Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 85 (2006) 643–647
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Delayed L-DOPA-induced hyperalgesia

T. Shimizu [a], S. Iwata [a,*], A. Miyata [a], T. Fukuda [a], M. Nomoto [b]

[a] Department of Pharmacology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima 890-8520, Japan
[b] Department of Clinical Pharmacology, Faculty of Medicine, Ehime University, Matsuyama 791-0295, Japan

Received 1 March 2006; received in revised form 28 October 2006; accepted 30 October 2006
Available online 4 December 2006

Abstract

Previously we reported on L-DOPA's antinociceptive effect on substance P-induced nociceptive behaviors in mice [Shimizu T, Iwata S, Morioka H, Masuyama T, Fukuda T, Nomoto M. Antinociceptive mechanism of L-DOPA. Pain 2004;110;246-9.]. Since significant hyperalgesia was noted following antinociception, our study was designed to investigate the mechanism of this hyperalgesia. Nociceptive behaviors were enhanced 2 h after L-DOPA administration. L-DOPA induced hyperalgesia occurred after conversion to dopamine because co-administration of benserazide, a DOPA decarboxylase inhibitor, completely abolished the L-DOPA-induced hyperalgesia. The D2 receptor agonist, quinpirole, depressed these behaviors entirely, while the D1 antagonist, SCH23390, inhibited the enhancement of these behaviors by L-DOPA. The D2 receptor antagonist, sulpiride, which induced hyperalgesia of the substance P-induced behaviors in naive mice, did not have any effects on L-DOPA-induced hyperalgesia. Spinal cord dopamine content increased rapidly after L-DOPA administration, exhibiting levels 100 times greater than baseline, and then returned to control after 1 h. These results suggested that the dopaminergic inhibitory system for pain sensation was temporarily impaired by excess amounts of exogenous dopamine that were derived from L-DOPA and both D1 and D2 receptors were involved in L-DOPA-induced hyperalgesia.

Keywords: L-DOPA; Hyperalgesia; D1 receptor; D2 receptor; Dopamine

1. Introduction

L-DOPA has been used to treat pain associated with diabetic neuropathy (Duby et al., 2004; Ertas et al., 1998) and polyneuropathy (Sindrup and Jensen, 2000). Although L-DOPA has a powerful antinociceptive action in experimental animals (Shimizu et al., 2004), hyperalgesia has occurred afterwards. Therefore, patients who were treated with L-DOPA can suffer from increased pain after an initial analgesia. As a result, we must develop a better understanding of this delayed hyperalgesia before L-DOPA can be used effectively to treat pain.

Some patients with Parkinson's disease who received L-DOPA experienced pain when L-DOPA was metabolized (Quinn et al., 1986). This "off-period pain" involved the spinal cord because spinal anesthesia, but not local blocking of the lumbar sympathetic chain or epidural blocking, reduced pain associated with the extremities (Sage et al., 1990). No effective drug treatment for off-period pain in Parkinsonian patients has been reported. Since L-DOPA-induced hyperalgesia in experimental animals resembles off-period pain in Parkinson's disease, our results will be important for controlling off-period pain.

Paalzow (1992) reported that L-DOPA's analgesia and hyperalgesia depended on both dose and time after treatment. Unfortunately, since treatments were given systemically in this study, the effects on the brain and spinal cord could not be differentiated. Therefore, we administered all drugs intrathecally (i.t.) to identify effects on pain conduction modification at the spinal cord level and substance P-induced nociceptive behaviors.

2. Materials and methods

Male ddY mice (Japan SLC Co., Hamamatsu, Japan) that weighed 25–35 g were housed in an environmentally controlled room (22–24 °C, 60–70% relative humidity, and a 12 h light/dark cycle with lights on at 7:00 a.m.) and given free access to standard food and water. All efforts were made to minimize animal

* Corresponding author. Tel.: +81 99 275 5256; fax: +81 99 265 8567.
E-mail address: shinichi@m.kufm.kagoshima-u.ac.jp (S. Iwata).

0091-3057/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2006.10.020

suffering and to reduce the number of animals used. Permission from the Committee of Animal Experimentation, Kagoshima University Graduate School of Medical and Dental Sciences was obtained prior to initiation of the present experiments.

Analgesic and hyperalgesic effects of L-DOPA were evaluated by nociceptive behaviors induced by intrathecal (i.t.) administration of substance P. After removing body hair around the lumbar portion, a 27-gauge needle was inserted between the L5 and L6 vertebrae. L6 is next to the pelvic bone, a needle can be inserted smoothly because of the physiological curvature of L5 and L6. Puncture of the dura was reliably indicated by a flick of the tail. Injections (5 μl) were administered using a 25-μl Hamilton microsyringe connected via polyethylene tubing. Doses administered included 10 pmol for substance P, and 10 nmol each for L-DOPA, benserazide, SKF82958, SCH23390 and sulpiride. As a result of poor solubility, only 1 nmol of quinpirole was given. Immediately after substance P administration, each mouse was placed in a glass cylinder (14 cm i.d. × 18 cm). Mice receiving substance P responded by biting or licking and scratching at the abdomen and hind portions of the body. The response lasted less than 1 min. These characteristic behaviors were not observed in artificial cerebrospinal fluid (ACSF)-treated controls. The intensity of the response elicited by substance P was quantified by counting the number of observed bites and scratches.

The time course of L-DOPA and its metabolites were studied in naïve mice that were not used in the behavioral study. Accordingly, mice were decapitated at 1, 5, 15, 60, 75 or 120 min after L-DOPA administration. The amounts of L-DOPA and dopamine in the lumbar segment of the spinal cord were determined by high performance liquid chromatography (HPLC). The sample was homogenized using ultrasonic cell disruptor (Model 185, Branson, Danbury, CT) in 200 vol ice-cold 0.1 M perchloric acid containing 5 mM EDTA and 3,4-dihydroxybenzylamine as an internal standard. After centrifugation at 28,000 ×*g* for 20 min at 4 °C, supernatant was filtered through 0.45-μm filter and a 20-μl aliquot was injected into a HPLC. The HPLC system was composed of a delivery pump (EP-10, Eicom, Kyoto, Japan), a sample injector (WISP 710B, Waters Associates, Inc., Milford, MA), a reverse-phase column (Eicopak MA-ODS), an electrochemical detector (ECD-100, Eicom, set at +0.80 V). The analytical column temperature was controlled at 40 °C. Mobile phase consisted of 7.35 g citric acid, 6.80 g sodium acetate, 0.003 g EDTA, and 0.215 g sodium octylsulfate in 850 ml distilled water; to this mixture methanol (130 ml) was added. The pH of this final solution was 3.90. Flow rate of the mobile phase was adjusted to 1 ml/min.

L-DOPA, benserazide, SKF82958, SCH23390, quinpirole and sulpiride were purchased from Sigma Chemical Co. (St. Louis, MO). Substance P was purchased from the Peptide Institute (Osaka, Japan). All drugs were dissolved in sterile ACSF (138 mM NaCl, 3 mM KCl, 1.25 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM D-glucose).

All groups consisted of 7–13 mice and statistical significance was determined by a one-way ANOVA with a subsequent post-hoc Fisher's PLSD test or Student's *t*-test.

3. Results

Mice exhibited scratching and licking behavior immediately after administration of substance P. These behaviors disappeared within 1 min. Significant depression and enhancement of substance P-induced nociceptive behaviors were observed 0 and 2 h after L-DOPA administration, respectively (Fig. 1). In order to study the hyperalgesic effect of L-DOPA, substance P was injected 2 h after L-DOPA. The enhancement of substance P-elicited nociceptive behaviors in L-DOPA-primed animals was abolished by concomitant administration of a DOPA decarboxylase inhibitor, benserazide, which indicated that L-DOPA enhanced the nociceptive behaviors after conversion to dopamine (Fig. 2A). In contrast, the D1 agonist, SKF82958, had no effects on substance P-elicited nociceptive behaviors in L-DOPA-primed animals (Fig. 2B). However, L-DOPA-enhanced substance P-elicited behaviors were suppressed by both the D1 receptor antagonist SCH23390 (Fig. 2C) and the D2 receptor agonist quinpirole (Fig. 2D). In contrast, the D2 antagonist sulpiride had no significant effect in L-DOPA-primed animals (Fig. 2E). In order to elucidate the effects of endogenous dopamine on substance P-elicited nociceptive behaviors, dopamine agonists or antagonists were co-administered with substance P in L-DOPA-naive mice. No significant effects were noted for either SKF82958 or SCH23390 (Fig. 3A, B). However, quinpirole depressed while sulpiride enhanced substance P-elicited nociceptive behaviors (Fig. 3C, D).

L-DOPA was extensively metabolized within 5 min. Dopamine content peaked at 5 min and by 60 min had returned to control levels (Table 1).

4. Discussion

The effect of L-DOPA on substance P-induced nociceptive behavior was biphasic. In addition, the effects of dopamine agonists and antagonists on L-DOPA's depression and enhancement of substance P-induced nociceptive behaviors were

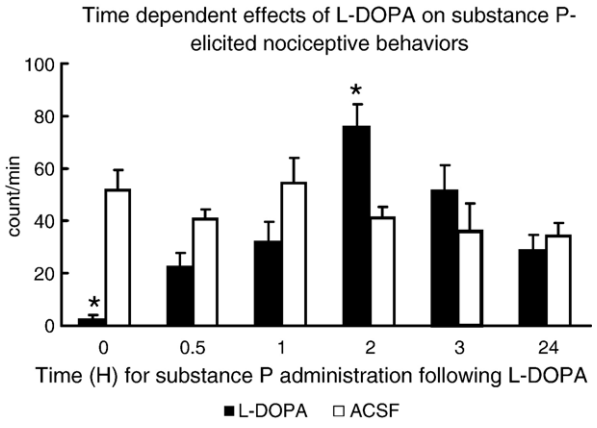

Fig. 1. Time dependent effects of L-DOPA on substance P-elicited nociceptive behaviors. Substance P was administered i.t. at 0, 0.5, 1, 2, 3 and 24 h after an administration of either L-DOPA, i.t. (closed column), or ACSF (open column). Immediately after substance P injection, nociceptive behaviors were evaluated for 1 min. Values represent mean ± SEM. (n = 7–13). *P < 0.05, as compared with values for ACSF-treated mice at each time point.

complex (Table 2). Since we previously reported on the mechanism for L-DOPA's depressive effect on substance P-induced nociceptive behaviors (Shimizu et al., 2004), only the mechanism for enhancement of substance P-induced nociceptive behaviors is discussed in this report.

Two hours after L-DOPA, when dopamine content in the spinal cord returned to control levels, L-DOPA enhanced substance P-elicited nociceptive behaviors. One hour after L-DOPA, when increased dopamine levels just returned to the control levels, no hyperalgesia was observed. In other words, L-DOPA-induced hyperalgesia required a 1 h drug-free period to produce hyperalgesia. L-DOPA pre-treatment enhanced L-DOPA-induced rotational behavior after a withdrawal (Carey et al., 1994). Therefore, sensitivity of dopaminergic receptors was augmented by L-DOPA, and a drug-free period was required to develop the receptor super-sensitivity. Although a repeated intermittent treatment is usually required to produce sensitization, a single exposure of amphetamine, which release dopamine from dopaminergic terminals, was sufficient to induce dopamine receptor super-sensitivity (Vanderschuren et al., 1999).

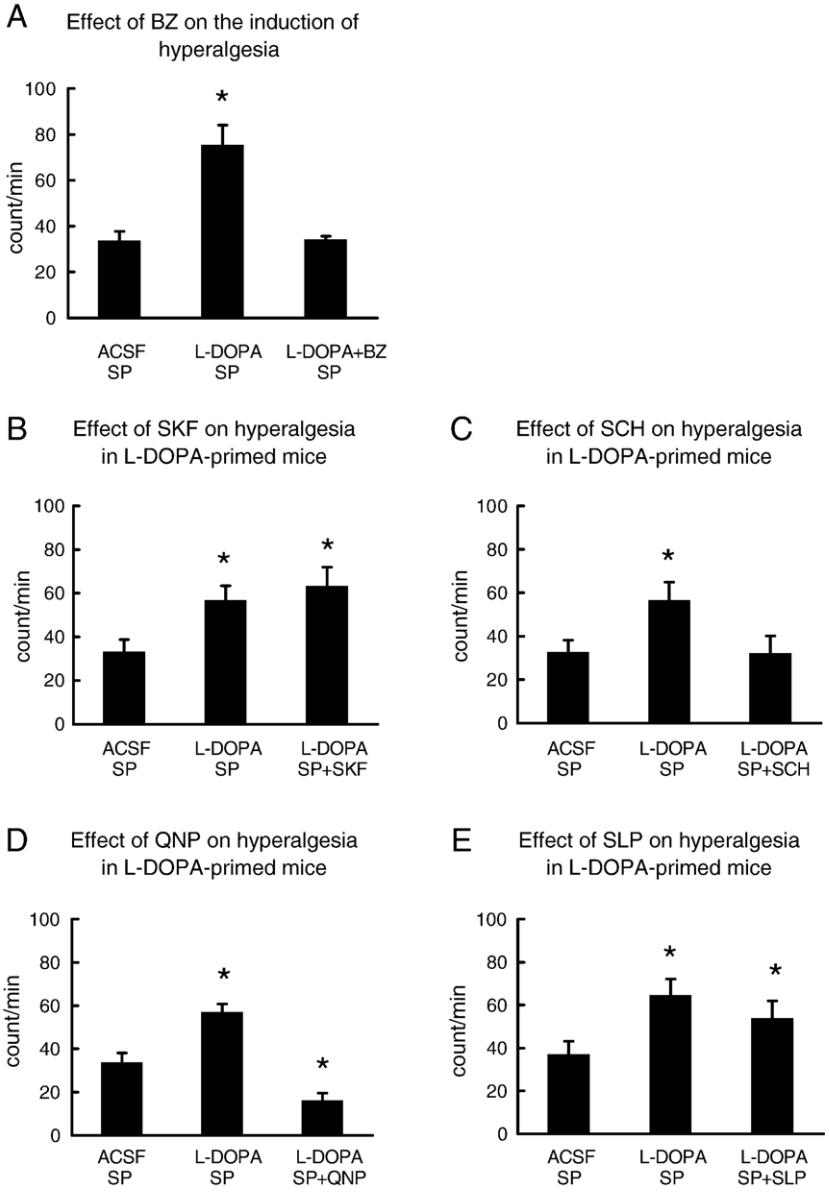

Fig. 2. (A) Effects of DOPA decarboxylase inhibitor, benserazide (BZ) on the induction of hyperalgesia. Two hours after administration of ACSF, L-DOPA, or L-DOPA + benserazide, substance P-elicited nociceptive behaviors were evaluated. (B–E) The effect of selective dopamine agonists or antagonists on substance P-elicited nociceptive behaviors in L-DOPA-primed mice. A D1 agonist (SKF82958; SKF; B), a D1 antagonist (SCH23390; SCH; C), a D2 agonist (quinpirole; QNP; D) or a D2 antagonist (sulpiride; SLP; E) was administered with substance P in mice that were pre-injected with L-DOPA. Left columns in figures B–E; substance P was administered without any dopamine agonists and antagonists in L-DOPA-naive mice. Central columns in figures B–E; substance P was administered without any dopamine agonists and antagonists in L-DOPA-primed mice. Right columns in figures B–E; substance P was administered with one of the dopamine agonists or antagonists in L-DOPA-primed mice. Values represent mean ± SEM. ($n = 7–9$). *$P < 0.05$, as compared with values for substance P 2 h after administration of ACSF (left column in each figure).

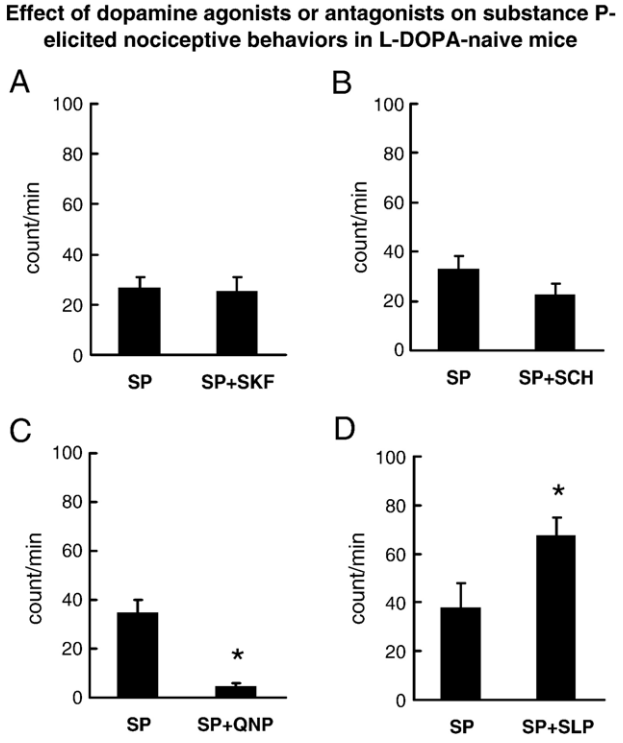

Fig. 3. Effects of co-administered dopamine agonists or antagonists on substance P-elicited nociceptive behaviors in L-DOPA-naive mice. SKF82958 (SKF; A), SCH23390 (SCH; B), quinpirole (QNP; C), or sulpiride (SLP; D) was co-administered with substance P in naive mice. Values represent mean±SEM. (n=7–9). *P<0.05, as compared with values for substance P without any drugs.

The result that quinpirole suppressed the nociceptive behaviors in L-DOPA-primed animals suggests that D2 receptors played a role in hyperalgesia. However, since sulpiride, which induces hyperalgesia in L-DOPA-naive mice, failed to enhance L-DOPA-induced hyperalgesia, suggesting that the endogenous dopaminergic inhibitory system of pain sensation was impaired. Thus, the mechanism of the hyperalgesia may be associated with impairment of the dopaminergic inhibitory system to pain sensation. SCH23390 significantly depressed the enhancement of substance P-induced nociceptive behaviors (Fig. 2C), suggesting that D1 receptors were involved in hyperalgesia. A dopaminergic descending system from the A10 and A11 to the dorsal horn of the spinal cord is present in mice (Qu et al., 2006), and this system is reported to be responsible for inhibiting pain conduction through D2 receptor (Tamae et al., 2005). In addition, D1, as well as D2 receptors, are present in the spinal cord (Levant and McCarson, 2001; Venugopalan et al., 2006). These anatomical results support the hypothesis

Table 2
Summary table as to the effect of dopamine agonists and antagonists on substance P-induced nociceptive behavior, analgesic effect of L-DOPA, and hyperalgesic effect of L-DOPA on substance P-induced nociceptive behaviors

Dopaminergic drugs	L-DOPA-naive mice (Fig. 3)	Analgesic effect of L-DOPA (Shimizu et al., 2004)	Hyperalgesic effect of L-DOPA (Fig. 2)
SKF82958 (D1 agonist)	No significant effect	Not measured	No significant effect
SCH23390 (D1 antagonist)	No significant effect	No significant effect	Antagonize
Quinpirole (D2 agonist)	Depress	Not measured	Antagonize*
Sulpiride (D2 antagonist)	Enhance	Antagonize	No significant effect

*It is not clear that quinpirole antagonizes hyperalgesic part of L-DOPA-induced hyperalgesia, since quinpirole completely depressed substance P-induced nociceptive behaviors.

that D1 receptors are involved in pain modification. D1 receptors modify the pain conduction only in L-DOPA-primed animals, not in the L-DOPA-naive animals. This suggests that D1 receptors only work in pathological conditions. In the physiological condition, D1 receptors may slightly facilitate the conduction of pain sensation and this effect can be suppressed by an activation of D2 receptors. After a withdrawal of L-DOPA, effect of D1 receptors to facilitate pain conduction could become apparent.

Paalzow (1992) reported that L-DOPA induces a multiphasic analgesia and hyperalgesia. These effects varied depending upon both the amount of time after L-DOPA administration and the doses given. However, this multiphasicity may be associated with the route of administration, as all of the drugs administered in the study were given intraperitoneally. Therefore, drug concentrations gradually increased, peaked and then gradually decreased within the spinal cord. In contrast, in our experiment, we administered all drugs i.t., which resulted in very rapid increases followed by gradual decreases in drug concentrations. Even with our simple animal pain model, biphasic L-DOPA effects were observed, thus making it very difficult to identify the mechanism by which L-DOPA was able to modify nociceptive transmissions after systemic drug administrations. Furthermore, since systemic drug administrations must have an effect on dopaminergic systems that are responsible for pain modification in the brain; this further complicates attempts to determine drug effects. Another potential reason for the multiphasicity observed could be due to the nonselective effects of L-DOPA on D1 and D2 receptors. In the present study, we

Table 1
L-DOPA and dopamine contents in the lumbar portion of the spinal cord

		Time after intrathecal L-DOPA administration						
ng/g tissue	Control	1 min	5 min	15 min	30 min	60 min	75 min	120 min
L-DOPA	19±4 (7)	115±279 (5)*	nd	nd	26±5 (5)	nd	nd	nd
Dopamine	16±8 (8)	1552±549 (5)*	1628±204 (5)*	470±69 (11)*	141±21 (5)*	50±10 (11)	71±4 (5)	45±4 (7)

Results are expressed as the mean±SEM. (number of mice). *P<0.05. nd : not detected.

observed a functional difference between the D1 and D2 receptors in substance P-induced nociceptive behaviors in L-DOPA-naive and in L-DOPA-primed animals. Additionally, expression of nociceptive behaviors was further complicated by the nonselective action on dopaminergic receptors by dopamine converted from L-DOPA.

In conclusion, the present results suggest that dopaminergic inhibitory system for pain conduction, in which D2 receptors are mainly involved, is impaired after L-DOPA priming, and D1 receptors take part in the pain conduction system in this pathological condition.

References

Carey RJ, Pinheiro-Carrera M, Tomaz C, Huston JP. Early L-dopa treatment initially retards but later enhances dopamine receptor supersensitivity following unilateral dopamine denervation. Brain Res 1994;658:145–54.

Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: an intensive review. Am J Health Syst Pharm 2004;61:160–73.

Ertas M, Sagduyu, Arac N, Uludag B, Ertekin C. Use of levodopa to relieve pain from painful symmetrical diabetic polyneuropathy. Pain 1998;75:257–9.

Levant B, McCarson KE. D(3) dopamine receptors in rat spinal cord: implications for sensory and motor function. Neurosci Lett 2001;303:9–12.

Paalzow GH. L-dopa induces opposing effects on pain in intact rats: (−)-sulpiride, SCH 23390 or alpha-methyl-D L-*p*-tyrosine methylester hydrochloride reveals profound hyperalgesia in large antinociceptive doses. J Pharmacol Exp Ther 1992;263:470–9.

Qu S, Ondo WG, Zhang X, Xie WJ, Pan TH, Le WD. Projections of diencephalic dopamine neurons into the spinal cord in mice. Exp Brain Res 2006;168:152–6.

Quinn NP, Koller WC, Lang AE, Marsden CD. Painful Parkinson's disease. Lancet 1986;1:1366–9.

Sage JI, Kortis HI, Sommer W. Evidence for the role of spinal cord systems in Parkinson's disease-associated pain. Clin Neuropharmacol 1990;13:171–4.

Shimizu T, Iwata S, Morioka H, Masuyama T, Fukuda T, Nomoto M. Antinociceptive mechanism of L-DOPA. Pain 2004;110:246–9.

Sindrup SH, Jensen TS. Pharmacologic treatment of pain in polyneuropathy. Neurology 2000;55:915–20.

Tamae A, Nakatsuka T, Koga K, Kato G, Furue H, Katafuchi T, et al. Direct inhibition of substantia gelatinosa neurones in the rat spinal cord by activation of dopamine D2-like receptors. J Physiol 2005;568:243–53.

Vanderschuren LJ, Schmidt ED, De Vries TJ, Van Moorsel CA, Tilders FJ, Schoffelmeer AN. A single exposure to amphetamine is sufficient to induce long-term behavioral, neuroendocrine, and neurochemical sensitization in rats. J Neurosci 1999;19:9579–86.

Venugopalan VV, Ghali Z, Senecal J, Reader TA, Descarries L. Catecholaminergic activation of G-protein coupling in rat spinal cord: further evidence for the existence of dopamine and noradrenaline receptors in spinal grey and white matter. Brain Res 2006;1070:90–100.