Furthermore, our findings apparently showed that NTS induced compensatory as well as enhancing effects on the activities and emotional states of both sexes.

Pauk et al. [25] previously demonstrated that tactile stimulation with a camel hair brush during maternal deprivation for 2 h prevented the increase of serum corticosterone in response to maternal deprivation in infants rats, which is similar to the effect of NTS observed in the present study. Although further studies are needed to verify the efficacy of tactile stimulation, it is postulated that neonatal tactile stimulation may prevent the developmental disturbance of emotionality in response to early adversity. On the other hand, it is well known that maternal behavior plays an important role in physical and psychological development through mother–infant contact. For example, early adversity such as NI [3] or low maternal care [6] was reported to enhance activity of the HPA axis in response to stress in later life. In this context, it cannot be ruled out that the ability of NTS to reverse the effect of NI in these behavioral tests may be due to differences in maternal care for 23 h/day between the NI and NTS groups, since we did not measure maternal behaviors such as licking and grooming or arched-back nursing in this study.

In the contextual fear-conditioning test, whereas NTS led to a significant reduction in contextual freezing among both sexes as compared with sham-treatment, NTS reversed the NI-induced increase in contextual freezing in female but not male rats. The differential effect of NTS on contextual freezing in male and female rats may be due to differences in the effect of NI, since NI significantly enhanced contextual freezing in female but not male rats. The results of the present study are almost consistent with a previous study which showed that 1 h-neonatal isolation on PN days 2–9 enhances contextual fear in female Sprague–Dawley rats and impairs it in male Sprague–Dawley rats [11]. It has been suggested that contextual freezing behavior is fear and anxiety-related response, at least in part, mediated by the hippocampus [5,26]. We speculated, based on the results of these studies, that the intensity of hippocampal-dependent fear and anxiety responses in adulthood due to early adversity may differ between male and female rats.

There was no significant interaction between neonatal treatments and gender with respect to locomotor activities or rearing movements in the open-field test, anxiety-like behavior without head dippings in the elevated plus maze test, or pain sensitivity in the hot-plate test. In contrast, Rhees et al. [28] reported that females were more active than males in both the sham-treated and maternally separated groups. This difference may be due to differences in the experimental paradigms such as the breeding environment, age of animals, period of handling, etc.

It is noteworthy that the estrous cycle is an important factor for mediating stress responses in females. Unfortunately, in the present experiments, the behaviors of the female rats were not tested at any specified or consistent time in their estrous cycle. Rat behavior in an open-field test was reported to be independent of hormonal variations during the estrous cycle [33], and no significant difference in anxiety-like behavior was seen in the elevated plus maze test during proestrus and diestrus [23]. In contrast, female rats were reported to show higher % open arm times and % open arm entries during proestrus and estrus than during diestrus [22]. Furthermore, Severino reported that there was no significant interaction between neonatal handling and estrous cycle phases on the percentage of open arm time and percentage of open arm entries, but not total entries [31]. They reported that in diestrus the total number of arm entries in handled females was higher than in the nonhandled group in the same phase and also higher than in the handled females in estrus. Based on these findings, further studies examining the influence of the estrous cycle on anxiety-like behavior in the open arm test are required.

There are several limitations in this study that should be taken into consideration. First, the behavioral experiments in adulthood were undertaken during the light period but not the dark period of the dark/light cycle. As in our study, numerous studies examining the influence of neonatal manipulations on the adulthood behavioral responses to environmental stimuli, were performed during the light phase [2,13,24,28,30–32]. However, since the differences in the locomotor activity or anxiety-like behavior [7,8] between the light and dark phase were reported, assessment during both the light and dark phase is required to promote our understanding of behavioral responses. Secondly, the behaviors of the female rats were not tested at any specified or consistent time in their estrous cycle. Thirdly, although Kosten et al. [13] showed that 1 h of neonatal isolation during PN days 2–9 did not affect on estrous stage cyclicity, it cannot be ruled out that the neonatal handling may affect estrous stage cyclicity and may subsequently change the neuromodulatory role of gonadal steroids on the adulthood behavior.

In summary, the present study clearly indicated that whereas NI altered behavioral and emotional responses to environmental stimuli in later life, NTS could reverse the effect of NI and promote behavioral and emotional responses, to the same extent, in both sexes. In other words, an adequate tactile stimulation in early life plays an important role in the prevention of susceptibility to environmental stimuli induced by an early adverse experience. In addition, long-lasting behavioral and emotional changes, induced by an early adverse experience, differ somewhat between male and female rats in adulthood.

Acknowledgments

This study was supported by a grant-in-aid for general scientific research from the Ministry of Education, Science, and Culture of Japan, a Health Science Research Grant for Research on Brain Science from the Ministry of Health and Welfare of Japan. The authors thank Drs. T. Takahashi, Y. Iwamoto, T. Watanabe, for helpful advice.

References

[1] Beane ML, Cole MA, Spencer RL, Rudy JW. Neonatal handling enhances contextual fear conditioning and alters corticosterone stress responses in young rats. Horm Behav 2002;41:33–40.

[2] Cannizzaro C, Martire M, Cannizzaro E, Provenzano G, Gagliano M, Carollo A, et al. Long-lasting handling affects behavioural reactivity in adult rats of both sexes prenatally exposed to diazepam. Brain Res 2001;22:225–33.

[3] Erabi K, Morinobu S, Kawano KI, Tsuji S, Yamawaki S. Neonatal isolation changes the expression of IGF-IR and IGFBP-2 in the hippocampus in response to adulthood restraint stress. Int J Neuropsychopharmacol 2006:1–13.
[4] Erskine MS, Stern JM, Levine S. Effects of prepubertal handling on shock-induced fighting and ACTH in male and female rats. Physiol Behav 1975;14:413–20.
[5] Fanselow MS, Helmstetter FJ. Conditional analgesia, defensive freezing, and benzodiazepines. Behav Neurosci 1988;102:233–43.
[6] Fish EW, Shahrokh D, Bagot R, Caldji C, Bredy T, Szyf M, et al. Epigenetic programming of stress responses through variations in maternal care. Ann N Y Acad Sci 2004;1036:167–80.
[7] Jones N, King SM. Influence of circadian phase and test illumination on pre-clinical models of anxiety. Physiol Behav 2001;72:99–106.
[8] Kalinichev M, Easterling KW, Plotsky PM, Holtzman SG. Long-lasting changes in stress-induced corticosterone response and anxiety-like behaviors as a consequence of neonatal maternal separation in Long-Evans rats. Pharmacol Biochem Behav 2002;73:131–40.
[9] Kehoe P, Bronzino JD. Neonatal stress alters LTP in freely moving male and female adult rats. Hippocampus 1999;9:651–8.
[10] Knuth ED, Etgen AM. Long-term behavioral consequences of brief, repeated neonatal isolation. Brain Res 2007;1128:139–47.
[11] Kosten TA, Miserendino MJ, Bombace JC, Lee HJ, Kim JJ. Sex-selective effects of neonatal isolation on fear conditioning and foot shock sensitivity. Behav Brain Res 2005;157:235–44.
[12] Kosten TA, Miserendino MJ, Kehoe P. Enhanced acquisition of cocaine self-administration in adult rats with neonatal isolation stress experience. Brain Res 2000;875:44–50.
[13] Kosten TA, Sanchez H, Jatlow PI, Kehoe P. Neonatal isolation alters the estrous cycle interactions on the acute behavioral effects of cocaine. Psychoneuroendocrinology 2005;30:753–61.
[14] Kosten TA, Sanchez H, Zhang XY, Kehoe P. Neonatal isolation enhances acquisition of cocaine self-administration and food responding in female rats. Behav Brain Res 2004;151:137–49.
[15] Levine S. Infantile experience and resistance to physiological stress. Science 1957;126:405.
[16] Levine S, Otis LS. The effects of handling before and after weaning on the resistance of albino rats to later deprivation. Can J Psychol 1958;12: 103–8.
[17] Liu D, Caldji C, Sharma S, Plotsky PM, Meaney MJ. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinepherine release in the hypothalamic paraventricular nucleus. J Neuroendocrinol 2000;12:5–12.
[18] Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 2000;3:799–806.
[19] McIntosh J, Anisman H, Merali Z. Short- and long-periods of neonatal maternal separation differentially affect anxiety and feeding in adult rats: gender-dependent effects. Brain Res Dev Brain Res 1999;113:97–106.
[20] Meaney MJ. Maternal care, gene expression, and the transmission of individual differences in stress reactivity across generations. Annu Rev Neurosci 2001;24:1161–92.
[21] Meaney MJ, Aitken DH, Viau V, Sharma S, Sarrieau A. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. Neuroendocrinology 1989;50:597–604.
[22] Mora S, Dussaubat N, Diaz-Veliz G. Effects of the estrous cycle and ovarian hormones on behavioral indices of anxiety in female rats. Psychoneuroendocrinology 1996;21:609–20.
[23] Nomikos GG, Spyraki C. Influence of oestrogen on spontaneous and diazepam-induced exploration of rats in an elevated plus maze. Neuropharmacology 1988;27:691–6.
[24] Papaioannou A, Gerozissis K, Prokopiou A, Bolaris S, Stylianopoulou F. Sex differences in the effects of neonatal handling on the animal's response to stress and the vulnerability for depressive behaviour. Behav Brain Res 2002;129:131–9.
[25] Pauk J, Kuhn CM, Field TM, Schanberg SM. Positive effects of tactile versus kinesthetic or vestibular stimulation on neuroendocrine and ODC activity in maternally-deprived rat pups. Life Sci 1986;39:2081–7.
[26] Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274–85.
[27] Ploj K, Pham TM, Bergstrom L, Mohammed AH, Henriksson BG, Nylander I. Neonatal handling in rats induces long-term effects on dynorphin peptides. Neuropeptides 1999;33:468–74.
[28] Rhees RW, Lephart ED, Eliason D. Effects of maternal separation during early postnatal development on male sexual behavior and female reproductive function. Behav Brain Res 2001;123:1–10.
[29] Rodrigues AL, Arteni NS, Abel C, Zylbersztejn D, Chazan R, Viola G, et al. Tactile stimulation and maternal separation prevent hippocampal damage in rats submitted to neonatal hypoxia–ischemia. Brain Res 2004;1002:94–9.
[30] Romeo RD, Mueller A, Sisti HM, Ogawa S, McEwen BS, Brake WG. Anxiety and fear behaviors in adult male and female C57BL/6 mice are modulated by maternal separation. Horm Behav 2003;43:561–7.
[31] Severino GS, Fossati IA, Padoin MJ, Gomes CM, Trevizan L, Sanvitto GL, et al. Effects of neonatal handling on the behavior and prolactin stress response in male and female rats at various ages and estrous cycle phases of females. Physiol Behav 2004;81:489–98.
[32] Silveira PP, Portella AK, Clemente Z, Gamaro GD, Dalmaz C. The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. Int J Dev Neurosci 2005;23:93–9.
[33] Steiner M, Katz RJ, Carroll BJ. Behavioral effects of dopamine agonists across the estrous cycle in rats. Psychopharmacology (Berl) 1980;71:147–51.
[34] Stephan M, Helfritz F, Pabst R, von Horsten S. Postnatally induced differences in adult pain sensitivity depend on genetics, gender and specific experiences: reversal of maternal deprivation effects by additional postnatal tactile stimulation or chronic imipramine treatment. Behav Brain Res 2002;133:149–58.
[35] Stephan M, Straub RH, Breivik T, Pabst R, von Horsten S. Postnatal maternal deprivation aggravates experimental autoimmune encephalomyelitis in adult Lewis rats: reversal by chronic imipramine treatment. Int J Dev Neurosci 2002;20:125–32.
[36] Weinberg J, Levine S. Early handling influences on behavioral and physiological responses during active avoidance. Dev Psychobiol 1977;10:161–9.
[37] Wigger A, Neumann ID. Periodic maternal deprivation induces gender-dependent alterations in behavioral and neuroendocrine responses to emotional stress in adult rats. Physiol Behav 1999;66:293–302.

SYNAPSE 62:259–267 (2008)

Prior Neonatal Isolation Reduces Induction of NGF mRNA and Decreases GDNF mRNA in the Hippocampus of Juvenile and Adult Rodents Subjected to Immobilization Stress

KI-ICHIRO KAWANO,[1] SHIGERU MORINOBU,[1]* TAKUYA SAWADA,[1] SEIICHI TSUJI,[1] KAORI ERABI,[1] MANABU FUCHIKAMI,[1] TOSHIRO KOZURU,[1] SHIGETO YAMAWAKI,[1] KAZUE HISAOKA,[2] AND MINORU TAKEBAYASHI[2]

[1]*Division of Frontier Medicine, Department of Psychiatry and Neurosciences, Graduate School of Medical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan*

[2]*Division of Clinical Research, Department of Psychiatry, National Hospital Organization, Kure Medical Center, 3-1 Aoyama, Kure, 737-0023, Japan*

KEY WORDS neonatal isolation; immobilization; NGF; GDNF; NT-3; hippocampus

ABSTRACT Numerous studies have demonstrated that early adverse experiences are associated with the development of susceptibility to stress later in life. Although it is known that early experience of adversity, such as neonatal isolation, maternal separation, and low maternal care, enhances the activity of the hypothalamo-pituitary-adrenal axis in rodents, the detailed mechanism underlying stress susceptibility induced by early adversity remains to be elucidated. Since neurotrophins have been shown to have a neuroprotective effect, we examined the influence of repeated neonatal isolation on expression of nerve growth factor (NGF), glia cell-derived neurotrophic factor (GDNF), and neurotrophin-3 mRNA in the hippocampus of juvenile and adult rats subsequently exposed immobilization stress, using real-time quantitative PCR and in situ hybridization. Neonatal isolation did not affect the basal hippocampal expression of these neurotrophin mRNAs in either juvenile or adult rats not subsequently exposed to immobilization. Similarly, there was a significant interaction between neonatal isolation and immobilization that affected the expression of NGF and GDNF mRNAs. Neonatal isolation attenuated the induction of NGF mRNA in both groups of rats and decreased GDNF mRNA in juvenile rats in response to immobilization. The decreased induction of NGF mRNA and reduced GDNF mRNA in response to immobilization was found in the CA3 pyramidal cell layer and dentate gyrus granular cell layer in the hippocampus of adult rats that had been subjected to neonatal isolation. These findings suggest that susceptibility to stress arising from prior neonatal isolation might be a result of decreased neuroprotective support through NGF and GDNF. **Synapse 62:259–267, 2008.** ©2008 Wiley-Liss, Inc.

INTRODUCTION

Numerous preclinical studies have revealed that exposure to either early adversity and/or long-term stress in adulthood can lead to alterations in hippocampal structure and function. For example, chronic adulthood stresses, such as forced immobilization or restraint, have been reported to induce a significant decrease in the dendritic length and the number of branch points in hippocampal CA3 pyramidal neurons (Vyas et al., 2002), and to atrophy of the apical dendrites of CA3 pyramidal cells in the rat hippocampus (Magarinos et al., 1995, 1996; Watanabe et al., 1992). Similarly, chronic parental separation induces a significant decrease in the length of the dendrites of hippocampal CA1 pyramidal neurons, and a significant reduction of granular cell spine density in the hippocampal dentate gyrus (DG) in the juvenile degus

*Correspondence to: Shigeru Morinobu, MD, PhD, Division of Frontier Medicine, Department of Psychiatry and Neurosciences, Graduate School of Medical Sciences, Hiroshima University, 1-2-3 Kasumi minami-ku, Hiroshima 724-8551, Japan. E-mail: smoriob@hiroshima-u.ac.jp

Received 9 February 2007; Accepted 30 September 2007

DOI 10.1002/syn.20487

Published online in Wiley InterScience (www.interscience.wiley.com).

(Poeggel et al., 2003). In addition, there is a significant reduction of mossy fiber density in the hippocampus of adult rats subjected to repeated episodes of maternal separation (Huot et al., 2002). Moreover, juvenile rats subjected to repeated episodes of maternal separation experienced a significant decrease in the volume and total number of BrdU-positive cells within the DG as well as a significant increase in the number of TUNEL-positive cells within this region of the brain (Lee et al., 2001). Mild prenatal stress has been shown to decrease synaptic density in the hippocampus of affected offspring (Hayashi et al., 1998). Taken together, these morphological findings suggest that various types of stress affect synaptic structure. However, the precise mechanism by which exposure to stress induces morphological changes in the hippocampus remains to be elucidated.

Neurotrophic factors and growth factors play a critical role in the differentiation and maintenance of neurons (Folli et al., 1996; Maisonpierre et al., 1990), suggesting that these factors may be involved in stress-induced synaptic remodeling. A significant reduction of brain-derived neurotrophic factor (BDNF) mRNA was found in the hippocampus of adult rats subjected to single and repeated episodes of maternal separation (MacQueen et al., 2003; Roceri et al., 2002). In contrast, the level of BDNF protein in adult rats subjected to repeated episodes of maternal separation was higher than that in adult rats subjected to neonatal brief handling despite a similar level of BDNF mRNA in these 2 groups (Greisen et al., 2005). Furthermore, the level of BDNF mRNA in the hippocampus of offspring of mothers that exhibited a high degree of maternal care was significantly higher than that in offspring of mothers that exhibited a low degree of maternal care (Liu et al., 2000). In contrast, a single episode of maternal separation was reported to increase the level of nerve-growth factor (NGF) mRNA in the developing rat hippocampus (Cirulli et al., 1998, 2000). Recently, we demonstrated that repeated episodes of neonatal isolation led to a significant decrease in the level of insulin-like growth factor (IGF) 1 receptor mRNA and protein in the hippocampus of the corresponding adult rats (Erabi et al., 2007). Although the influences of early adverse experiences on the expression of neurotrophic and growth factors in the hippocampus has been recognized, further studies focusing on other factors such as NGF, glia cell-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT-3), are required to elucidate the involvement of these factors in the morphological changes in response to early adversity.

In this report, we examined the effect of repeated episodes of neonatal isolation on the levels of NGF, GDNF, and NT-3 mRNA in the hippocampus of juvenile (28-day-old) and adult (90-day-old) rats. In addition, epidemiological studies of mental disorders have suggested that early adverse experiences increase the susceptibility to mental disorders in response to stress. Therefore, we also examined the levels of these neurotrophic factors in response to a single episode of immobilization stress (SIS) in juvenile and adult animals that had been previously exposed to repeated episodes of neonatal isolation.

MATERIALS AND METHODS

Animals

Pregnant female Sprague-Dawley rats were purchased from Charles River Japan (Yokohama, Japan). The rats were housed individually in a breeding colony at constant room temperature (23°C ± 2°C) and humidity (60%) with a 12 h/12 h light/dark cycle (lights on 8 AM to 8 PM). Food and water were provided ad libitum. The litters were culled to 12 pups on postnatal day 1 (PN day 1).

Neonatal isolation paradigm

The mothers and pups of the nonisolated group were left undisturbed until weaning. Neonatal isolation was conducted according to the method of Kehoe and Bronzino (1999). Pups were isolated from the dam, nest, and siblings and placed in individual round containers for 1 h per day on PN days 2–9. All litters were weaned on PN day 21, separated on the basis of sex, and maintained with ad libitum access to food and water. Only the male rats were subjected to the following experimental procedure. All animal procedures were conducted in accordance with the Guiding Principles on Animal Experimentations in Research Facilities for Laboratory Animal Science Hiroshima University and approved by Hiroshima University Animal Care Committee.

Single immobilization stress paradigm

On PN day 28 (juvenile) or 90 (adult), half of both the isolated and nonisolated rats were subjected to a single restraint stress (SRS) for 2 h. The immobilization stress was conducted as described previously (Morinobu et al., 2003; Suenaga et al., 2004). Briefly, rats were immobilized in clear polyethylene disposable cone bags (Asahikasei, Tokyo, Japan), sized so that rats were equally immobilized. Animals were sacrificed by decapitation after completion of the immobilization stress. On PN day 28 or 90, isolated and nonisolated rats (sham) were sacrificed by decapitation. The hippocampus was isolated, immediately frozen, stored at −70°C, and used for real-time quantitative PCR analysis. In this procedure, the lateral choroid plexus was carefully removed from the hippocampus. For in situ hybridization, brains were

TABLE I. Primer and probe sequences used for real time PCR

NGF	Forward primer	AGCCCACTGGACTAAACTTCAGC
	Reverse primer	GGGCACTGCGGGCTC
	TaqMan prove	TTCCCTTGACACAGCCCTCCGC
GDNF	Forward primer	ACTCCCTCGGCCACCG
	Reverse primer	ATAATCTTCGGGCATATTGGAGTC
	TaqMan prove	CGTGCCCTTCGCGCTGACC
NT-3	Forward primer	GAGGCACCCAGAGAACCAGA
	Reverse primer	TTGCAATCATCGGCTGGA
	TaqMan prove	CAGGGAGAGGCCACCAGGTCAGAA

Forward primer: 5′ to 3′; Reverse primer: 5′ to 3′; TaqMan probe: 5′ [FAM] to 3′ [TAMRA].

removed rapidly, immediately frozen, and stored at −70°C.

Four groups of both juvenile and adult rats [subjected to neonatal isolation followed by SRS (NI + SRS); neonatal isolation alone (NI); SRS alone (SRS); or sham treatment (Sham)] were used for real-time quantitative PCR. No more than two rats per experimental group were from any single litter. A total of 24 juvenile and 51 adult rats were subjected to real-time quantitative PCR analysis and a different set of rats was used for each experiment (real-time quantitative PCR and hybridization).

Real-time quantitative PCR

Total RNA was extracted with an RNAqueous Phenol-free Total RNA Isolation Kit (Ambion, Austin, TX). After treatment with RNase-free DNase I (Takara, Kusatsu, Japan), real-time quantitative PCR was performed with an ABI PRISM 7700 sequence detection system (Applied Biosystems, Foster City, CA) to quantify relative mRNA levels in samples. Real-time quantitative PCR was performed to amplify the genes encoding NGF, GDNF, and NT-3. The primers and TaqMan hybridization probe were designed using Primer Express software (Applied Biosystems). Table I shows the sequences and associated fluorescent dye of each PCR primers and TaqMan probe. The TaqMan probes, which were designed to hybridize to the PCR products, were labeled with a fluorescent reporter dye at the 5′ end and a quenching dye at the 3′ end. PCR was carried out using the TaqMan Universal PCR Master Mix (Applied Biosystems). All standards and samples were assayed in triplicate. Each plate contained the same standard. Thermal cycling was initiated with an initial denaturation at 50°C for 2 min and 95°C for 10 min. After this initial step, 40 cycles of PCR (heating at 95°C for 15 s and 60°C for 1 min) were performed. The PCR assay for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed using TaqMan Rodent GAPDH Control Reagents (Applied Biosystems). The PCR assays on the test samples were performed simultaneously with the assays on the standard samples (rat brain tissue), which allowed us to construct a standard curve. The estimated relative expression of GAPDH and NGF, GDNF, or NT-3 mRNAs in the test samples were calculated using this standard curve and the ratio of the relative expression of NGF, GDNF, or NT-3 mRNAs was calculated relative to the expression of GAPDH.

Hybridization paradigm

Analysis of NGF and GDNF expression by in situ hybridization was conducted according to the method of Wetmore et al. (1992) with a minor modification. Oligonucleotide probes complementary to parts of the mRNAs encoding NGF and GDNF were synthesized and labeled with DIG at the 5′ end. The following antisense DNA oligonucleotides were used: NGF: 5′-CTG CGG GCT CTG CGG AGG GCT GTG TCA AGG GAA TGC TGA AGT TTA GTC CA-3′ (Nosrat et al., 1997) and GDNF: 5′-AGC CAC CAT CAA AAG ACT GAA AAG GTC ACC AGA TAA ACA AGC GGC GGC A-3′ (Lin et al., 1993).

Fresh-frozen coronal brain sections (15 μm) through the hippocampus were prepared using a cryostat, thaw-mounted onto slides (Matsunami, Kishiwada, Japan) and fixed with 4% paraformaldehyde for 5 min. Sections were hybridized for 18 h at 42°C in a humidified chamber with at least 0.5 ng probe/slide in a mixture of 4 × SSC (1 × SSC = 0.15 M NaCl/0.015 M sodium citrate), 50% formamide, 1 × Denhardt's solution (0.02% each of polyvinylpyrrolidone, bovine serum albumin, and Ficoll), 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulfate, 1 mg/ml of yeast RNA, and 200 mM dithiothreitol. Following hybridization, slides were rinsed for 5 × 15 min at 55°C in 2 × SSC, and allowed to reach room temperature. The sections were then incubated at 4°C overnight with an antidigoxigenin-alkaline phosphatase conjugate (Roche, Basel, Switzerland). After the incubation, the sections were extensively washed in PBT containing 0.2% BSA and 4 mM Levamisol, then washed in the reaction buffer (100 mM NaCl, 100 mM TrisHCl at pH 9.5, 50 mM $MgCl_2$, 0.1% Tween 20, and 4 mM Levamisol), and incubated with chromagen containing nitroblue tetrazolium salt (337 mg/ml) and 5-bromo-4-chloro-3-indoyl phosphate (175 mg/ml) according to the manufacturer's protocol (Roche). After color development (DIG labeling is visualized as a blue reaction product under light microscopy), slides were photographed under light microscopy (Model BZ-8000) (Keyence, Osaka, Japan). The mean density of sections was measured in the CA1 and CA3 regions (400 μm × 400 μm), and granule cell layer of the DG (400 μm × 200 μm) using the NIH Scion Image analysis program (Figs. 3a and 4a).

Statistical analysis

Results were expressed as mean ± SEM. The results of real-time quantitative PCR were analyzed

by two-way analysis of variance (ANOVA) (neonatal isolation × single immobilization stress), and posthoc comparisons were performed using Scheffe's test. For hybridization analyses, the results of experiments containing two groups of rats were analyzed by the Mann-Whitney U test. Significance was set at $P < 0.05$.

RESULTS

We first performed real-time quantitative PCR analysis to measure NGF, GDNF, and NT-3 mRNA in RNA isolated from the hippocampus of juvenile and adult rats subjected to either neonatal isolation followed by SIS (NI + SIS), neonatal isolation alone (NI), SIS alone (SIS) or sham treatment. In both juvenile and adult rats, two-way ANOVA showed no significant effect of NI [juvenile: $F(1, 23) = 2.652$, $P = 0.119$; adult: $F(1, 23) = 4.257$, $P = 0.051$] on the level of NGF mRNA, but revealed a significant effect of SIS [juvenile: $F(1, 23) = 7.093$, $P = 0.015$; adult: $F(1, 23) = 4.926$, $P = 0.037$], and a significant interaction between NI and SIS [juvenile: $F(1, 23) = 12.437$, $P = 0.002$; adult: $F(1, 23) = 6.605$, $P = 0.017$] (Figs. 1a and 2a). Posthoc analysis revealed that the levels of NGF mRNA in the SIS group was significantly higher ($P < 0.05$) than those in the Sham or NI + SIS group for both juveniles and adults (Figs. 1a and 2a). However, there was no significant difference between the level of NGF mRNA in the NI + SIS and Sham groups. In situ hybridization analysis demonstrated that the level of NGF mRNA in the CA3 pyramidal cell layer ($P = 0.022$) and the DG granular cell layer ($P = 0.002$) of the NI + SIS group was significantly lower than that in the SIS group in adulthood (Figs. 3a and 3b).

In the analyses of GDNF mRNA in juvenile rats, we observed no significant effect of SIS on the level of GDNF mRNA [$F(1, 23) = 0.877$, $P = 0.360$], but we did observe a significant effect of NI [$F(1, 23) = 8.865$, $P = 0.007$]. We found the trend toward significant interaction between NI and SIS [$F(1, 23) = 4.328$, $P = 0.051$] (Fig. 1b). Posthoc analysis revealed that the level of GDNF mRNA ($P < 0.05$) in the NI + SIS group was significantly lower than that in the SIS group (Fig. 1b). In adulthood, we observed no significant effect of NI on the level of GDNF mRNA [$F(1, 23) = 0.017$, $P = 0.935$], but we did observe a significant effect of SIS [$F(1, 23) = 8.172$, $P = 0.009$], as well as a significant interaction between NI and SIS [$F(1, 23) = 12.143$, $P = 0.002$] (Fig. 2b). Posthoc analysis revealed that the level of GDNF mRNA ($P < 0.05$) in the NI + SIS group was significantly lower than that in the NI group (Fig. 2b). The levels of GDNF mRNA in the Sham group also tended to be lower than that in the NI group, although the difference did not reach statistical significance ($P = 0.096$).

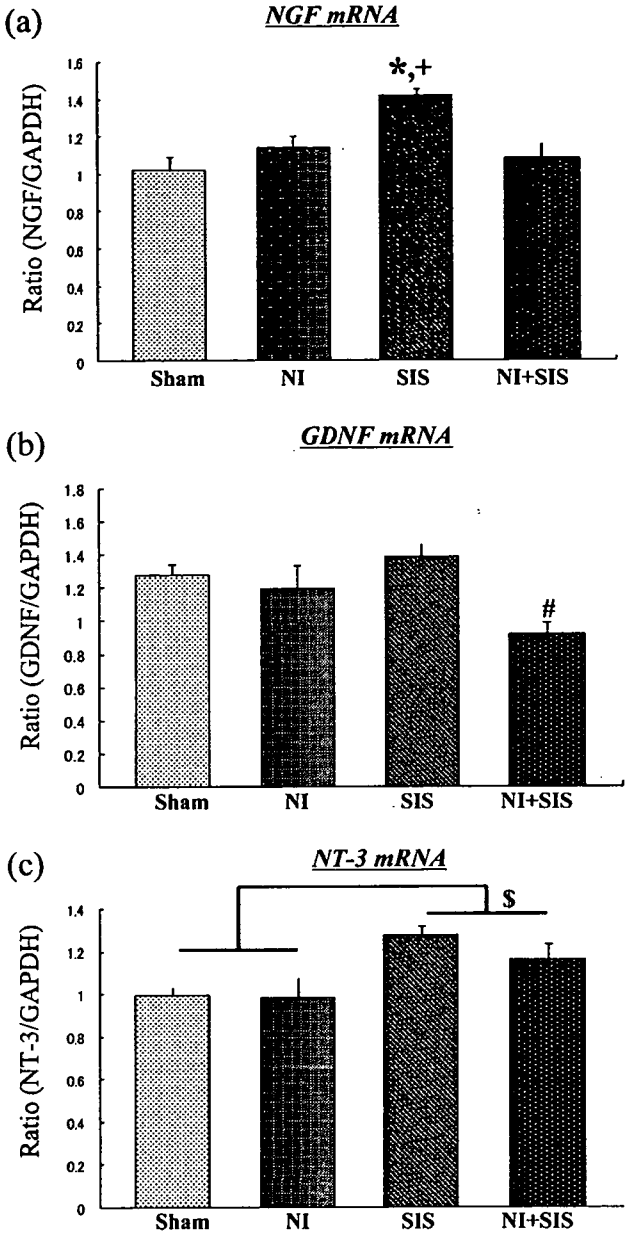

Fig. 1. Expression of (a) NGF, (b) GDNF, and (c) NT-3 mRNA in the hippocampus of juvenile rats subjected to sham treatment (Sham), neonatal isolation alone (NI), single immobilization stress alone (SIS), and neonatal isolation followed by a single immobilization stress (NI + SIS). Results are expressed as the ratio of the concentration of the target neurotrophin to that of GAPDH (target neurotrophin/GAPDH) percentage of Sham levels. The mean ± SEM ($n = 6$) is shown. $^{*}P < 0.05$ compared with sham, $^{\#}P < 0.05$ compared with SIS, $^{+}P < 0.05$ compared with NI + SIS, $^{\$}P < 0.05$ compared with Sham and NI (two-way ANOVA followed by Scheffe's test).

In situ hybridization analysis demonstrated that the level of GDNF mRNA in the CA1 ($P = 0.004$) and CA3 ($P = 0.025$) pyramidal cell layers and the DG ($P = 0.010$) granular cell layer of the NI + SIS group was significantly lower than that in the NI group (Fig. 4).

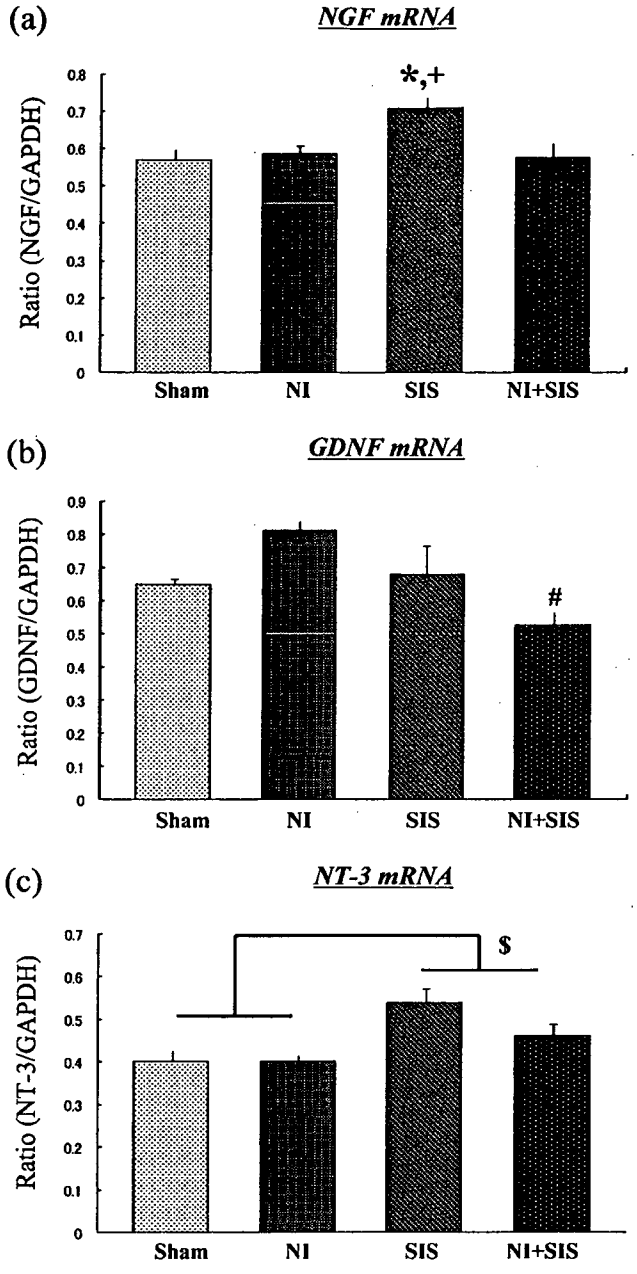

Fig. 2. Expression of (a) NGF, (b) GDNF, and (c) NT-3 mRNA in the hippocampus of adult rats subjected to sham treatment (Sham), neonatal isolation alone (NI), single immobilization stress alone (SIS), and neonatal isolation followed by a single immobilization stress (NI + SIS). Results are expressed as the ratio of the concentration of the target neurotrophin to that of GAPDH (target neurotrophin/GAPDH) percentage of Sham levels. The mean ± SEM (n = 6–8) is shown. $^{*}P < 0.05$ compared with sham, $^{\#}P < 0.05$ compared with NI, $^{+}P < 0.05$ compared with NI + SIS, $^{\$}P < 0.05$ compared with Sham and NI (two-way ANOVA followed by Scheffe's test).

In both juvenile and adult rats, two-way ANOVA showed no significant effect of NI on the level of NT-3 mRNA [juvenile: $F(1, 23) = 1.084$, $P = 0.309$; adult: $F(1, 23) = 2.539$, $P = 0.125$], but did show a significant effect of SIS [juvenile: $F(1, 23) = 13.285$, $P = 0.001$; adult: $F(1, 23) = 15.136$, $P = 0.001$], but no significant interaction between NI and SIS [juvenile: $F(1, 23) = 0.611$, $P = 0.443$; adult: $F(1, 23) = 2.319$, $P = 0.141$] (Figs. 1c and 2c). Posthoc analysis revealed that the level of NT3 mRNA in rats subjected to SIS was significantly higher ($P < 0.05$) than that in juvenile and adult rats not subjected to SIS.

DISCUSSION

The results of the present study demonstrate that repeated episodes of neonatal isolation do not affect the basal levels of NGF, GDNF, or NT-3 mRNA in the hippocampus of juvenile or adult rats not subsequently subjected to immobilization stress. However, we did observe a significant effect of NI on the basal level of GDNF mRNA in juvenile rats. The neonatal isolation paradigm used in this study has been reported to alter plasma corticosterone responses to restraint stress in juveniles and adults (Erabi et al., 2007; McCormick et al., 2002). This paradigm has also been reported to alter the levels of insulin-like growth factor-I (IGF-1) receptor and IGF binding protein-2 in the hippocampus in response to adulthood restraint stress (Erabi et al., 2007). Thus, it is likely that this neonatal isolation paradigm can affect the expression of neurotrophins in response to immobilization stress later in life.

Two studies conducted by Crelli et al. (1998, 2000) examined the influence of early adverse experiences on the expression of NGF in the rat hippocampus. The former study showed that a single brief episode of maternal separation (45 min) on PN day 3 significantly increased the level of NGF mRNA in the hippocampus of 3-day-old rats (Cirulli et al., 1998). Similarly, the latter study indicated that a single 1-h episode of maternal separation on PN day 9 or a single 3-h episode of maternal separation on PN day 16 significantly increased NGF mRNA in the DG (Cirulli et al., 2000). However, we found no significant influence of neonatal isolation on expression of NFG mRNA in the hippocampus of 28- or 90-day-old rats, and it may be that the effects of early adverse experience may no longer manifest at PN day 28. Further studies are necessary to determine whether there is a specific period of time during which neonatal isolation can affect the levels of NGF mRNA in the hippocampus. In contrast to studies using NGF (Cirulli et al., 1998, 2000), there has been no previous study examining the influence of early adversity on the basal levels of GDNF and NT-3 mRNA in the adult rat hippocampus. We have shown here that the level of neither GDNF nor NT-3 mRNA is altered in the hippocampus of 28- or 90-day-old rats that had been subjected to repeated episodes of neonatal isolation. In contrast to what has been reported for BDNF (Roceri et al., 2002, 2004), we did not find any change in the basal level of

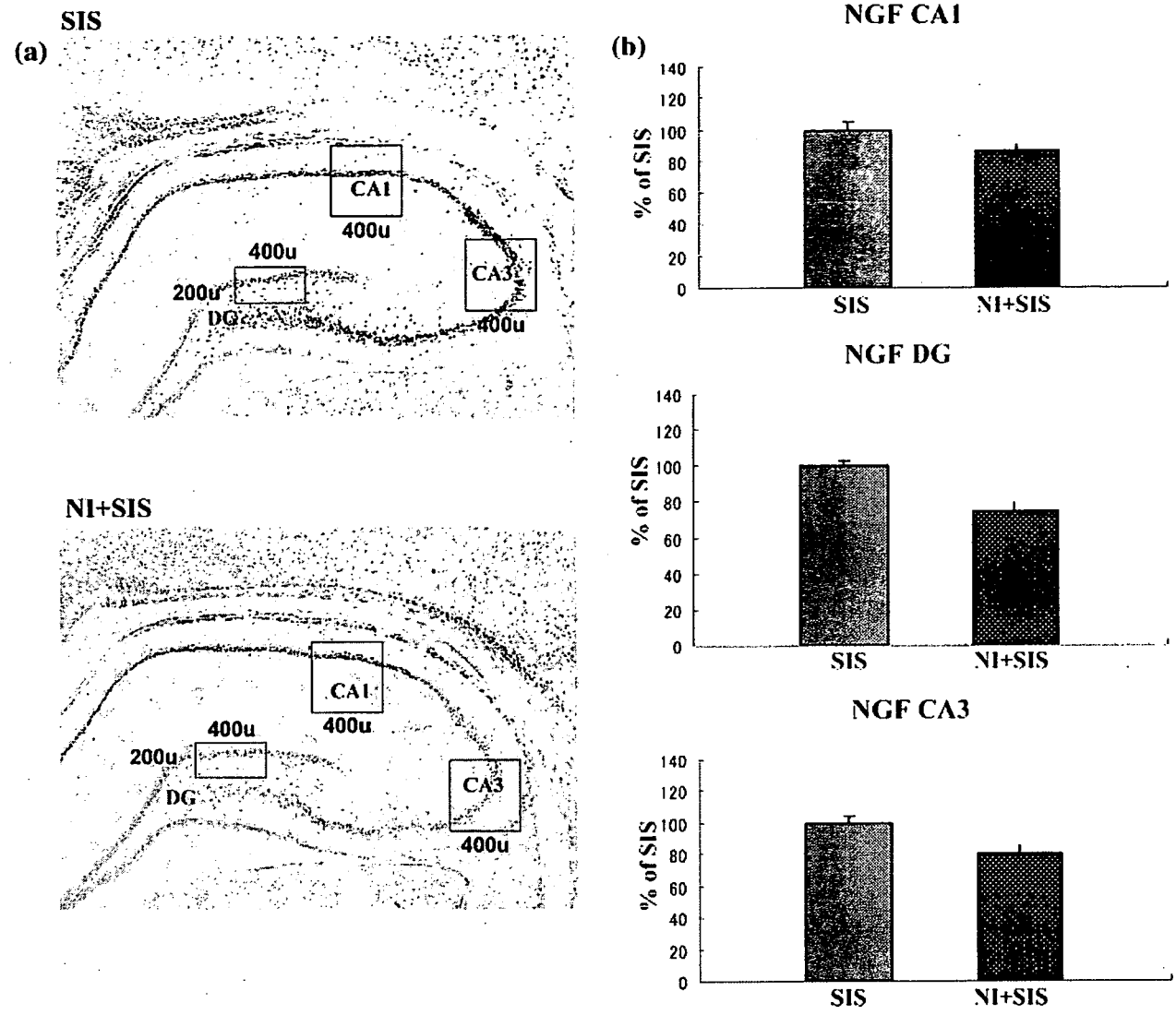

Fig. 3. Analysis of NGF mRNA expression by *in situ* hybridization in the adult rat hippocampus. (a) NGF mRNA expression in the rat hippocampus. SIS, single immobilization stress, NI + SIS, neonatal isolation followed by a single immobilization stress. (b) Mean density of NGF mRNA in the CA1 and CA3 pyramidal cell layers, and the dentate gyrus granular cell layer in the hippocampus of adult rats subjected to single immobilization stress (SIS) and neonatal isolation followed by a single immobilization stress (NI + SIS). Results are expressed as the percentage of SIS levels. The mean ± SEM (SIS: $n = 7$; NI + SIS: $n = 6$) is shown. $^*P < 0.05$ compared with SIS (Mann-Whitney U test). CA, cornu anmonis; DG, dentate gyrus.

NGF, GDNF, or NT-3 mRNA in the hippocampus of juvenile or adult rats that had been subjected solely to repeated episodes of neonatal isolation.

In this study, we also observed that exposure of rats to neonatal isolation abrogated the increase in the level of NGF mRNA that subsequently occurs in the same rats exposed to a single episode of immobilization as juveniles or adults. Furthermore, the exposure of rats to neonatal isolation decreased the level of GDNF mRNA in response to a single immobilization in juvenile rats. Recently, maternal separation (from Day PN 2 to PN day 14) has been shown to affect the level of NGF and NT-3 in the hippocampus of rats in response to single swim stress on PN day 35 and PN day 60 (Faure et al., 2006). Faure et al., (2006) demonstrated that adolescent and adult rats subjected to the swim stress expressed significantly higher levels of NGF and NT-3 in the hippocampus if they had been previously subjected to repeated episodes of maternal separation. Unfortunately, since they did not evaluate the influence of swim stress on the levels of NGF and NT-3 in the hippocampus of adult rats that had not been subjected to maternal separation as neonates, it is difficult to determine the effect of repeated episodes of maternal separation on the response of NGF and NT-3 to swim stress during adulthood.

Whereas subjection of adult rats to a single 8-h episode of immobilization stress leads to a significant

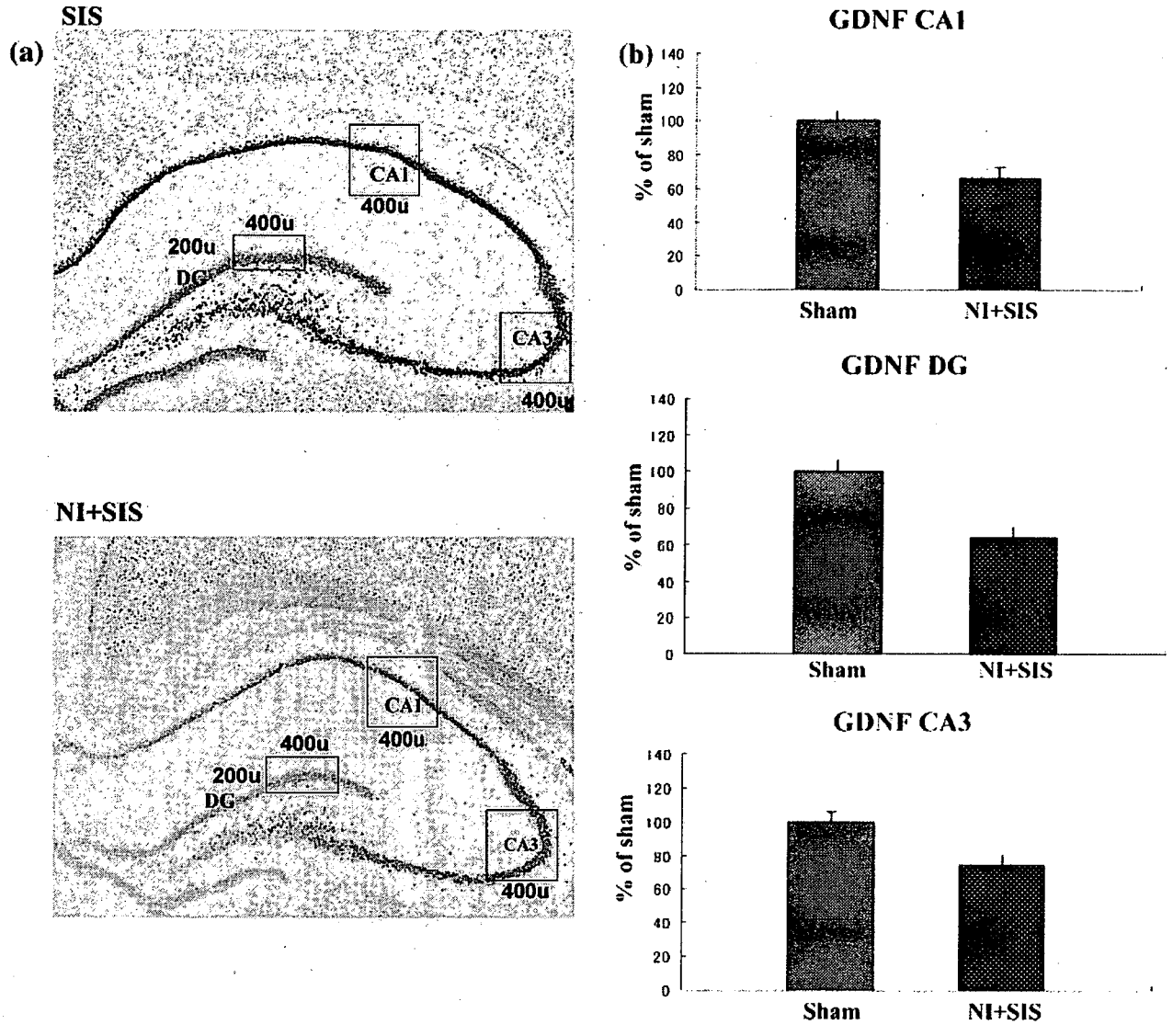

Fig. 4. Analysis of GDNF mRNA expression by in situ hybridization in the adult rat hippocampus. (a) GDNF mRNA expression in the rat hippocampus. NI, neonatal isolation; NI + SIS, neonatal isolation followed by a single immobilization stress. (b) Mean density of GDNF mRNA in the CA1 and CA3 pyramidal cell layers, and the dentate gyrus granular cell layer in the hippocampus of adult rats subjected to neonatal isolation (NI) and neonatal isolation followed by a single immobilization stress (NI + SIS). Results are expressed as the percentage of the SIS levels. The mean ± SEM (NI: $n = 7$; NI + SIS: $n = 6$) is shown. $^*P < 0.05$ compared with NI (Mann-Whitney U test). CA, cornu anmonis; DG, dentate gyrus.

reduction in the level of NGF in the hippocampus (Ueyama et al., 1997), traumatic brain injury (Grundy et al., 2001), subchronic cold stress (Foreman et al., 1993), immobilization stress (Smith and Clazza, 1996; Smith et al., 1995), and glucocorticoids (Mocchetti et al., 1996; Sun et al., 1993) significantly increase the expression of NGF in the rat hippocampus. These data indicating that stress-induced activation of the hypothalamo-pituitary-adrenal (HPA) axis upregulates the expression of NGF in the hippocampus, are consistent with the present findings. On the basis of the functional properties of NGF (Semkova and Kriegsten, 1999; Sofroniew et al., 2001; Tabakman et al., 2005), the induction of NGF in response to stress may be associated with neuroprotective responses. As such, the decreased response of NGF induction in both juvenile and adult rats and the decreased level of GDNF in juvenile, but not adult, rats in response to immobilization stress in the adult rats subjected to neonatal isolation may be, at least in part, involved in the stress susceptibility in later life due to neonatal isolation.

It is unclear how neonatal isolation attenuates the increased expression of NGF and GDNF in response to adulthood immobilization stress. Since activation of the HPA axis increases NGF expression in the rat

brain (Mocchetti et al., 1996; Sun et al., 1993), it is hypothesized that the decreased response of the HPA axis to adulthood immobilization may account for the decreased induction of NGF in the rats subjected to neonatal isolation. However, we have recently reported that the corticosterone response following a single 2-h restraint stress in adult rats previously subjected to neonatal isolation was significantly enhanced compared with the effect in adult rats not previously subjected to neonatal isolation (Erabi et al., 2007). On the basis of our recent finding, it is unlikely that the HPA axis is involved in attenuation of the induction of NGF in adult rats that had been previously subjected to neonatal isolation.

Early adverse experiences have been suggested to alter epigenetic programming through DNA methylation and histone acetylation (Weaver et al., 2004). Weaver et al. (2004) demonstrated that low levels of maternal care markedly induced cytosine methylation in the promoter of the glucocorticoid receptor (GR). This increased methylation inhibited the stimulation of GR mRNA expression in response to restraint stress in adult rats, due to inhibition of the binding of NGF1A to the GR promoter. In analogy, the induction of cytosine methylation in transcription-factor binding regions of the promoters of NGF or GDNF genes following neonatal isolation might lead to a reduction of the induction of NGF and GDNF in response to immobilization stress in adult rats. Further studies examining the effect of neonatal isolation on cytosine methylation in the promoters of these genes are needed to elucidate the mechanism of the inhibition of gene induction.

In summary, repeated episodes of neonatal isolation inhibit the induction of NGF in the hippocampus of juvenile and adult rats, and decrease the levels of GDNF in the hippocampus of juvenile rats in response to a SIS, but have no significant effect on the basal expression of these mRNAs in the absence of immobilization stress. These findings suggest that the susceptibility to stress derived from experience of neonatal isolation might play a role in decreased neuroprotective support in response to subsequent exposure to stress later in file through the down regulation of NGF induction and decreased expression of GDNF.

REFERENCES

Cirulli F, Micera A, Alleva E, Aloe L. 1998. Early maternal separation increases NGF expression in the developing rat hippocampus. Pharmacol Biochem Behav 59:853–858.

Cirulli F, Alleva E, Antonelli A, Aloe L. 2000. NGF expression in the developing rat brain: Effects of maternal separation. Brain Res Dev Brain Res 123:129–134.

Erabi K, Morinobu S, Kawano K, Tsuji S, Yamawaki S. 2007. Neonatal isolation changes the expression of IGF-IR and IGFBP-2 in the hippocampus in response to adulthood restraint stress. Int J Neuropsychopharmacol 10:369–381.

Faure J, Uys JDK, Marals L, Stein DJ, Daniels WMU. 2007. Early maternal separation followed by later stressors leads to dysregulation of the HPA-axis and increases in hippocampal NGF and NT-3 levels in arat model. Metab Brain Dis 22:183–195.

Folli F, Ghidella S, Bonfanti L, Kahn CR, Merighi A. 1996. The early intracellular signaling pathway for the insulin/insulin-like growth factor receptor family in the mammalian central nervous system. Mol Neurobiol 13:155–183.

Foreman PJ, Taglialatela G, Angelucci L, Turner CP, Perez-Polo JR. 1993. Nerve growth factor and p75NGFR factor receptor mRNA change in rodent CNS following stress activation of the hypothalamo-pituitary-adrenortical axis. J Neurosci Res 36:10–18.

Greisen MH, Altar CA, Bolwig TG, Whitehead R, Wortwein G. 2005. Increased adult hippocampal brain-derived neurotrophic factor and normal levels of neurogenesis in maternal separation rats. J Neurosci Res 79:772–778.

Grundy PL, Patel N, Harbuz MS, Lightman SL, Sharples PM. 2001. Glucocorticoids modulate the NGF mRNA response in the rat hippocampus after traumatic brain injury. Brain Res 892:386–390.

Hayashi A, Nagaoka M, Yamada K, Ichitani Y, Miake Y, Okado N. 1998. Maternal stress induces synaptic loss and developmental disabilities of offspring. Int J Dev Neurosci 16:209–216.

Huot RL, Plotsky PM, Lenox RH, McNamara RK. 2002. Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. Brain Res 950:52–63.

Kehoe P, Bronzino JD. 1999. Neonatal stress alters LTP in freely moving male and female adult rats. Hippocampus 9:651–658.

Lee HJ, Kim JW, Yim SV, Kim MJ, Kim SA, Kim YJ, Kim CJ, Chung JH. 2001. Fluoxetine enhances cell proliferation and prevents apoptosis in dentate gyrus of maternally separated rats. Mol Psychiatry 6:725–728.

Lin LF, Doherty DH, Lile JD, Bektesh S, Collins F. 1993. GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. Science 260:1130–1132.

Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. 2000. Maternal care, hippocampal synaptogenesis and cognitive development in rats. Nat Neurosci 3:799–806.

MacQueen GM, Ramakrishnan K, Ratnasingan R, Chen B, Young LT. 2003. Desipramine treatment reduces the long-term behavioural and neurochemical sequelae of early-life maternal separation. Int J Neuropsychopharmacol 6:391–396.

Magarinous AM, McEwen BS. 1995. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Comparison of stressors. Neuroscience 69:83–88.

Magarinos , McEwen BS, Flugge G, Fuchs E. 1996. Chronic psychosocial stress caises apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. J Neurosci 16: 3534–3540.

Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, Lindsay RM, Yancopoulos GD. 1990. NT-3. BDNF, and NGF in the developing rat nervous system: Parallel as well as reciprocal patterns of expression. Neuron 5:501–509.

McCormick CM, Kehoe P, Mallinson K, Cecchi L, Frye CA. 2002. Neonatal isolation alters stress hormone and mesolimbic dopamine release in juvenile rats. Pharmacol Biochem Behav 73:77–85.

Mocchetti I, Spiga G, Hayes VY, Isackson PJ, Colangelo A. 1996. Glucocorticoids diffrentoally increase nerve growth factor and basic fibroblast factor expression in the rat brain. J Neurosci 16: 2141–2148.

Morinobu S, Fujimaki K, Kawano K, Tanaka K, Takahashi J, Ohkawa M, Yamawaki S, Kato N. 2003. Influence of immobilization stress on the expression and phosphatase activity of protein phosphatase 2A in the rat brain. Biol psychiatry 54:1060–1066.

Nosrat CA, Fried K, Lindskog S, Olson L. 1997. Cellular expression of neurotrophin mRNAs during tooth development. Cell Tissue Res 290:569–580.

Poeggel G, Helmeke C, Abraham A, Schwabe T, Friedrich P, Braun K. 2003. Juvenile emotional experience alters synaptic composition in the rodent cortex, hippocampus, and lateral amygdala. Proc Natl Acad Sci USA 100:16137–16142.

Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA. 2002. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. Mol Psychiatry 7:609–616.

Roceri M, Cirulli F, Pessina C, Peretto P, Racagni G, Riva MA. 2004. Postnatal repeated maternal deprivation produces age-dependent changes of brain-derived neurotrophic factor expression in selected rat brain regions. Biol Psychiatry 55:708–714.

Semkova I, Krieglstein J. 1999. Neuroprotection mediated via neurotrophic factors and induction of neurotrophic factors. Brain Res Brain Res Rev 30:176–188.

Smith MA, Clzza G. 1996. Stress-induced changes in brain-derived neurotrophic factor expression are attenuated in aged Fischer 344/N rats. Neurobiol Aging 17:859–864.

Smith MA, Makino S, Kvetnansky R, Post RM. 1995. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. J Neurosci 15:1768–1777.

Sofroniew MV, Howe CL, Mobley WC. 2001. Nerve growth factor signaling, neuroprotection, and neural repair. Annu Rev Neurosci 24:1217–1281.

Suenaga T, Morinobu S, Kawano K, Sawada T, Yamawaki S. 2004. Influence of immobilization stress on the levels of CaMKII and phospho-CaMKII in the rat hippocampus. Int J Neuropsychopharmacol 7:299–309.

Sun FY, Costa E, Mocchetti I. 1993. Adrenal steroids mediate the increases of hippocampal nerve growth factor biosynthesis following bicuculline convulsions. Neuropharmacol 8:219–225.

Tabakman P, Jiang H, Shahar I, Arien-Zakay H, Levine RA, Lazarovici P. 2005. Neuroprotection by NGF in the PC12 in vitro OGD odel: Involvement of mitogen-activated protein kinases and gene expression. Ann N Y Acad Sci 1053:84–96.

Ueyama T, Kawai Y, Nemoto K, Sekimoto M, Tone S, Senba E. 1997. Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. Neurosci Res 28:103–110.

Vyas A, Mitra R, Rao S, Chatterji S. 2002. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. J Neurosci 22:6810–6818.

Watanabe Y, Gould E, McEwen BS. 1992. Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. Brain Res 588:341–345.

Weaver IC, Cervoni N, Champagne FA, D'Alessio AC, Sharma S, Seckl JR, Dymov S, Szyf M, Meaney MJ. 2004. Epigenetic programming by maternal behavior. Nat Neurosci 7:847–854.

Wetmore C, Bygdeman M, Olson L. 1992. High and low affinity NGF receptor mRNAs in human fetal spinal cord and ganglia. Neuroreport 3:689–692.

Palliative and Supportive Care (2007), 5, 3–9. Printed in the USA.

DOI: 10.1017/S1478951507070022

ORIGINAL ARTICLES

Can psychiatric intervention improve major depression in very near end-of-life cancer patients?

KEN SHIMIZU, M.D., PH.D.,[1] TATSUO AKECHI, M.D., PH.D.,[2,3] MASAYA SHIMAMOTO, M.D.,[4] MASAKO OKAMURA, M.D., PH.D.,[5] TOMOHITO NAKANO, M.D.,[1] TADASHI MURAKAMI, M.D.,[4] TATSUHIKO ITO, M.D., PH.D.,[4] AKIRA OBA, M.A.,[6] MAIKO FUJIMORI, M.A., PH.D.,[3] NOBUYA AKIZUKI, M.D., PH.D.,[3,4] MASATOSHI INAGAKI, M.D., PH.D.,[3,4] AND YOSUKE UCHITOMI, M.D., PH.D.[3,4]

[1]Psychiatry Division, National Cancer Center Hospital, Chuou-ku, Tokyo, Japan
[2]Department of Psychiatry and Cognitive-Behavioral Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, Aichi, Japan
[3]Psycho-Oncology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwa, Chiba, Japan
[4]Psycho-oncology Division, National Cancer Center Hospital East, Kashiwa, Chiba, Japan
[5]Psycho-Oncology Division, Cancer Institute Hospital, Tokyo, Japan
[6]Psycho-Oncology Division, Shizuoka Cancer Center Hospital, Shizuoka, Japan

(RECEIVED July 22, 2006; ACCEPTED August 27, 2006)

ABSTRACT

Objective: Although depression is a prevalent and burdensome psychiatric problem in end-of-life cancer patients, little is known about its susceptibility to treatment, especially when patients reach very close to the end of life. This study was conducted to evaluate response rate of that end-of-life depression to psychiatric intervention and to assess the feasibility of conventional evidence-based pharmacological therapy for depression.

Methods: The medical records of 20 patients who were referred to the psychiatry division for major depressive disorder and died within 3 months after the referral were reviewed. The Clinical Global Impression–Improvement (CGI-I) Scale was used for each case, and responders were defined as patients whose scores were much or very much improved. All pharmacological treatments were extracted, and the doses of the antidepressant prescribed were compared to their evidence-based-defined therapeutic doses.

Results: Of the 20 patients, seven were responders, but no response was achieved when the survival time was less than 3 weeks. Most patients were treated with antidepressants, but the doses prescribed were far less than the defined doses, especially the doses of the tricyclic antidepressants (TCAs).

Significance of results: These results suggested that patients' survival time largely determines susceptibility to psychiatric treatment, and it is hard to achieve response in patients whose survival time was less than about 1 month. Implementation of conventional evidence-based pharmacological treatment is difficult, especially with TCAs, and various antidepressants, which can be administrated by other routes, are needed when oral intake is impossible.

KEYWORDS: Terminally ill, End of life, Cancer, Major depression, Therapeutics

Corresponding author: Yosuke Uchitomi, Psycho-Oncology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba, 277-8577 Japan. E-mail: yuchitom@east.ncc.go.jp

INTRODUCTION

Depression is the most prevalent and distressing psychiatric issue in terminally ill patients with advanced cancer. The prevalence of depression in such patients deduced by rigorous methods (e.g., structured clinical interview) has been reported to be 5%–26%, with a median value of 15% (Hotopf et al., 2002) Several studies have indicated that depression can have a serious negative impact on terminally ill patients with advanced cancer, including reducing their quality of life (Grassi et al., 1996) causing severe suffering (Cherny et al., 1994), causing a desire for early death and requests for physician-assisted suicide and/or euthanasia (Brown et al., 1986; Chochinov et al., 1995; Breitbart et al., 2000; Akechi et al., 2002), and suicide (Henriksson et al., 1995) as well as psychological distress in family members (Cassileth et al., 1985).

Treatment of depression in patients very near the end of life is very challenging. The illness trajectory of cancer shows steady progression in the beginning, but a very rapid decline in the final few months. In about the last 3 months of the cancer journey, patients must confront one major stressful event after another, such as weight loss, reduction in performance status, and impaired ability for self care (Lunney et al., 2003; Murray et al., 2005). Pain and other burdensome physical symptoms may also exacerbate depression (Chochinov et al., 1995). Management that combines psychosocial and pharmacological intervention is recommended for end-of-life depression (Wilson et al., 2000; Pessin et al., 2003), and in our institution, psychiatrists specializing in psycho-oncology have tried to treat such depression aggressively. However, because of the very few empirical studies concerning its susceptibility to treatment, we have wondered if patients benefit from aggressive treatment, which may cause adverse events, as many palliative care physicians have been reluctant to prescribe antidepressants (Lawrie et al., 2004).

Focusing on pharmacotherapy, which is a key treatment for depression, to achieve conventional pharmacotherapy, which is defined based on evidence from physically healthy patients, may be difficult for these patients in regard to duration and dose prescribed. Some randomized controlled trials have shown that antidepressants can improve depression in advanced cancer patients (Costa et al., 1985; Holland et al., 1998; Fisch et al., 2003) However, there is not enough time to treat patients near the end of life, because, although side effects appears early, it takes 6–8 weeks to achieve full symptom reduction with antidepressants (Gabbard, 2000). Moreover, prescribing evidence-based therapeutic doses may be difficult in end-of-life cancer patients because of side effects, especially those of tricyclic antidepressants (TCAs; Popkin et al., 1985; Lloyd-Williams et al., 1999).

In this study, we reviewed our treatment experience and discussed future clinical implications. The primary aim of the study was to determine whether or not depression in very near end-of-life cancer patients is treatable, and we assessed the response of patients to psychiatric intervention. The secondary aim of the study was to determine the feasibility of pharmacotherapy, especially in regard to duration and dose prescribed.

METHODS

Study Sample

All psychiatric consultations referred to the Psychiatry Division, National Cancer Center Hospital East (NCCH-E), Japan, between August 2002 and December 2003 were reviewed. All psychiatric consultations were recorded in a computerized database (Akechi et al., 2001), and we constructed another database for consultations of major depression in advanced cancer patients in this period as a part of a pharmacological treatment algorithm study (Akizuki et al., 2002). This major depression database include patients' demographic factors, medical factors such as cancer site, performance status, and pain intensity, starting date of psychiatric consultation, selected antidepressant and its dose, and date of death. Using the above two databases, we extracted cases that met the following inclusion criteria: (1) clinical diagnosis of a major depressive disorder based on DSM-IV and (2) cancer death within 90 days of the start of psychiatric treatment. Patients were excluded from the study if follow-up was impossible for any reason, such as transfer. Because this study was a retrospective review of clinical practices, written consent and institutional review board approval were not obtained.

Psychiatric Intervention

Psychiatric intervention was mainly composed of psychotherapy, pharmacotherapy, family support, and recommendation of physical symptom management from the standpoint of depression management. Each component is described in detail below.

Psychotherapy

Psychotherapy was individualized and modified for each patient. Supportive psychotherapy consisted of active listening with supportive verbal inter-

vention and the occasional interpretation is the fundamental element. Psychiatrists maintained ongoing contact and allowed patients to talk about anything they wanted, for example, their life, experience, death, and so on. Cognitive-behavioral interventions, such as relaxation and distraction with pleasant imagery, were also used. A psychoeducational approach with realistic assurance was used for patients who felt anxiety or hopelessness because of any misunderstandings (Wilson et al., 2000; Pessin et al., 2003).

Pharmacotherapy

We had previously developed a pharmacological treatment algorithm (Nakano et al., 1999), and the second version of the algorithm was used during the study period. One of two TCA antidepressants, clomipramine or amitriptyline, was chosen for patients unable to take drugs by mouth because these were the only antidepressants available in parenteral formula in Japan. Alprazolam or methylphenidate was chosen because of their rapid onset of action for patients with mild depression. Drugs were chosen because of their side effect profile for the other patients. For example, SSRI was avoided because it exacerbates nausea in patients with nausea. All drugs were started at the lowest possible dose, and the dose was escalated over the following 3–6 days till the optimal dose.

Family Support

Depression in cancer patients is closely associated with psychological distress in family members (Cassileth et al., 1985), and psychological distress in the family may cause low social support, which is associated with patient's depression (Wilson et al., 2000). We also evaluated family distress and gave family members some advice. When a family member's distress was severe, we sometimes recommended consultation in our psycho-oncology clinic.

Recommendation of Physical Treatment

Physical symptoms, such as pain and fatigue, are closely associated with depression. If we concluded that patients' physical symptoms affected the patients' depression, we recommend that the attending oncologist treat the symptom or sometime to consult with a specialist in physical symptom management.

Judgment of Improvement

A structured clinical interview based on the DSM-IV criteria (employing an inclusive approach) was used to diagnose major depressive disorder, and nine symptoms for major depressive episodes were routinely evaluated and recorded on the medical charts. Final determination of the psychiatric diagnoses and the follow-up evaluation of the patients were discussed at a weekly meeting of the psychiatric division. Treatment outcome in the present study was retrospectively rated by two independent psychiatrists (K.S., M.S.) according to the Clinical Global Impression-Improvement (CGI-I) Scale (Guy, 1976), and we assessed the change from baseline to the point when the greatest improvement was achieved. We used an anchor point that had been used in another study of terminal major depression (Table 1; Macleod, 1998). A "response" was defined when a patient's rating on the CGI-I was "very much improved" or "much improved," even in a short period. The reliability (kappa coefficient) of the rating for whether response had been achieved was 0.66. Whenever the raters disagreed, disagreements were discussed and a final rating was made.

Duration and Dose of Psychotropic Drugs Prescribed

We assumed that conventional evidence-based treatments for depression in noncancer patients were applicable (Berney et al., 2000), while accepting that further research is required to establish this. All pharmacological treatment to alleviate depressive symptoms was extracted from the medical charts. We evaluated "prescribed duration," which is the number of days the antidepressant was prescribed, and "% optimal dose," which is the dose administered as a percentage of the minimal optimal dose defined by the Japanese Ministry of Health, Labor and Welfare, based on results of clinical trials. If the dose prescribed was above the minimal optimal dose but within the defined range of the

Table 1. *Anchor points of the Clinical Global Impression–Improvement Scale*

Very much improved: a complete or nearly complete remission of all depressive symptoms.
Much improved: improvement in several symptoms but without complete remission.
Minimally improved: minor improvement in mood without improvement in other symptoms.
No change or worse: other than those above.

optimal dose, the "% optimal dosage" was evaluated as 100%. However, because of recent evidence suggesting that the TCAs prescribed may be effective at a dose of 75 mg (Furukawa et al., 2002), that dose was defined as the optimal dose for these agents. If pharmacotherapy was discontinued, the reason was also extracted.

Statistical Analysis

All patients who were prescribed psychotropic drugs were dichotomized into TCAs group and non-TCAs group, and "% optimal dose" was compared by the Mann–Whitney *U* test. All analyses were performed using SPSS 12.0 J for Windows statistical software (SPSS Japan Institute).

RESULTS

During the study period, 65 patients were referred to the psychiatry division and diagnosed with major depressive episodes. Nine patients were excluded from the analysis because follow-up was impossible due to transfer. Of the remaining 56 patients, 20 died within 90 days of the psychiatric referral and were included in this study. Their demographic characteristics, tumor sites, length of survival, pain intensity, and performance status as defined by Eastern Cooperating Oncology Group are listed in Table 2.

Table 3 shows the detailed course of treatment of all 20 patients. "Very much improved" was not achieved in any of the patients, but "much improved" was achieved in 7 (35%), and they were recorded as "response." Minimal improvement was achieved in three of the other 13 patients, and 10 showed no improvement at all. A "response" was not achieved in any of the eight patients who died within 3 weeks of the beginning of psychiatric treatment.

As shown in Table 3, one patient was treated by psychotherapy alone, and 19 patients received pharmacotherapy from start to the end of their psychiatric treatment for depression. The "prescribed duration" was shorter than survival time in most patients, and in four patients, it was shorter than 10 days, even when the survival time was more than 1 month. One patient received terminal sedation, but treatment for depression was stopped in the other 18 patients because of the development of terminal delirium.

Only five of the 19 patients were treated with the optimal dose of the psychotropic agent, and 11 of the 19 patients were treated with less than half that of the minimal optimal dose. All seven patients who were treated with antidepressant intravenously were chosen amitriptyline or clomipramine because oral intake was impossible. The median "percent of optimal dose" in the nine patients who were prescribed TCAs (amitriptyline, clomipramine, and nortriptyline) was 16.7%, and significantly lower than that of the other drugs (median 83.9%; $p = .007$).

Table 2. *Characteristics of patients*

Patient number	20
Age (mean ± *SD*, median)	59 ± 11, 60.5
Gender	10 men, 10 women
Primary tumor site	Lung (4), stomach (4), esophagus (4), colon (3), others (5)
Survival length (mean ± *SD*)	41 ± 29 days
Current pain intensity	
0 (none)	7
1 (a little)	6
2 (tolerable)	5
3 (intolerable)	2
Performance status (ECOG)	
0	0
1	1
2	5
3	11
4	3

DISCUSSION

This is a preliminary study whose results provide information on whether major depression in terminally ill cancer patients is treatable by psychiatric intervention or not. The results suggest the therapeutic potential of psychiatric intervention, because one third showed considerable improvement of their depressive symptoms, even though no patient could achieve complete remission. Another important finding was that no responses were achieved in the patients whose survival time was less than 3 weeks.

The closer end-of-life cancer patients approach death, the more untreatable their depression may become. Macleod et al. assessed the efficacy of methylphenidate for major depression in the terminally ill and observed a response in 50% of those who survived more than 6 weeks as opposed in only 7% of those who survived less than 6 weeks (Macleod, 1998). In our study, also dividing patients by 6 weeks of survival time, 54.5% (6/11) of those who survived more than 6 weeks showed a response, as opposed to only 11.1% of those who survived less than 6 weeks. Our colleagues previously reported successful antidepressant treatment of major depression in six end-of-life patients whose median survival was 4 weeks (Kugaya et al., 1999), but

Table 3. *Improvement after Psychiatric Intervention*

Case	Age	Sex	PS	Pain	Cancer site	Survival length (days)	CGI-I	Drug	Prescribed duration (days)	Administrated dose (mg)	% Optimal dose (%)
1	57	M	2	0	Bile duct	6	No change or worse	Methylphenidate	6	10	50
2	47	F	4	3	Colon	7	Minimally improved	Clomipramine i.v.[b]	6	6	8
3	67	F	4	1	Primary unknown	8	No change or worse	None	—	—	—
4	68	F	4	1	Stomach	8	No change or worse	Amitriptyline i.v.[b]	8	10	13.3
5	72	M	3	0	Lung	11	No change or worse	Milnacipran	7	15	30
6	58	F	3	1	Colon	13	No change or worse	Amoxapine	10	50	100
7	32	F	3	0	Breast	14	No change or worse	Trazodone	13[c]	150	100
8	45	F	3	0	Stomach	16	Minimally improved	Milnacipran	5	30	60
9	61	F	3	3	Colon	26	Much improved[a]	Amitriptyline i.v.[b]	25	10	13.3
10	36	M	3	2	Liposarcoma	48	No change or worse	Amitriptyline i.v.[b]	6	10	13.3
11	50	M	2	2	Esophagus	53	Much improved[a]	Clomipramine i.v.[b]	50	12.5	16.7
12	72	F	3	2	Stomach	55	Much improved[a]	Amitriptyline i.v.[b]	44	15	20
13	64	F	3	2	Lung	58	Much improved[a]	Nortriptyline[b]	26	25	33.3
14	63	M	3	2	Esophagus	63	No change or worse	Mianserin	7	10	33.3
15	71	M	1	0	Lung	64	Much improved[a]	Paroxetine	41	30	100
16	66	F	2	0	Lung	66	Much improved[a]	Amoxapine	43	25	100
17	60	M	3	1	Stomach	67	No change or worse	Nortriptyline[b]	67	35	46.7
18	57	M	3	1	Esophagus	73	No change or worse	Trazodone	9	50	67.7
19	59	M	2	0	Esophagus	82	Much improved[a]	Amitriptyline i.v.[b]	80	20	26.7
20	65	M	3	1	Pancreas	86	Minimally improved	Alprazoram	58	1.2	100

PS: Performance status as defined by Eastern Cooperative Oncology Group; CGI-I: Clinicl Grobal Impression-Improvement Scale; % Optimal Dosage: the administrated dose/the minimal optimal dose.
[a]Recorded as "response"
[b]Tricyclic Antidepressants
[c]Stopping pharmacotherapy due to continuous sedation

these cases may be a minority in whole treated very near end-of-life depression. Although both our result and Macleod's are preliminary, they suggest that patients' survival time largely determines treatability, and about 1 month may be the turning point. Aggressive pharmacotherapy often induces adverse effects in cancer patients (Popkin et al., 1985), so setting impossible goals of treatment may cause them unnecessary suffering. Treatment by the usual strategy for depression may be harmful to patients whose life expectancy is estimated to be less than 1 month. For those patients, alternative approaches should be considered, including withholding administration of antidepressants.

As a proactive approach, early detection and treatment may be the key to overcoming end-of-life depression. There exists the condition of the underrecognition and the introduction of treatment too late in end-of-life depression, and this may lead to missing the chance to treat (Lloyd-Williams et al., 1999). Screening end-of-life patients for depression can be an important approach (Hotopf et al., 2002). In addition, development of novel methods of preventiving major depression among end-of-life cancer patients is needed.

The results of our study suggested that pharmacotherapy for end-of-life depression is difficult. The short survival time restricts the duration of treatment, and the development of delirium prevents continuation of pharmacotherapy. The psychotropic agents, especially the TCAs, were prescribed in fairly low doses, and a previous study showed results similar to ours in a British palliative care setting (Lloyd-Williams et al., 1999). Even with specialized knowledge of psychopharmacology, it was hard to achieve the optimal dose, which was defined based on physically healthy subjects. Delirium in the terminally ill occurs in most patients (Lawlor et al., 2000), and psychiatrists must be very cautious about treating with drugs such as TCAs that sometimes induce delirium (Degner et al., 2004). TCAs may be a difficult choice for depression in the terminally ill, but about half the patients in our study were prescribed TCAs in very low doses. Terminally ill cancer patients often lack a functioning gastrointestinal tract, and psychiatrists chose intravenous TCAs in such a case. A variety of antidepressants that can be administered by alternate routes are needed when oral intake is impossible (Koelle & Dimsdale, 1998).

There were several limitations in this study. First, it was based on a retrospective chart review of clinical practice at a single teaching cancer center hospital. Because there were biases due to patient selection and physician's influence, caution is required in terms of generalizing the results. Second, a problem of assessment existed concerning diagnosis and treatment course. Although we made the diagnoses and evaluation according to structured diagnostic interview based on the DSM-IV criteria, there exists difficulty in evaluating major depression in the terminally ill (Wilson et al., 2000; Pessin et al., 2003). Third, some important information that may be associated with depression, such as physical distress, other treatment in parallel, past history of psychiatric disorders, social support, and coping style, was not included.

Although our study was not based on a rigorous design, some highly suggestive results emerged as a clue to the next step. Further research is needed to elucidate proper treatment strategies for major depression at the end of life according to the patient's prognosis. Also, it is hard to prescribe TCAs in conventional doses for end-of-life patients, and alternative, nonoral formulations of antidepressants are needed.

REFERENCES

Akechi, T., Nakano, T., Okamura, H., et al. (2001). Psychiatric disorders in cancer patients: Descriptive analysis of 1721 psychiatric referrals at two Japanese cancer center hospitals. *Japanese Journal of Clinical Oncology, 31*, 188–194.

Akechi, T., Okamura, H., Nishiwaki, Y., et al. (2002). Predictive factors for suicidal ideation in patients with unresectable lung carcinoma. *Cancer, 95*, 1085–1093.

Akizuki, N., Okamura, H., Akechi, T., et al. (2002). Clinical experience of the pharmacological treatment algorithm for major depression in advanced cancer patients: Preliminary study. *International Journal of Psychiatry in Clinical Practice, 6*, 83–89.

Berney, A., Stiefel, F., Mazzocato, C., et al. (2000). Psychopharmacology in supportive care of cancer: A review for the clinician. III. Antidepressants. *Supportive Care in Cancer, 8*, 278–286.

Breitbart, W., Rosenfeld, B., Pessin, H., et al. (2000). Depression, hopelessness, and desire for hastened death in terminally ill patients with cancer. *JAMA, 284*, 2907–2911.

Brown, J.H., Henteleff, P., Barakat, S., et al. (1986). Is it normal for terminally ill patients to desire death? *American Journal of Psychiatry, 143*, 208–211.

Cassileth, B.R., Lusk, E.J., Strouse, T.B., et al. (1985). A psychological analysis of cancer patients and their next-of-kin. *Cancer, 55*, 72–76.

Cherny, N.I., Coyle, N., & Foley, K.M. (1994). Suffering in the advanced cancer patient: A definition and taxonomy. *Journal of Palliative Care, 10*, 57–70.

Chochinov, H.M., Wilson, K.G., Enns, M., et al. (1995). Desire for death in the terminally ill. *American Journal of Psychiatry, 152*, 1185–1191.

Costa, D., Mogos, I., & Toma, T. (1985). Efficacy and safety of mianserin in the treatment of depression of women with cancer. *Acta Psychiatrica Scandinavica, 320*(Suppl.), 85–92.

Degner, D., Grohmann, R., Kropp, S., et al. (2004). Severe adverse drug reactions of antidepressants: Results of

the German multicenter drug surveillance program AMSP. *Pharmacopsychiatry, 37*(Suppl. 1), S39–45.

Fisch, M.J., Loehrer, P.J., Kristeller, J., et al. (2003). Fluoxetine versus placebo in advanced cancer outpatients: A double-blinded trial of the Hoosier Oncology Group. *Journal of Clinical Oncology, 21*, 1937–1943.

Furukawa, T.A., McGuire, H., & Barbui, C. (2002). Meta-analysis of effects and side effects of low dosage tricyclic antidepressants in depression: Systematic review. *British Medical Journal, 325*, 991.

Gabbard, G.O. (2000). Mood disorders. In *Comprehensive Textbook of Psychiatry*, Sadock, B.J. & Sadock, V.A. (eds.), pp. 1328–1440. Philadelphia: Lippincott Williams & Wilkins.

Grassi, L., Indelli, M., Marzola, M., et al. (1996). Depressive symptoms and quality of life in home-care-assisted cancer patients. *Journal of Pain and Symptom Management, 12*, 300–307.

Guy, E. (1976). *ECDEU Assessment Manual for Psychopharmacology*. Rockville, MD: U.S. Department of Health, Education, and Welfare.

Henriksson, M.M., Isometsa, E.T., Hietanen, P.S., et al. (1995). Mental disorders in cancer suicides. *Journal of Affective Disorders, 36*, 11–20.

Holland, J.C., Romano, S.J., Heiligenstein, J.H., et al. (1998). A controlled trial of fluoxetine and desipramine in depressed women with advanced cancer. *Psychooncology, 7*, 291–300.

Hotopf, M., Chidgey, J., Addington-Hall, J., et al. (2002). Depression in advanced disease: A systematic review. Part 1. Prevalence and case finding. *Palliative Medicine, 16*, 81–97.

Koelle, J.S. & Dimsdale, J.E. (1998). Antidepressants for the virtually eviscerated patient: Options instead of oral dosing. *Psychosomatic Medicine, 60*, 723–725.

Kugaya, A., Akechi, T., Nakano, T., et al. (1999). Successful antidepressant treatment for five terminally ill cancer patients with major depression, suicidal ideation and a desire for death. *Supportive Care in Cancer, 7*, 432–436.

Lawlor, P.G., Gagnon, B., Mancini, I.L., et al. (2000). Occurrence, causes, and outcome of delirium in patients with advanced cancer: A prospective study. *Archives of Internal Medicine, 160*, 786–794.

Lawrie, I., Lloyd-Williams, M., & Taylor, F. (2004). How do palliative medicine physicians assess and manage depression. *Palliative Medicine, 18*, 234–238.

Lloyd-Williams, M., Friedman, T., & Rudd, N. (1999). A survey of antidepressant prescribing in the terminally ill. *Palliative Medicine, 13*, 243–248.

Lunney, J.R., Lynn, J., Foley, D.J., et al. (2003). Patterns of functional decline at the end of life. *JAMA, 289*, 2387–2392.

Macleod, A.D. (1998). Methylphenidate in terminal depression. *Journal of Pain and Symptom Management, 16*, 193–198.

Murray, S.A., Kendall, M., Boyd, K., et al. (2005). Illness trajectories and palliative care. *British Medical Journal, 330*, 1007–1011.

Nakano, T., Kugaya, A., Akechi, T., et al. (1999). Algorithm for the treatment of major depression in patients with advanced cancer. *Psychiatry and Clinical Neurosciences, 53*(Suppl), S61–65.

Pessin, H., Potash, M., & Breitbart, W. (2003). Diagnosis, assessment, and treatment of depression in palliative care. In *Psychosocial Issues in Palliative Care*, Lloyd-Williams, M. (ed.), pp. 81–103. New York: Oxford University Press.

Popkin, M.K., Callies, A.L., & Mackenzie, T.B. (1985). The outcome of antidepressant use in the medically ill. *Archives of General Psychiatry, 42*, 1160–1163.

Wilson, K.G., Chochinov, H.M., Faye, B.J.d., et al. (2000). Diagnosis and management of depression in palliative care. In *Handbook of Psychiatry in Palliative Medicine*, Chochinov, H.M. & Breitbart, W. (eds.), pp. 25–49. New York: Oxford University Press.

ELSEVIER

Journal of Affective Disorders 99 (2007) 231–236

JOURNAL OF AFFECTIVE DISORDERS

www.elsevier.com/locate/jad

Brief report

Regional cerebral glucose metabolism in patients with secondary depressive episodes after fatal pancreatic cancer diagnosis

Masatoshi Inagaki [a], Eisho Yoshikawa [b], Makoto Kobayakawa [c], Yutaka Matsuoka [d], Yuriko Sugawara [e], Tomohito Nakano [f], Nobuya Akizuki [a], Maiko Fujimori [a], Tatsuo Akechi [g], Taira Kinoshita [h], Junji Furuse [i], Koji Murakami [j], Yosuke Uchitomi [a,*]

[a] *Psycho-Oncology Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, Kashiwanoha 6-5-1, Kashiwa, Chiba 277-8577, Japan*

[b] *Division of Psycho-Oncology, Shizuoka Cancer Center, Shizuoka, Japan*

[c] *Department of Psychiatry, Hiroshima University Hospital, Hiroshima, Japan*

[d] *Division of Adult Mental Health, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan*

[e] *Department of Psychiatry and Behavioral Science, Tokai University School of Medicine, Kanagawa, Japan*

[f] *Department of Psychiatry, Kitasato Institute Hospital, Tokyo, Japan*

[g] *Department of Psychiatry and Cognitive-Behavioral Medicine, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan*

[h] *Division of Upper Abdominal Surgical Oncology, National Cancer Center Hospital East, Chiba, Japan*

[i] *Division of Hepatobiliary and Pancreatic Oncology, National Cancer Center Hospital East, Chiba, Japan*

[j] *PET Center, Dokkyo University School of Medicine, Tochigi, Japan*

Received 6 July 2006; received in revised form 9 August 2006; accepted 14 August 2006
Available online 20 September 2006

Abstract

Background: Secondary depression is common in the clinical oncology setting after pancreatic cancer diagnosis, following which the patients have to face the fact that they have a cancer with an extremely poor prognosis. However, the specific pathophysiology remains unclear. The present study examined the regional cerebral glucose metabolism using F18-fluorodeoxyglucose (F18-FDG) positron emission tomography (PET) in antidepressant-naïve pancreatic cancer patients with a depressive episode after their cancer diagnosis and before their cancer treatment.

Methods: Regional cerebral glucose metabolism in pancreatic cancer patients without any antidepressant medication after the cancer diagnosis was measured with F18-FDG PET. A depressive episode after the cancer diagnosis was defined as including major and minor depressive episodes, and was diagnosed using the Diagnostic and Statistical Manual, Fourth Edition (DSM-IV). The prefrontal and limbic regions were the primary regions-of-interest, and an uncorrected value of $p<0.005$ was used as significant.

Results: Six of 21 pancreatic cancer patients were diagnosed as having a depressive episode. Significantly higher glucose metabolism in depressed patients was found in the subgenual anterior cingulate cortex (sACC) (uncorrected $p=0.002$).

Limitations: There was a small number of subjects, and there were no healthy controls.

Conclusions: The higher metabolism in the sACC may be associated with the pathophysiology of secondary depressive episodes in patients following pancreatic cancer diagnosis.

Keywords: Depression; Anterior cingulate cortex; Positron emission tomography; Pancreatic cancer; Regional cerebral metabolic rate of glucose

* Corresponding author. Tel.: +81 4 7134 7013; fax: +81 4 7134 7026.
E-mail address: yuchitom@east.ncc.go.jp (Y. Uchitomi).

0165-0327/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jad.2006.08.019

1. Introduction

Pancreatic cancer is a refractory malignant tumor, and has a 5-year survival rate of only 4%. More than 30,000 patients died from pancreatic cancer in the year 2006 in the USA. Psychiatric symptoms, especially depression and anxiety, in pancreatic cancer patients have been focused on by clinicians, as several textbooks describe the association of psychiatric symptoms with pancreatic cancer. Previous studies reported 18–50% of pancreatic cancer patients experienced depression (Bernhard and Hurny, 1998; Green and Austin, 1993).

Although several serum substrates secreted by pancreatic cancer have been postulated to be involved in the pathophysiology of depression (Bernhard and Hurny, 1998; Green and Austin, 1993), no clear associations have so far been made in human. On the other hand, depressive episodes in pancreatic cancer patients may reflect the psychological response to the knowledge to having a tumor whose extremely poor prognosis is well known. However, brain neuron-circuitry related to depressive episodes in patients after pancreatic cancer diagnosis has been unclear.

Recent developments in neuroimaging techniques have revealed the involvement of neural mechanisms in a depressive episode. Previous studies have shown the involvement of the prefrontal and limbic regions (Drevets, 2001) and corticolimbic dysregulation in depression (Mayberg et al., 1999). Although findings have been variable, decreased dorsolateral and subgenual prefrontal and increased amygdala cerebral metabolic rates tend to be seen in patients with depression. A previous review article showed that prefrontal hypometabolism may be a common pathway to depressive symptoms in primary (psychiatric) and secondary (to neurological disorders) depression (Mayberg, 1994). However, potential differences in the depression subtype may cause different patterns of neural activity. An involvement of the potential serum substrates secreted by pancreatic cancer with depressive episodes may suggest a different pathophysiology. In addition, the depressive episodes in cancer patients have generally been assumed to be reactive and of short duration (Chochinov, 2001), suggesting that they have a different pathophysiology.

Although serum substrates secreted by pancreatic cancer and/or psychological stressor have been postulated to be associated with the depressive episodes in pancreatic cancer patients, details of the mechanisms have been unclear. Investigation of the neural circuitry is one of the useful strategies to reveal the possible mechanisms of depressive episodes in pancreatic cancer patients. In the present study, we performed a preliminary investigation using positron emission tomography (PET) to examine the regional cerebral metabolic rate of glucose (rCMRgluc) in the prefrontal and limbic regions among pancreatic cancer patients with a depressive episode after their cancer diagnosis.

2. Methods

2.1. Subjects

This study was approved by the Institutional Review Board and the Ethics Committee of the National Cancer Center, Japan, and was performed after obtaining written informed consent from the patients. The inclusion criterion of the subject sampling was clinically diagnosed pancreatic cancer patients after disclosure of the diagnosis before anticancer treatment who were going to undergo positron emission tomography (PET) to evaluate the clinical stage of the cancer as a clinical investigation. Exclusion criteria were (1) <18 years old; (2) having neurological and/or Axis I psychiatric disorders defined by the Diagnostic and Statistical Manual, Fourth Edition (DSM-IV) except for mood and anxiety disorders; (3) having a mass legion in the brain; (4) having a blood sugar level higher than 120 mg/dl; (5) receiving a hypoglycemic agent; (6) having any physical function that interfered with daily life, as assessed by performance status; and (9) a cognitive impairment defined as a score of less than 24 on the Mini-Mental State Examination (Mori et al., 1985).

2.2. Procedure

Subject sampling was performed among inpatients who were going to undergo PET for pancreatic cancer before their treatment in the National Cancer Center Hospital East. Diagnosis of pancreatic cancer was done as a clinical diagnosis, but not as a pathological diagnosis. After their attending physician had booked their PET examination for clinical staging of their pancreatic cancer and at least within 2 days before the PET examination, trained psychiatrists (EY, MK) performed an interview including the Structured Clinical Interview for DSM-IV-Axis I Disorder to determine whether the subjects had a depressive episode. We defined the *depressive episode* on the basis of criteria of the major and/or minor depression of the DSM-IV, because, not only major depression, but a minor depressive episode can also have a negative impact in the same manner as a major depressive episode (Evans et al., 1999). In addition, a study into depressive symptomatology has indicated continuity of minor depressive episodes to major depressive episodes (Kendler and Gardner, 1998).