of ionotropic glutamate receptor subunits during the development of transplanted fetal ventral mesencephalic neurons, Brain Res. 940 (2002) 79–85.

[17] J.F.R. König, R.A. Klippel, The Rat Brain. A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem, Williams and Wilkins, Baltimore, 1963.

[18] N. Lindefors, E. Brodin, U. Tossman, J. Segovia, U. Ungerstedt, Tissue levels and in vivo release of tachykinins and GABA in striatum and substantia nigra of rat brain after unilateral striatal dopamine denervation, Exp. Brain Res. 74 (1989) 527–534.

[19] M. Manier, D.N. Abrous, C. Feuerstein, M. Le Moal, J.P. Herman, Increase of striatal methionin enkephalin content following lesion of the nigrostriatal dopaminergic pathway in adult rats and reversal following the implantation of embryonic dopaminergic neurons: a quantitative immunohistochemical analysis, Neuroscience 42 (1991) 427–439.

[20] J. Mokry, Experimental models and behavioural tests used in the study of Parkinson's disease, Physiol. Res. 44 (1995) 143–150.

[21] H. Nishino, T. Hashitani, M. Kumazaki, H. Sato, F. Furuyama, Y. Isobe, N. Watari, M. Kanai, S. Shiosaka, Long-term survival of grafted cells, dopamine synthesis/release, synaptic connections, and functional recovery after transplantation of fetal nigral cells in rats with unilateral 6-OHDA lesions in the nigrostriatal dopamine pathway, Brain Res. 534 (1990) 83–93.

[22] G. Paxinos, C. Watson, The Rat Brain in Stereotaxic Coordinates, Academic Press, San Diego, 1997.

[23] N.F. Saadé, S.F. Atweh, N.B. Bahuth, S.J. Jabbur, Augmentation of nociceptive lesions of either dopaminergic terminals or midbrain dopaminergic neurons, Brain Res. 751 (1997) 1–12.

[24] R. Sandyk, C.R. Bamford, R.P. Iacono, Pain and sensory symptoms in Parkinson's disease, Int. J. Neurosci. 39 (1988) 15–25.

[25] M. Savasta, F. Mennicken, M. Chritin, D.N. Abrous, C. Feuerstein, M. Le Moal, J.P. Herman, Intrastriatal dopamine-rich implants reverse the changes in dopamine

D_2 receptor densities caused by 6-hydroxydopamine lesion of the nigrostriatal pathway in rats: an autoradiographic study, Neuroscience 46 (1992) 729–738.
[26] R.K.W. Schwarting, J.P. Huston, The unilateral 6-hydroxydopamine lesion model in behavioral brain research. Analysis of functional deficits, recovery and treatments, Prog. Neurobiol. 50 (1996) 275–331.
[27] G. Skagerberg, A. Björklund, O. Lindvall, R.H. Schmidt, Origin and termination of the diencephalo-spinal dopamine system in the rat, Brain Res. Bull. 9 (1982) 237–244.
[28] J.A. Smith, F.M. Leslie, R.S. Broide, S.E. Loughlin, Long-term changes in striatal opioid systems after 6-hydroxydopamine lesion of rat substantia nigra, Neuroscience 55 (1993) 935–951.
[29] R. Takeda, T. Ikeda, F. Tsuda, H. Abe, H. Hashiguchi, Y. Ishida, T. Nishimori, Unilateral lesions of mesostriatal dopaminergic pathway alters the withdrawal response of the rat hindpaw to mechanical stimulation, Neurosci. Res. 52 (2005) 31–36.