ELSEVIER

Neuroscience Letters 359 (2004) 119–123

Neuroscience Letters

www.elsevier.com/locate/neulet

Activation of the primary and association auditory cortex by the transition of sound intensity: a new method for functional examination of the auditory cortex in humans

Tomohisa Okada[a], Manabu Honda[a], Jun Okamoto[b], Norihiro Sadato[a,c,*]

[a] *National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki, 444-8585, Japan*
[b] *Siemens-Asahi Medical Technologies, Tokyo, Japan*
[c] *JST/RISTEX, 2-5-1, Atago, Minato-ku, Tokyo, 105-6218, Japan*

Received 26 November 2003; received in revised form 26 January 2004; accepted 5 February 2004

Abstract

During functional MRI image acquisition, the scanning equipment generates substantial auditory noise, the effects of which are usually ignored. To investigate the neural activity in response to the transition of noise, we measured cerebral responses to short silent periods (1 and 5 s) during which the slice readout gradients were switched off. In all 15 normal volunteers, the 1 s silence bilaterally activated the primary and association auditory cortex. Subtraction of the response to the 1 s silent period from that to the 5 s silent period revealed the activation related to the onset (transition of sound from OFF to ON) event, indicating that the 1 s response is offset (transition of sound from ON to OFF) related. The complex response of the auditory cortex to the transition of the noise should be considered in designing functional MRI with auditory tasks.

Keywords: Functional MRI; Auditory cortex; Sound intensity change; Acoustic noise

The adaptation of functional MRI (fMRI) to neuroscience studies was facilitated by echo planar imaging (EPI) acquisition, a scanning process associated with substantial auditory noise. This is caused by a Lorenz force that is generated during the rapid switching of a large gradient, causing deformation of the gradient coil, and a corresponding vibration similar to that generated in a loudspeaker [16]. Although essential to the rapid acquisition of image data, this noise risks interfering with task presentation and perception [12,17] and task-related neuronal activity [2], confounding experimental results. This is particularly critical in studies of auditory cortical function [6]. To reduce acquisition noise for clear auditory task presentation, the EPI sequence has been modified to skip volume acquisition by switching off the slice readout gradients [19], generating an 'OFF' (silent) period. Previous electrophysiological studies in humans [5] and animals [7,10], however, have shown that transition of sound elicits a response in the auditory cortex. Hence, we predicted that the periodic reduction of fMRI acquisition noise would induce transition-related neural activity and its clarification is required to determine the application and limitation of this method. To further differentiate the physiological response to 'offset' (the transition of sound from ON to OFF) and 'onset' (the transition from OFF to ON), we alternated the length of the OFF period between 1 and 5 s.

EPI images were captured (TR 1 s, TE 30 ms, FA 62 degrees, FOV 19.2 cm, 64 × 64 matrices, 16 slices of 6 mm thickness and 1.2 mm gap covering the whole cerebrum) with a 3T scanner (Allegra; Siemens, Erlangen, Germany). The images were acquired continuously through the repeat (TR) period (Fig. 1).

We modified the image acquisition protocol by turning off slice readout gradients for 1 s periods; during these silent 'OFF' periods, we provided radiofrequency pulses in order to keep the magnetization constant (Fig. 1). Throughout the study, scanning noise was measured with a sound pressure meter (LA-4350; Ono Sokki, Tokyo, Japan) inside the

* Corresponding author. National Institute for Physiological Sciences, 38 Nishigonaka, Myodaiji, Okazaki, 444-8585, Japan. Tel.: +81-564-55-7874; fax: +81-564-55-7786.
E-mail address: sadato@nips.ac.jp (N. Sadato).

0304-3940/03/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.02.018

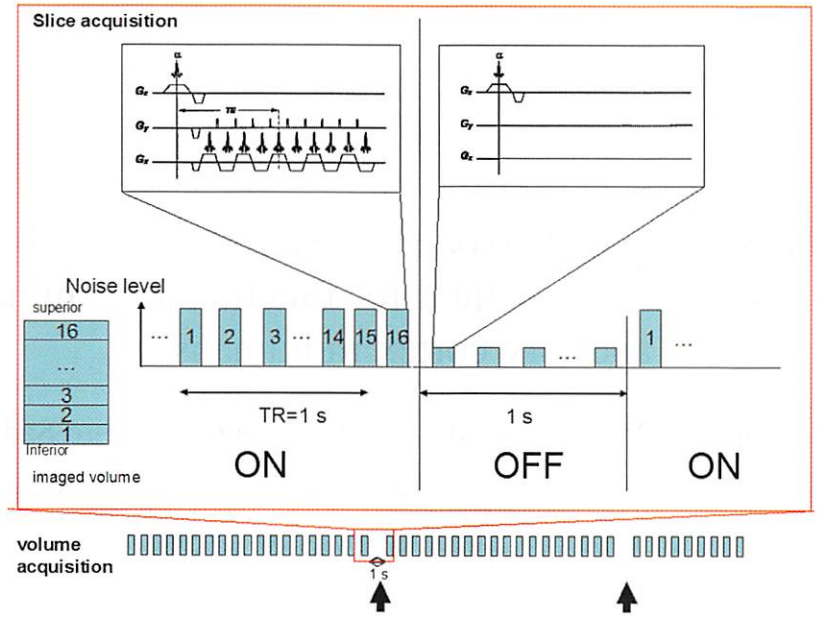


Fig. 1. Task design of event-related fMRI of transition of sound intensity. Single shot gradient echo EPI for acquisition of volume data consisting of 16 contiguous slices. Repetition time (TR) = 1 s, echo time (TE) = 30 ms. After a single radio frequency (RF) excitation (alpha) with slice selection (by means of the gradient of Gz), an oscillatory gradient was applied along the frequency-encoding direction (Gx; readout gradient) to generate multiple echoes. These echoes were phase-encoded independently (by Gy using a blipped phase encoding gradient; phase encoding gradient) and scanned the entire k-space to form a slice image (upper left). During volume acquisition, large scanner noise was generated which was mainly due to Gx. During the OFF condition for 1 s, Gx was turned off whereas RF excitation, slice selection, and phase encoding were conducted. Residual noise was mainly caused by slice selection (Gz) and phase encoding (Gy) gradients. Sudden reduction of the noise was utilized as the auditory stimulus (Off events, arrows).

magnet. As depicted in the spectrogram (Fig. 2), turning off the readout gradient reduced the frequency components from 107.2 to 88.8 dB (A-weighted measurement). The sound pressure was lowered to 1/10 and the sound intensity to 1/100 of the original levels, representing relatively silent periods during scans. Using awake non-human primates, Liang et al. [10] found that an amplitude modulation of 50% was sufficient to induce responses in the primary auditory cortex to both onset and offset of pure tone stimuli. Hence, our scan method will elicit both onset and offset responses.

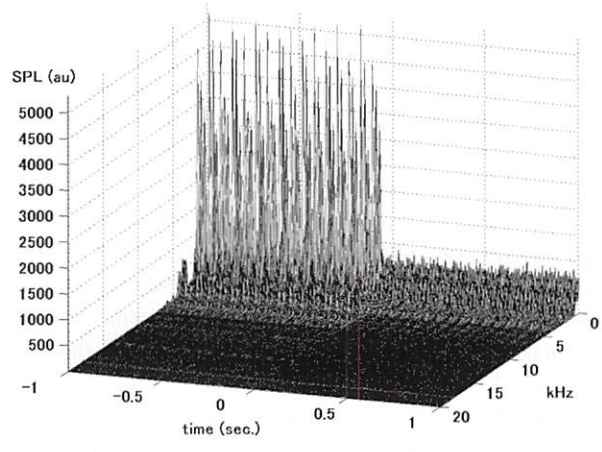


Fig. 2. Spectrogram of the scanner noise for 1 s both before and after extinction of the readout gradient (0 s). After extinction of the readout gradient, most of the scan sound was under 5 kHz, representing a marked reduction in intensity. The vertical axis represents the sound pressure level (SPL) in arbitrary units (au).

Our study was conducted on 15 normal subjects (seven men and eight women, mean age 26.9 ± 6.5 years) who had no history of neurological or psychiatric illness or developmental disorders. The ethics committee of the National Institute for Physiological Sciences approved the protocol, and all subjects provided written informed consent. Lying on the scanner table with both ears plugged, each subject experienced 44 trials of 1 s silent periods. Trials were divided into four sessions lasting 3 min each, with an inter-trial interval (ITI) of 13–17 s. To maintain alertness throughout the session, subjects were required to press a button every 3 s when an eye-fixation cross (+) changed to minus (−). Twelve of the 15 subjects also performed a second variation of the fMRI experiment, which was similar to the first except that it incorporated longer silent periods of 5 s each. In this latter case, 40 trials were divided into five sessions, each lasting 3 min, and separated by ITIs of 12–15 s. This second experiment was implemented to compensate for the low temporal resolution of fMRI studies. Two events must be separated by several seconds in order to differentiate their hemodynamic responses; therefore, the longer silent period enabled a distinction between offset- and onset-related physiological activities.

Imaging data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, London, UK) and in-house programs implemented in Matlab (Mathworks, Sherborn, MA). In the case of 1 s silences, the missing image volumes were linearly interpolated with volumes immediately preceding and following the OFF period. The EPI

images were realigned, spatially normalized into a stereotaxic space, and smoothed with an isotropic Gaussian kernel of 6 mm full width at half maximum.

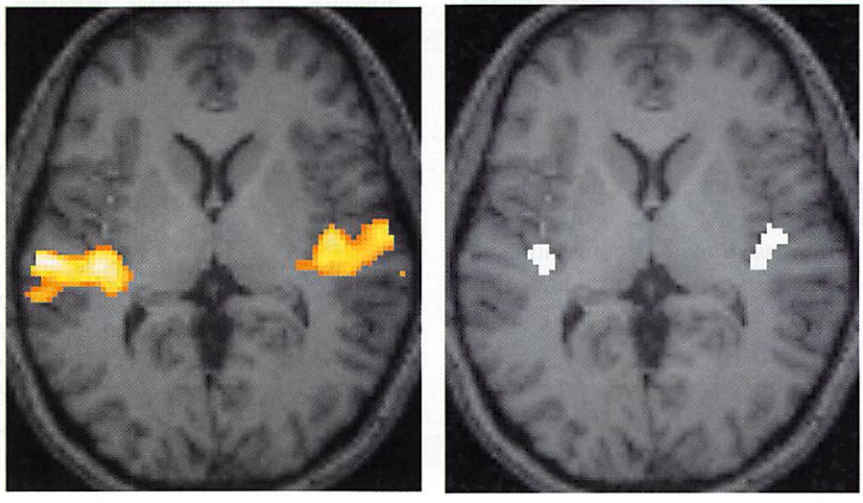
The effect of 1 s silent events was estimated using a general linear model for every individual voxel. The resulting set of voxel values constituted a statistical parametric map of the t statistic, SPM{t}. The statistical threshold was $P < 0.05$, corrected for multiple comparisons at the cluster level (a representative result is shown in Fig. 3). In order to present the activation common to the subject group as a whole, random effect analysis was also performed ($P < 0.05$, corrected for multiple comparisons at the voxel level, i.e. more stringent than the individual level; Table 1).

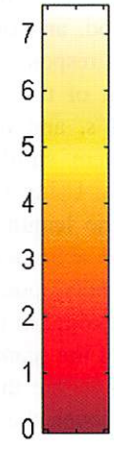
The bilateral transverse temporal gyri were identified in each subject using MRIcro (http://www.psychology.nottingham.ac.uk/staff/cr1/mricro.html). The overlapping areas were defined as significantly activated primary auditory areas (A1) [20], and the averaged time-course data were extracted for both left and right areas in each subject. The time-course signal changes after each 1 or 5 s silent period were calculated as percentage changes from

Table 1
Event-related activation due to the 1 s silent period

Location	Side	Cluster size	Local maximum (mm)			t	P (corrected)
			x	y	z		
A1	L	118	−42	−26	6	12.18	0.002
	R	139	52	−14	4	13.26	0.001

The result of group analysis conducted with a random effect model after correction for multiple comparisons at each voxel level. A1: primary auditory cortex.





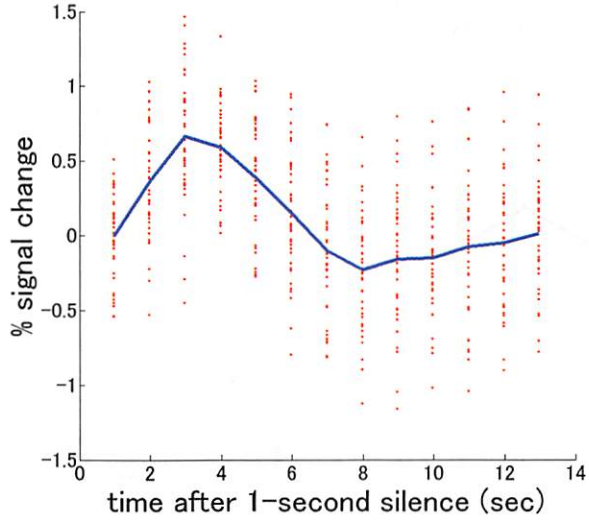


Fig. 3. (Left) A representative statistical parametric map (24-year-old female) of activated areas displaying a significant transient increase in activity during the 1 s silent period, superimposed on the T1-weighted high-resolution MRI of the same subject. The statistical threshold was $P < 0.05$ with a correction for multiple comparisons. The color bar indicates the t scores. (Right) The activated areas that overlap with the anatomically defined primary auditory cortex (white area; 6 mm above the AC-PC plane in Montreal Neurological Institute (MNI) space). (Bottom) Averaged signals across 44 trials at the voxel showing the maximum t score in the right A1 (Talairach's coordinate of $x = 46$, $y = -22$, $z = 6$) in a representative subject.

the average signal of the preceding two time points to the periods.

In the first fMRI experiment, the 1 s silence activated the primary and adjacent association auditory cortex bilaterally in the region overlying the transverse temporal gyri (Fig. 3). This was true of all subjects, and group analysis confirmed the activation (Table 1). Hemodynamic response curves extracted from the activated areas began after the cessation of the silent period for 1 s, peaked 3–5 s later, and returned nearly to the baseline at 13 s. This is a typical hemodynamic response to the neural activities of short duration.

With 12 subjects who underwent both the first and second experiments, we subtracted the response to the 1 s silent period from that to the 5 s silent period (Fig. 4). The response was almost identical regarding the hemodynamic delay and amplitude. The peak activity was observed 4 s after the cessation of the silent periods. The averaged maximal % signal change to the 1 s silent period was 0.57 ± 0.12 (mean $\pm$ SD, $N = 12$) in the left A1, and 0.57 ± 0.13 in the right A1. The averaged maximal % signal change of the subtracted response was 0.57 ± 0.12 in the left A1, and 0.57 ± 0.14 in the right A1. Initial activity was slightly higher in the subtracted response.

The similarity between the response to the 1 s silent period and the subtracted response suggests that both are related to events of short duration, i.e. the transition of the sound. As the subtracted response is mainly representing the onset-related activity considering the long preceding silent period of 5 s, the response to the 1 s silent period should mainly represent offset-related activity.

The transition of sound intensity at both ON to OFF (offset) and OFF to ON (onset) events activated the primary and association auditory cortex. This is consistent with findings by Harms and Melcher [6], who used a single-slice fMRI with a long TR to eliminate scan noise. They found that in the auditory cortex, a fast rate auditory burst stimulus (>10/s) elicited a phasic hemodynamic response that included prominent peaks just after train onset and offset.

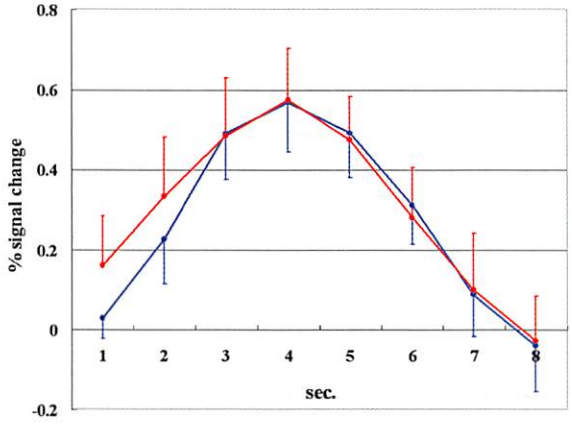


Fig. 4. The average responses at the left primary auditory cortex of 12 subjects. The blue line is the response to the 1 s silent periods. The red line is the subtraction of the response to the 1 s silent period from that to the 5 s silence. The *x*-axis shows the time after the cessation of 1 or 5 s silent periods. Error bars indicate the standard deviations.

The offset response may be related to electrophysiological 'off-responses' observed intracortically in animals following the termination of stimulus trains [3,18]. In humans, extracranial evoked potential [8,14] and magnetic field recordings [5,9,11,13] have also been used to detect off-responses following prolonged noise or tone bursts (with durations ranging from 0.5 to 10 s). Both the electrophysiological and fMRI offset responses imply increased activity in auditory cortical neurons following sound offset [1,4,7,10,15].

Regarding onset-related activity, Harms and Melcher [6] suggest that the underlying mechanism involves burst-to-burst adaptation in neuronal activity. This is based on the observation that the initial onset response to a high-frequency stimulus train (e.g. 10/s) tended to diminish as the signal persisted, and the degree of inhibition was proportional to the stimulus frequency. In the present study, the frequency of the auditory sound train was 16/s (corresponding to the number of MR slices obtained per second); therefore, initial phasic onset responses were expected to recede as the high-frequency scanner noise continued.

Slightly higher initial activity in the subtracted responses than the response to the 1 s silent period may be due to the fact that the response to the 1 s silent period is not identical to the offset-related activity during the 5 s silence condition. They have different durations of the preceding ON period; 12–16 s for the 1 s silent period, and 7–10 s for the 5 s silent period. The response to the 1 s silent period also contains the onset response with a short preceding OFF period of 1 s. A previous magnetoencephalographic study showed that the magnitude of onset responses is related to the duration of the preceding OFF period, and offset response to that of the ON period [5]. Both responses increased in amplitude with increasing duration of the preceding ON period or OFF periods up to 8.8 s, and the magnitudes of onset and offset responses were similar when the preceding durations were the same. Using fMRI, Harms also found a correlation between the length of the ON period, and the size of offset-related activities [6].

Background scanner noise potentially confounds fMRI studies of auditory processing; in this study we utilized the extinction and ignition of the scanner itself as the auditory stimulus, enabling us to isolate the neural response to the transition of sound. Moreover, there was no need for active participation of the subjects in this protocol; this would enable its adaptation to the pediatric age group. Finally, the present method can be used to monitor the responsiveness of the auditory cortex simultaneous to activation by other non-auditory tasks, facilitating evaluation of a cross-modal interaction.

Acknowledgements

This study was supported by a Grant-in Aid for Encouragement of Young Scientists # 14780608 (T.O.), and a Grant-in Aid for Scientific Research B#14380380 (N.S.) from Japan Society for the Promotion of Science, and Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, the Japanese Government.

References

[1] M. Abeles, M.H.J. Goldstein, Responses of single units in the primary auditory cortex of the cat to tones and to tone pairs, Brain Res. 42 (1972) 337–352.
[2] P.A. Bandettini, A. Jesmanowicz, J. Van Kylen, R.M. Birn, J.S. Hyde, Functional MRI of brain activation induced by scanner acoustic noise, Magn. Reson. Med. 39 (1998) 410–416.
[3] G.E. Chatrian, M.C. Petersen, J.A. Lazarte, Responses to clicks from the human brain: some depth electrographic observations, Electroenceph. clin. Neurophysiol. 12 (1960) 479–489.
[4] M.H.J. Goldstein, J.L. Hall, B.O. Butterfield, Single-unit activity in the primary auditory cortex of unanesthetized cats, J. Acoust. Soc. Am. 43 (1968) 444–455.
[5] R. Hari, M. Pelizzone, J.P. Makela, J. Hallstrom, L. Leinonen, O.V. Lounasmaa, Neuromagnetic responses of the human auditory cortex to On- and Offsets of noise bursts, Audiology 26 (1987) 31–43.
[6] M.P. Harms, J.R. Melcher, Sound repetition rate in the human auditory pathway: representations in the waveshape and amplitude of fMRI activation, J. Neurophysiol. 88 (2002) 1433–1450.
[7] J. He, T. Hashikawa, H. Ojima, Y. Kinouchi, Temporal integration and duration tuning in the dorsal zone of cat auditory cortex, J. Neurosci. 17 (1997) 2615–2625.
[8] S.A. Hillyard, T.W. Picton, ON and OFF components in the auditory evoked potential, Percept. Psychophys. 24 (1978) 391–398.
[9] S.L. Joutsiniemi, R. Hari, V. Vilkman, Cerebral magnetic responses to noise bursts and pauses of different durations, Audiology 28 (1989) 325–333.
[10] L. Liang, T. Lu, X. Wang, Neural representations of sinusoidal amplitude and frequency modulations in the primary auditory cortex of awake primates, J. Neurophysiol. 87 (2002) 2237–2261.
[11] K. Noda, M. Tonoike, K. Doi, I. Koizuka, M. Yamaguchi, R. Seo, N. Matsumoto, T. Noiri, N. Takeda, T. Kudo, Auditory evoked off-response: its source distribution is different from that of on-response, NeuroReport 9 (1998) 2621–2625.
[12] K.N. O'Connor, M.L. Sutter, Global spectral and location effects in auditory perceptual grouping, J. Cogn. Neurosci. 12 (2000) 342–354.
[13] C. Pantev, C. Eulitz, S. Hampson, B. Ross, L.E. Roberts, The auditory evoked "off" response: sources and comparison with the "on" and the "sustained" responses, Ear Hear. 17 (1996) 255–265.
[14] A. Pfefferbaum, M. Buchsbaum, J. Gips, Enhancement of the average evoked response to tone onset and cessation, Psychophysiology 8 (1971) 332–339.
[15] G.H. Recanzone, Response profiles of auditory cortical neurons to tones and noise in behaving macaque monkeys, Hear. Res. 150 (2000) 104–118.
[16] F. Schmitt, W. Irnich, H. Fischer, Physiological side effects of fast gradient switching, in: F. Schmitt, M.K. Stehling, R. Turner (Eds.), Echo-Planar Imaging, Springer, Berlin, 1998, pp. 201–252.
[17] N.J. Shah, L. Jancke, M.L. Grosse-Ruyken, H.W. Muller-Gartner, Influence of acoustic masking noise in fMRI of the auditory cortex during phonetic discrimination, J. Magn. Reson. Imaging 9 (1999) 19–25.
[18] M. Steinschneider, D.H. Reser, Y.I. Fishman, C.E. Schroeder, J.C. Arezzo, Click train encoding in primary auditory cortex of the awake monkey: evidence for two mechanisms subserving pitch perception, J. Acoust. Soc. Am. 104 (1998) 2935–2955.
[19] P.F. Van De Moortele, H.G. Le Clec, S. Dehaene, D. Le Bihan, Improving auditory comprehension in fMRI: insertion of silent intervals in multi-slice EPI, Neuroimage 7 (1998) S554.
[20] R.J. Zatorre, P. Belin, V.B. Penhune, Structure and function of auditory cortex: music and speech, Trends Cogn. Sci. 6 (2002) 37–46.

ELSEVIER

Neuroscience Letters 359 (2004) 124–126

Neuroscience Letters

www.elsevier.com/locate/neulet

Erythropoietin receptor is expressed on adult rat dopaminergic neurons and erythropoietin is neurotrophic in cultured dopaminergic neuroblasts

Marie Csete[a,*], Luis Rodriguez[b], Melissa Wilcox[a], Seetharam Chadalavada[c]

[a]*Department of Anesthesiology, Program in Neurosciences, Emory University School of Medicine, Research Labs, 1462 Clifton Road NE, Room 420, Atlanta, GA 30322, USA*

[b]*Neurosurgery, University of Michigan Medical Center, 2128B Taubman Center, Ann Arbor, MI 48109-0338, USA*

[c]*University of Michigan School of Literature, Science and the Arts, 4412 Kresge III, 200 Zina Pitcher Place, Ann Arbor, MI 48109-0585, USA*

Received 22 September 2003; received in revised form 5 January 2004; accepted 8 January 2004

Abstract

Exogenous erythropoietin (EPO) is a potent neurotrophic factor in vivo, protective against neuronal death in animal models of brain ischemia and human stroke. To date, reports on the distribution of EPO receptor in brain suggest that it is expressed mostly on capillaries. This receptor pattern suggests an indirect effect of EPO on neurons. In these studies, we show that EPO receptor is abundantly expressed on adult dopaminergic neurons, suggesting a direct effect of EPO on neurons. Furthermore, we show that EPO mediates the classic neurotrophic effects of proliferation, differentiation and maintenance in a dopaminergic cell line. The biology of therapeutically administered EPO in brain is a function of its receptor distribution, and the neuronal expression of EPO receptor on adult CNS neurons is consistent with EPO's potent neurotrophic function in vivo.

Keywords: Dopaminergic neurons; Dopamine transporter; Erythropoeitin; Parkinson's disease; Tyrosine hydroxylase

Erythropoietin (EPO), the growth factor for red cell precursors, has received considerable recent attention as neuroprotective therapy for ischemic and traumatic CNS injuries. In animal models of stroke, seizure, and autoimmune CNS injury [2], spinal cord trauma [10] and human stroke [8]. EPO given peripherally protects against neuronal loss. The distribution of EPO receptor in brain was reported to be mostly in capillaries, suggesting that peripherally administered EPO crosses the blood–brain barrier by facilitated transport [2]. In the mouse model of Parkinson's disease induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, EPO is also highly neuroprotective when injected directly into brain; peripheral administration of EPO is not discussed in this study [9].

EPO is emerging as a classical neurotrophic factor, regulating neuronal development and differentiation, and postnatal neuronal maintenance. The EPO protein contains a neurotrophic sequence that has no effect on erythropoeisis [3]. Targeted deletion of the EPO receptor confirms that EPO is essential for developmental neurogenesis [14]. EPO promotes dopaminergic differentiation from CNS stem cells in vitro and may be more potent than glial cell line derived neurotrophic factor in this regard [13]. In adult brain, EPO is produced mostly by astrocytes [4,7] though one study suggests that some neuronal populations may produce EPO [1]. The aim of this study was to characterize the neurotrophic effects (proliferation, protection, differentiation) of EPO in dopaminergic neurons.

We used two antibodies raised against distinct epitopes of the EPO receptor to characterize its localization in the adult rat brain substantia nigra. Male Sprague–Dawley rats (~300 g) were euthanized and brains removed and fixed in 30% sucrose-10% formalin for 4 days. Sections were cut to 30 μm and fixed with 10% formalin. Block for non-specific staining was done with 10% donkey serum for 30 min at room temperature, and primary antibodies (in PBS-TBA) incubated at 4 °C for 48 h, followed with washes with PBS-T. Secondary antibodies were Cy-3 (red) (Jackson Labs, ME) and Alexa Fluor 488 (green) conjugates (Molecular Probes, OR), incubated for 2 h at room temperature.

Dopaminergic neuron expression of the EPO receptor was further confirmed at the message and protein levels using the rat mesencephalic dopaminergic cell line, $1RB_3AN_{27}$ [6]. Polymerase chain reaction (PCR) was used to assay EPO and

* Corresponding author. Tel.: +1-404-712-2588; fax: +1-404-712-2585.
E-mail address: marie_csete@emoryhealthcare.org (M. Csete).

0304-3940/03/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2004.01.068