Neuroscience 235 (2013) 1–9

C-FOS ACTIVITY MAPPING REVEALS DIFFERENTIAL EFFECTS OF NORADRENALINE AND SEROTONIN DEPLETION ON THE REGULATION OF OCULAR DOMINANCE PLASTICITY IN RATS

K. NAKADATE, [a] **K. IMAMURA** [b*] **AND Y. WATANABE** [c]

[a] *Department of Basic Biology, Educational and Research Center for Pharmacy, Meiji Pharmaceutical University, Kiyose-shi, Tokyo 204-8588, Japan*

[b] *Department of Systems Life Engineering, Maebashi Institute of Technology, Maebashi-shi, Gunma 371-0816, Japan*

[c] *RIKEN Center for Molecular Imaging Science, Kobe-shi, Hyogo 650-0047, Japan*

Abstract—The roles of the central noradrenergic and serotonergic system in the activity-dependent regulation of ocular dominance plasticity have been a contentious issue. Using c-Fos activity mapping, we have developed a new, straightforward method to measure the strength of ocular dominance plasticity: the number of c-Fos-immunopositive cells in layer IV of rat visual cortex (Oc1B), ipsilateral to the stimulated eye, is a sensitive and reliable measure of the effects of monocular deprivation. Applying this new method, here we studied the unique modification of the degree of c-Fos expression induced in the visual cortex, in that endogenous noradrenaline (NA) and serotonin (5HT) in the cortex were significantly reduced, respectively by specific pharmacological agents. Intraperitoneal injections of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4) and p-chlorophenylalanine (pCPA) selectively impair NA- and 5HT-containing nerve terminals and fibers, respectively. In the visual cortex with strongly reduced NA, the number of c-Fos-immunopositive cells was found remaining significantly decreased in response to stimulation of the deprived eye, while by open eye stimulation the expected increase in c-Fos-immunoreactivity was strongly suppressed, showing values not different from those obtained by monocular stimulation in the normal rats. In contrast, in the visual cortex with strongly reduced 5HT no expected decrease was found in response to stimulation of the deprived eye, while, as is usually the case for the normal animals, a significant increase was still induced in response to open eye stimulation. These findings suggest that the noradrenergic and serotonergic system regulate ocular dominance (OD) plasticity differently: in the NA-depleted cortex the expected increase in c-Fos expression by open eye stimulation was not seen due to strong suppression, whereas in 5HT-depletion, the expected decrease in c-Fos expression was not materialized due to strong suppression. The present findings with c-Fos activity mapping method indicated a novel possibility of the differential regulation of OD plasticity by two types of common monoaminergic systems.

Key words: ocular dominance plasticity, 5HT, NA, primary visual cortex, immediate-early gene, c-Fos induction.

INTRODUCTION

Experience-dependent changes in synaptic efficacy or synaptic plasticity are an important characteristic of the brain. One of the extensively studied, experimental models of this kind of synaptic plasticity is ocular dominance (OD) plasticity in the primary visual cortex of immature mammals (Wiesel et al., 1974; Hubel et al., 1977; Issa et al., 1999). Previous studies have collectively indicated that OD plasticity is regulated by neuronal activity in the visual cortex, since various forms of experimental manipulation of cortical excitability invariably result in changes in the shift in the OD distribution expected following monocular deprivation, suggesting the impairment of OD plasticity (Greuel et al., 1987; Muller et al., 1993; Fonta et al., 2000; Mataga et al., 2002; Wong-Riley and Jacobs, 2002). In addition to this activity-dependent mechanism, it is recognized that a few neuromodulators, such as noradrenaline (NA) and serotonin (5HT), are *causally* involved in the OD plasticity regulation (Kasamatsu and Pettigrew, 1976; Pettigrew and Kasamatsu, 1978; Kasamatsu and Pettigrew, 1979; Gu and Singer, 1995).

Pharmacological depletion of cortical NA in the kitten visual cortex prevents the OD shift from taking place following monocular deprivation (Kasamatsu and Pettigrew, 1976). Although some later studies reported contradictory findings, i.e., no effects of NA depletion (Daw et al., 1984, 1985; Trombley et al., 1986), a tome of accumulated evidence supports the original thesis and its expansion that the NA, β-adrenoceptor, cyclic adenosine monophosphate (cAMP), and cAMP-responsive element-binding protein (CREB) system plays critical roles in the regulation of OD plasticity (Pettigrew and Kasamatsu, 1978; Kasamatsu and Pettigrew, 1979; Kasamatsu and Shirokawa, 1985; Shirokawa and Kasamatsu, 1986; Imamura and Kasamatsu, 1988; Shirokawa et al., 1989; Mataga et al., 1992; Muguruma et al., 1997; Mower et al., 2002).

*Corresponding author. Tel: +81-27-265-0111x670; fax: +81-27-265-3837.
E-mail address: imamurak@maebashi-it.ac.jp (K. Imamura).

Abbreviations: c-fos, protooncogene c-fos; c-Fos, protein product of c-fos gene; DβH, dopamine-β-hydroxylase; DSP4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; IEG, immediate-early gene; LTP, long-term potentiation; NA, noradrenaline; Oc1B, binocular subfields of rodent primary visual cortex; OD, ocular dominance; pCPA, p-chlorophenylalanine methyl ester; TTX, tetrodotoxin; 5HT, serotonin.

0306-4522/12 $36.00
http://dx.doi.org/10.1016/j.neuroscience.2013.01.013

Similarly, later studies suggest that 5HT is also involved in the regulation of OD plasticity (Gu and Singer, 1995; Wang et al., 1997; Baroncelli et al., 2010). Moreover, recent morphological studies have shown that NA and 5HT both facilitate the formation and the maintenance of synapses in rat visual cortex (Matsukawa et al., 2003; Nakadate et al., 2006).

In electrophysiological experiments, it is assumed that the refinement of cortical circuits is attained by strengthening some synaptic connections and weakening others, depending on the activity pattern of cortical neurons at a given moment, and that long-term potentiation (LTP) and depression (LTD) of synaptic transmission mediate the initial phase of these strengthening and weakening processes, respectively (Stent, 1973; Bear et al., 1987; Singer, 1995; Katz and Shatz, 1996; Zhang and Poo, 2001; Jang et al., 2010). It was reported that NA and 5HT facilitate both LTP and OD plasticity, and it is possible that LTP in the visual cortex is a basis for the experience-dependent enhancement of visual responses of cortical neurons during the critical period (Inaba et al., 2009).

Released from the axon terminals of lateral geniculate nucleus cells, glutamate is a neurotransmitter within the visual cortex. Following the activation of glutamatergic receptors in the visual cortex, transcription factors of immediate-early genes (IEGs) are rapidly and transiently activated (Sheng and Greenberg, 1990). IEGs including c-fos are considered to play important roles in activity-dependent plasticity of developing animals (Kaczmarek and Chaudhuri, 1997; Kaczmarek et al., 1999). In fact, the expression of these genes in the primary visual cortex is modulated by intraocular injections of tetrodotoxin (TTX) or by i.p.-injections of glutamate receptor ligands, such as kainate (agonist) and dizocilpine maleate (antagonist) (Kaminska et al., 1995; Yamada et al., 1999; Mataga et al., 2001). Importantly, in the visual cortex of monkeys, cats, and rats, previous studies have demonstrated differences in the expression pattern between c-fos and zif268 genes, or their proteins, both at the constitutive level and after visual deprivation or stimulation (Kaplan et al., 1996; Chaudhuri et al., 1997; Kaczmarek et al., 1999; Yamada et al., 1999; Mower and Kaplan, 2002; Zangenehpour and Chaudhuri, 2002). The response of c-Fos is rapid and tangent while that of zif268 is relatively long-lasting (Kaczmarek et al., 1999).

In a recent study, we have found that monocular deprivation during the postnatal sensitive period significantly and selectively changes both the amount and pattern of c-Fos expression upon monocular stimulation of either eye, deprived or non-deprived. And, a quantitative measurement revealed that the number of immunopositive cells in layer IV of binocular subfields of rodent primary visual cortex (Oc1B) ipsilateral to the stimulated eye was found to be a dependable index of the effects of monocular deprivation (Nakadate et al., 2012). In our previous study (Nakadate et al. 2012), we examined c-fos expressions in all layers of the binocular region of the visual cortex (Oc1B). We found that the number of immunopositive neurons in the *layer IV* of the binocular region of visual cortex *ipsilateral* to the stimulated eye is the best index for the assessment of OD plasticity. We initially examined all layers and both hemispheres of the visual cortex before obtaining this conclusion. Now, we have applied this finding to examine pharmacological treatments on the OD plasticity.

Moreover, administration of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP4), a selective noradrenergic neurotoxin, suppressed the basal expression of c-fos messenger RNA, and the response of c-fos expression to visual stimulation was also significantly lowered in the visual cortex of DSP4-treated animals (Yamada et al., 1999). However, it is not yet clear how activity-dependent plasticity as related to the IEG expression is regulated by classical neuromodulators such as NA and 5HT.

In the present study, to anatomically re-evaluate whether NA and 5HT would regulate OD plasticity and if so, to determine how such regulation is carried out, we studied the c-Fos expression in layer IV of Oc1B in response to monocular stimulation of either the visually deprived or non-deprived, open eye of rats, in which endogenous NA and 5HT, respectively, in the cortex was sufficiently depleted by specific pharmacological agents.

EXPERIMENTAL PROCEDURES

Animals

In this study, we used a total 18 Long–Evans strain, male rats. Each of the four experimental groups consisted of four animals per group, and an additional two animals served as control (Table 1). All rats were kept in a room under the usual rearing conditions at 24 °C (room temperature), on a light/dark cycle of 16-h light and 8-h dark. They were maintained on *ad libitum* food and water. All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the animal research committee of the pertinent institute. Every effort was made to minimize animal suffering and to reduce the number of animals used in the present study.

Pharmacological procedures

Pharmacological procedures are shown in Fig. 1A as a schematic protocol. Pharmacological means were used to examine an impact of monoamine depletion on the effects of monocular deprivation as described previously (Matsukawa et al., 1997, 2003). Briefly, to deplete NA, on postnatal day 12 rat pups were intraperitoneally injected with 25 mg/kg body weight of DSP4 (a selective neurotoxin of NA; RBI, MA, USA) twice 1 h apart. To deplete 5HT, 100 mg/kg body weight of p-chlorophenylalanine methyl ester (pCPA; a selective depletor of brain 5HT, SIGMA, MO, USA) was intraperitoneally injected on postnatal days 12, 13, 15, 17, 19, 21, 23, 25, and 27.

Monocular deprivation and visual activation

Experimental procedure from monocular deprivation to light exposure is shown in Fig. 1B as a schematic protocol. Initial monocular deprivation and subsequent visual activation were both performed according to the protocol established in our previous study (Nakadate et al., 2012). Rats at postnatal day