が少ない結果が得られた[84]．さらに，Stage 1 大うつ病患者に対するリチウム治療の前後で DEX/CRH 負荷試験を行ったが，リチウムに対する反応者と非反応者で負荷後の血中コルチゾールや ACTH 濃度に差はみられなかった[85]．しかし，その後の研究で，Stage 1 大うつ病患者でリチウム併用前の DEX/CRH 試験における血中コルチゾール/ACTH 濃度比がリチウムに対する非反応者で反応者よりも高いことを報告した[86]．なお，DEX/CRH 試験における HPA 系の過大反応は抗うつ薬による改善と共に通常正常化するが[69,70]，リチウム併用後は，治療効果の有無にかかわらず HPA 系の反応はむしろより大きくなる(すなわちむしろ非抑制となる)点は，DEX/CRH 試験の実施上注意を要する[85]．

c. 甲状腺ホルモン併用(保険適用外)

T_3 による効果増強について，8 試験(292 例の Stage 1 の大うつ病および双極性うつ病患者．ただし，大部分は大うつ病)のメタ解析が報告された[6]．絶対ベネフィット増加は 23.2%(95% 信頼区間 4.5～41.9%)，治療効果発現必要症例数は 4.3 人であった．しかし，そのうち 4 つの RCT の結果を解析すると，T_3 による効果増強は有意とはいえず，治療効果発現必要症例数は 12.5 人であった．このメタ解析は，T_3 による効果増強について，無作為化対照試験がさらに必要であることを指摘した．

メタ解析にも含まれていた研究のうち，示唆に富む 2 研究を紹介する．Joffe らは，Stage 1 の非精神病性大うつ病 50 例を対象に，偽薬あるいは T_3 37.5 μg/日，炭酸リチウム 900 mg/日を抗うつ薬に併用して 2 週間の RCT を行った[87]．反応率は偽薬群 19%，T_3 群 59%，リチウム群 53% であり，T_3 とリチウムの効果は有意であった．さらに，Joffe と Singer は，Stage 1 の大うつ病患者(ただし，抗うつ薬の治療期間は 3 週間であり不十分)を対象に T_3 37.5 μg/日と L-サイロキシン(T4)150 μg/日の 3 週間の二重盲検比較試験を行った[88]．T_3 に対する反応率は 59% であり，T_4 の 19% に比べて有意に優れていた．

以上のように，T_3 の難治性うつ病に対する効果の方が多く報告されているが，T_4 の Stage 2 難治性うつ病に対する有効性もオープン試験で報告されている[89]．双極性うつ病 12 例，大うつ病 5 例を対象として，抗うつ薬に T_4 を平均 482 μg/日と極めて高用量追加したところ，8 週間で 8 例(47%)が反応かつ寛解した(双極性うつ病では 50%，大うつ病では 60% が寛解)．高用量の T_4 で，freeT_4 濃度は正常値の上限の平均 1.5 倍となったが，発汗，振戦以外に重篤な副作用はなかったという．筆者らは，Stage 2 の難治性うつ病患者に対する T_4 併用の効果を報告したが[11]，最長 11 年までの長期経過観察後の最終診断に基づいて T_4 の有効率を再解析したところ，有効率は大うつ病で 12.5%(8 例中 1 例)，双極性うつ病で 67%(6 例中 4 例)であり，双極性で高い傾向がみられた[55]．

d. ドパミンアゴニスト併用(保険適用外)

難治性うつ病に対するドパミンアゴニストの効果については前方視的なオープン試験，症例報告，後方視的調査が 10 編ほど報告されている[55]．残念ながら，難治性うつ病に対する偽薬との二重盲検比較試験は行われていないので，ドパミンアゴニストの効果のエビデンス・レベルは低い．難治性大うつ病を対象とした研究ではないが，プラミペキソールの非精神病性大うつ病に対する二重盲検比較試験が偽薬と fluoxetine を対照として行われた[90]．プラミペキソール(1 mg)と fluoxetine (20 mg)はともに偽薬と比べて有意にうつ症状を改善した[90]．したがって，ドパミンアゴニストは抗うつ作用を有することから，抗うつ薬に併用した時の効果は，増強効果というよりはむしろ併用効果である．

筆者らは Stage 2 の難治性うつ病患者に対するドパミンアゴニスト(ブロモクリプチン)併用の効果を報告したが[11]，最長 11 年までの長期経過観察後の最終診断に基づいてドパミンアゴニストの有効率を再解析したところ，有効率は大うつ病では 62.5%(15 例中 9 例)，双極性うつ病では 62.5%

(8例中5例)であり，ドパミンアゴニストは両病型で有効であった[55]．

e. 非定型抗精神病薬併用(保険適用外)

ドパミンアゴニストの結果と一見矛盾した効果のようにも思われるが，抗うつ薬と非定型抗精神病薬の併用が難治性うつ病の治療に有効であることが報告されている．Barbee らは，後方視的調査で，Stage 1 以上の非精神病性大うつ病に対する非定型抗精神病薬併用の有効率を，オランザピン 57%，リスペリドン 50%，クエチアピン 33%，ziprasidone 10% と報告した[91]．2006 年にわが国でも発売されたアリピプラゾールの Stage 1 以上の非精神病性大うつ病に対する併用効果も，最近多くのオープン試験で検討され有効性が報告された(引用は最新の文献のみとする)[92]．

非定型抗精神病薬のうちオランザピン併用は大規模な二重盲検比較試験で Stage 2 の非精神病性大うつ病に有効であることが報告された[93]．8 週間の fluoxetine 治療に対する非反応者 605 例をオランザピン/fluoxetine 併用群，fluoxetine 継続群，オランザピン単独群の 3 群に無作為割り付けし 8 週間試験したところ，併用群はそれぞれの単独群に比べて有意に改善し，反応率，寛解率ともに有意に優れていた[93]．

一方，オランザピン/fluoxetine 併用療法が Stage 2 の難治性非精神病性大うつ病に有効ではないという大規模な二重盲検比較試験(500 例)も報告された[94]．SSRI 非反応者のうち，ノルトリプチリンで 7 週間治療してやはり非反応であった症例をオランザピン/fluoxetine 併用群，fluoxetine 単独群，オランザピン単独群，ノルトリプチリン継続群の 4 群で 8 週間比較したところ，反応率は 4 群に差はみられなかった[94]．しかし，オランザピンによる抗うつ薬の効果増強を期待するのであれば，むしろオランザピン/ノルトリプチリン併用群をオランザピン単独群，ノルトリプチリン継続群と比較すべきだったのではないか．この点が，上記の研究[93]との方法上の大きな相違点である．

f. ピンドロール併用(保険適用外)

1990 年代より，β 遮断薬であるとともに，セロトニン 1A/1D 受容体アンタゴニストであるピンドロールとセロトニン再取り込み阻害薬(SSRI とクロミプラミン)の併用がセロトニン再取り込み阻害薬の効果を増強するのではないかと注目され，多くの臨床試験が行われてきた．実験的にピンドロールは前シナプスの受容体を遮断し，SSRI との併用でシナプス間隙セロトニン濃度をさらに上昇させることから両者の併用効果が期待された．しかし，最近の二重盲検比較試験では，セロトニン再取り込み阻害薬非反応者に対してピンドロール併用は無効であることが報告されている[95]．

g. 反復性経頭蓋磁気刺激法 repetitive transcranial magnetic stimulation (TMS) (rTMS) (保険適用外)

大うつ病に対する rTMS 群と偽治療(Sham)群の二重盲検比較試験がいくつか行われた．初期の研究では，Stage 1 あるいは Stage 2 の難治性大うつ病を対象として左前頭前野への rTMS を行ったが，Sham 群との差がみられないか[96]，Sham 群との差がみられても改善度・寛解率は軽度であり[97]，あまり有望とはいえない結果であった．しかし，最近報告された 2 試験では，Stage 2 難治性うつ病(主に大うつ病だが，20～30% の双極性うつ病を含む)を対象とした Sham 群との二重盲検比較試験で rTMS 群は有意に高い反応率(44% vs 8%[98]；61% vs 6%[99])あるいは寛解率(36% vs 0%[98])を示した．初期の研究との施行方法の違いは，Rossini らの試験では左前頭前野への rTMS でより強い強度(100% motor threshold, MT)を用いたことであり，初期の研究[96,97]で採用された左前頭前野への 80% MT の rTMS と比べても 100% MT の rTMS は有意に有効であることを報告した[99]．また，Fitzgerald らの試験では，以上の 3 試験とは rTMS の刺激部位が異なり，右前頭前野への低頻度 rTMS と左前頭前野への高頻度 rTMS を組み合わせた治療を行っ

た[98]．いずれも抗うつ薬あるいは気分安定薬に併用してrTMSを施行した．最近の2試験では双極性うつ病を対象に含んでいるが，躁転はみられなかった．

h. 迷走神経刺激 vagus nerve stimulation (VNS)（国内未承認）

新しいうつ病治療法としてVNSが最近注目されている．Stage 2の非精神病性難治性大うつ病と非精神病性難治性双極性うつ病(双極性うつ病は全対象の10.4%で，気分安定薬あるいは抗うつ薬による2種類の十分な治療に非反応と定義された)合わせて210例に対して，抗うつ薬あるいは気分安定薬に併用して，VNS群と偽治療(Sham)群との10週間の二重盲検比較試験が行われた[100]．VNS群の反応率はSham群と差はみられなかった．なお，大うつ病と双極性うつ病では反応率の差はみられず，病型にかかわらずVNSは難治性うつ病に有効とはいえない．

i. 脳深部刺激療法 deep brain stimulation (DBS)（国内未承認）

4種類以上の抗うつ薬に難治性の非精神病性大うつ病6例(5例ではECTにも難治性)に対して膝下野(Brodmann 25野，subgenual cingulate)に電極を置き，高頻度電気刺激を慢性的に加えたところ，治療開始2カ月後に6例中5例で反応がみられ，治療開始6カ月後では6例中4例が反応し，3例で寛解が得られた[101]．Mayberg らは難治性うつ病では膝下野の高活動が認められ(非難治性大うつ病の研究では低活動が報告されているが[73])，DBSによりこの部位の活動が低下し，同時に他の脳部位の活動が刺激されることが，抗うつ効果と関連することを示唆している[101]．

j. lamotrigine 併用

Stage 2の難治性非精神病性大うつ病症例へのlamotrigine併用の有効性がオープン試験で報告された[102]．本章-2で紹介したように，現在わが国で抗てんかん薬として申請中のlamotrigineは双極性うつ病に有効性であることはエビデンスとなっているが[26]，難治性大うつ病への効果も期待できる．

k. 精神療法

大うつ病の治療において精神療法は必須であり，これまで紹介した種々の薬物・身体療法は精神療法と併用して行われるべきものである．小精神療法[3]の他，認知行動療法，対人関係療法などが行われている他に，大うつ病について深く理解することを助ける心理教育も精神療法的である．多くの二重盲検比較試験では偽薬に対する大うつ病の反応率が26～49%[103](Janicak, 2001)と報告されているが，このことは単に偽薬効果を意味するだけでなく，精神科医による関わりが薬物療法なしでも治療的に有効であることを示していると筆者らは考える．

難治性大うつ病の薬物・心理的治療についてのStimpsonらによる系統的レビューは，難治性大うつ病に対する精神療法の効果についての十分なRCTはないと評価した[43]．その後，Stimpsonらが指摘した限界を踏まえたうえで，McPhersonらは組み入れ基準をゆるめて12の試験(ほとんどが認知行動療法．うち4つが比較試験)についての系統的レビューを発表した[104]．多くの研究はStage 1の大うつ病を対象としているが，難治性うつ病の定義が十分ではないこと，臨床試験としての質の高いものがないことなどが指摘され，やはり難治性うつ病に対する精神療法の効果に関するエビデンスが欠如していることが明らかになった．今後，質の高いRCTによって精神療法の効果が難治性うつ病で検討されることが必要である．

8 難治性双極性うつ病に対する治療

本章-2でも紹介したが，双極性うつ病の治療ガイドラインはまだ流動的であり，双極性うつ病の第一選択薬として非定型抗精神病薬が注目される一方，抗うつ薬の有効性については疑問視する意見もある[21]．双極性うつ病に対する第一選択薬

のラインアップが確立されないかぎりは，難治性双極性うつ病の定義も完成されないであろう．定義が確立されていないということは，その治療に関する研究もまだ十分にされていない，あるいはできないということを意味する．

以上のような現状であるが，難治性双極性うつ病の治療に関する少数の研究が発表されている．気分安定薬と1種類の抗うつ薬併用による十分な治療に反応しない難治性双極性うつ病に対して，オープン試験でアリピプラゾール併用(全例で気分安定薬に併用．一部症例で抗うつ薬・非定型抗精神病薬も併用)の有効性が報告された[25]．同様の定義の難治性双極性うつ病に対するlamotrigineとリスペリドン，inositolのオープン試験(3群あるいは任意の2群に無作為割り付けのため，一次的解析は2群間の比較となる)が気分安定薬・抗うつ薬に併用して最近行われた[24]．回復率(完全寛解に相当)に統計学的に有意な差はみられなかったが，lamotrigine群でより症状改善する傾向がみられた[24]．

1種類の抗うつ薬に非反応(大うつ病の分類ではStage 1に該当)の双極II型障害のうつ病患者(リチウムかバルプロ酸を服用中)に対してプラミペキソール(最終与薬量0.375～4.5 mg/日．平均1.7 mg/日)が有効であることが偽薬との二重盲検比較試験で報告された(反応率はプラミペキソール群60%，偽薬群9%)[105]．同じ年に，別のグループも難治性双極性うつ病(定義は気分安定薬に併用して2種類以上の抗うつ薬による十分な治療に非反応であること．約2/3は双極I型)に対して，気分安定薬に併用してプラミペキソール(平均最高用量1.7 mg/日)と偽薬の二重盲検比較試験を行い，プラミペキソールの有効性(反応率はプラミペキソール群67%，偽薬群20%)を報告した[23]．

本章-5で紹介したように，難治性大うつ病の一部は偽性単極性うつ病であると筆者らは報告した[55]．躁・軽躁病相が出現するまで偽性単極性うつ病(潜在性双極性うつ病)症例は，双極性うつ病の治療ガイドラインとはまったく異なる大うつ病の治療ガイドラインに準じた治療を受けることになり，当然治療抵抗性(むしろ偽性治療抵抗性というべきかもしれない)となりうる可能性がある．したがって，難治性の潜在性双極性うつ病を疑う症例では，双極性うつ病の治療ガイドラインに準じて治療することが，難治性の解決につながると思われる．偽性単極性うつ病(潜在性双極性うつ病)と大うつ病の鑑別が，症状レベル(困難と思われるが)あるいは生物学的マーカーによって可能になるまでは，この問題は常に一定の割合(非難治性で12.5%[14]，難治性で29%[55])で起こりうることを常に認識すべきである．

9 予後

a. 筆者らの長期予後研究：難治性うつ病は寛解するか？

これまでStage 2の難治性うつ病の研究は少なかったが，最近徐々にStage 2の難治性うつ病の治療に関する大規模な比較試験が発表されてきている[93, 94, 100]．一方，Stage 2の難治性うつ病の長期予後についてはまったく報告されてこなかった．「そもそも難治性うつ病のうつ病相は寛解するのだろうか？　寛解するとしたらいつ，どのような治療で寛解するのか？」という臨床的疑問に答えるために，筆者らは1995年に調査した難治性うつ病症例[11]のうち，1年以上経過を追うことができた26症例(双極性うつ病5例，大うつ病21例)を最長7年間当科で診療を継続し，診断・重症度・薬物療法について長期転帰調査を行い報告した[42]．その後観察期間をさらに11年間まで延長して，診断・重症度・寛解に寄与した薬物療法の他，病型別の各種増強治療の効果，発症時の病相，遺伝負因について報告した[55]．

長期経過観察期間中に，観察開始時の単極性うつ病21例のうち5例(28.6%)が双極性障害に移行したが，気分障害以外の病名に変更となる症例はなかった[55]．1995年の調査開始時の大うつ病エピソードは，1995年の調査時点で既に平均5.1年と慢性に続いていた[11]．うつ病相が自然に寛解することはKraepelinの教科書[15]にも詳細に記載されているが，Kraepelinは14年間うつ病が続

いた症例も紹介しており[15]，実際に難治性うつ病が寛解するかどうかを確認することは重要である．1995年調査時の難治性うつ病相のうち，完全寛解(症状が消失し，機能も回復した状態)した症例を調査したところ，最終診断が大うつ病の症例では15例中9例が完全寛解となった(残り6例は最終観察時は軽症であった)[55]．一方，最終診断が双極性うつ病の症例では，11例中9例が完全寛解となった(残りの症例は最終観察時に軽症1例，中等症1例であった)．寛解までのうつ病相期間は大うつ病で平均5.6年，双極性うつ病で平均4.8年であった．

b. 筆者らの長期予後研究：難治性うつ病の再発と治療薬

筆者らの研究では，大うつ病の寛解例9例中4例(3例は患者自身の判断で中断)，双極性うつ病の寛解例9例中6例(5例は患者自身の判断で中断)は薬物治療中止となったが，薬物療法中止後経過を十分に追跡できた症例では大うつ病で2例，双極性で4例再発した[55]．再発率が高率であることから，両病型において薬物療法は寛解後も長期に継続すべきであると思われる．1995年調査時点の難治性うつ病相の寛解に寄与したと思われる治療を調査したところ，寛解した大うつ病9例全例でドパミンアゴニストが有効であり，1例でリチウム併用も有効であった．寛解した双極性うつ病9例においても，6例でドパミンアゴニストが，4例でリチウムが，2例で甲状腺ホルモン(L-サイロキシン)が有効であり，寛解に寄与したと考えられた[55]．自然史的な観察で薬剤の効果を評価したため，自然寛解との区別をできない点が本研究の限界点であるが，これらの結果を今後RCTで確認する必要がある

c. Dunnerらの長期予後研究

筆者らの報告と同時期に，Dunnerらが難治性の大うつ病あるいは双極性うつ病患者124人を2年間経過観察し，報告した[16]．彼らの報告によると，種々の作用機序の抗うつ薬や気分安定薬，抗精神病薬，リチウム，ホルモン，ECTなどのtreatment as usual(従来型の経験論的な治療)を行っても持続的な寛解に至る症例はほとんどなく，観察開始1年後に寛解していた患者は112例中4例であり，そのうち2年後まで寛解が持続した症例はわずか1例であった[16]．彼らの報告を読むと失望するかもしれないが，筆者らの追跡調査と同様により長期に観察を続けることによって，あるいは，彼らが試みていない治療法の導入によって，今後もっと高い寛解率が得られると期待する．

本稿では実際の症例を呈示しなかったが，個々の難治性うつ病症例は実に多様である．すべてのうつ病患者が回復することがわれわれ精神科医の目標であるが，なかなか目標に達しないことが多い．そのような時に，まず鑑別診断・併存疾患・性格要因を再検討しなくてはならないし，本稿で指摘したように偽性単極性うつ病の可能性も考慮に入れる必要がある．大うつ病という診断が間違いないとしても，心理社会的要因が大きくて，環境調整や精神療法的関わりの工夫が功を奏する場合もある．さらに，難治性かなと思っても，他の抗うつ薬への変更で劇的に回復する症例もしばしばみられる．診断から治療まで実に多くのハードルがあり，そのハードルを1つ1つ確実にクリアしていくことが難治性うつ病の診療では肝要である．そして，たくさんあるハードルの存在を常に忘れないことと，そのハードルの乗り越え方を知っていることが，ゴールに到達する条件でもある．このようなハードルにたとえられる診断・治療上の多くの難治化要因を科学的に明らかにし，論文として発表し，精神医学の知識として蓄積することはわれわれの務めである．多数の難治性うつ病研究と称する研究はあるが，真に難治性うつ病(Stage 2以上)を対象とした研究は数えるほどしかないのが現状であり，難治性うつ病を十分に定義するだけで多くの研究が可能となる．今後，Stage 2以上(可能ならばStage 5)の難治性うつ病にできるだけ焦点を絞って，多角的に研究成果を蓄積していくことが求められている．

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(井上 猛・小山 司)

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

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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.**

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

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(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 ($P = 0.096$).

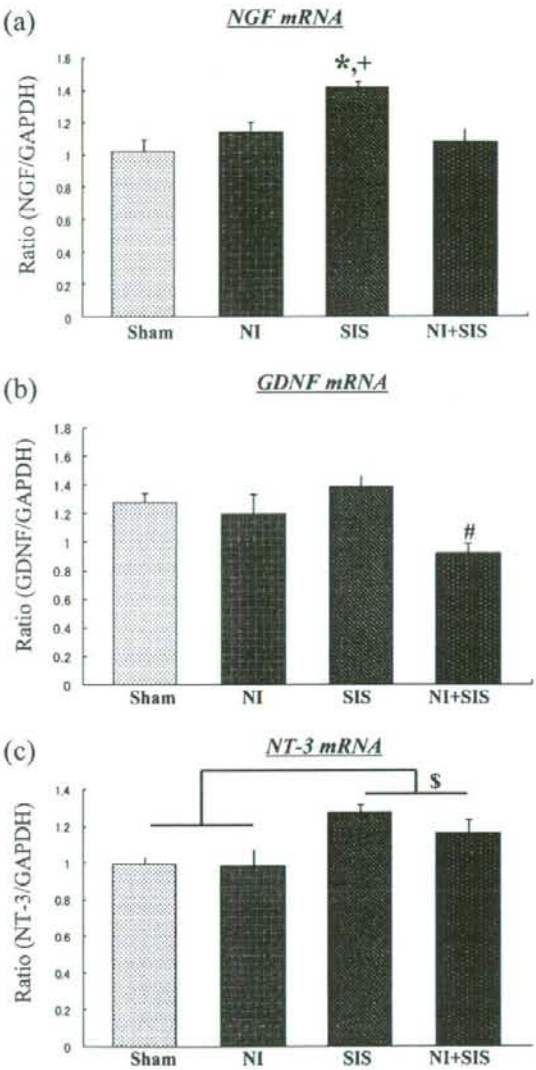

Fig. 1. Expression of (a) NGF, (b) GDNF, and (c) NT-3 mRNA in the hippocampus of juvenile rats subjected to sham treatment (Sham), neonatal isolation alone (NI), single immobilization stress alone (SIS), and neonatal isolation followed by a single immobilization stress (NI + SIS). Results are expressed 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).

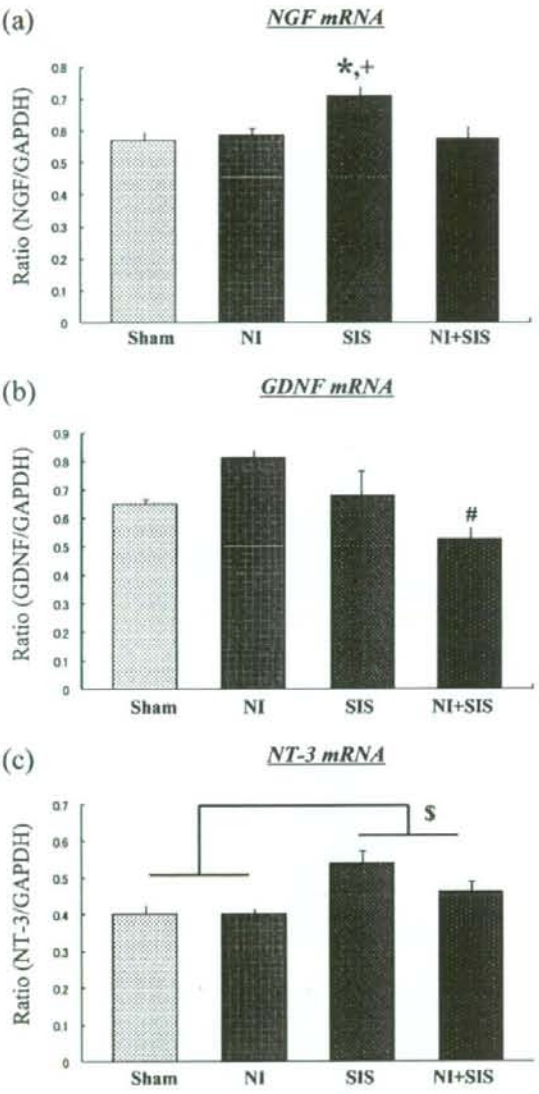

Fig. 2. Expression of (a) NGF, (b) GDNF, and (c) NT-3 mRNA in the hippocampus of adult rats subjected to sham treatment (Sham), neonatal isolation alone (NI), single immobilization stress alone (SIS), and neonatal isolation followed by a single immobilization stress (NI + SIS). Results are expressed 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

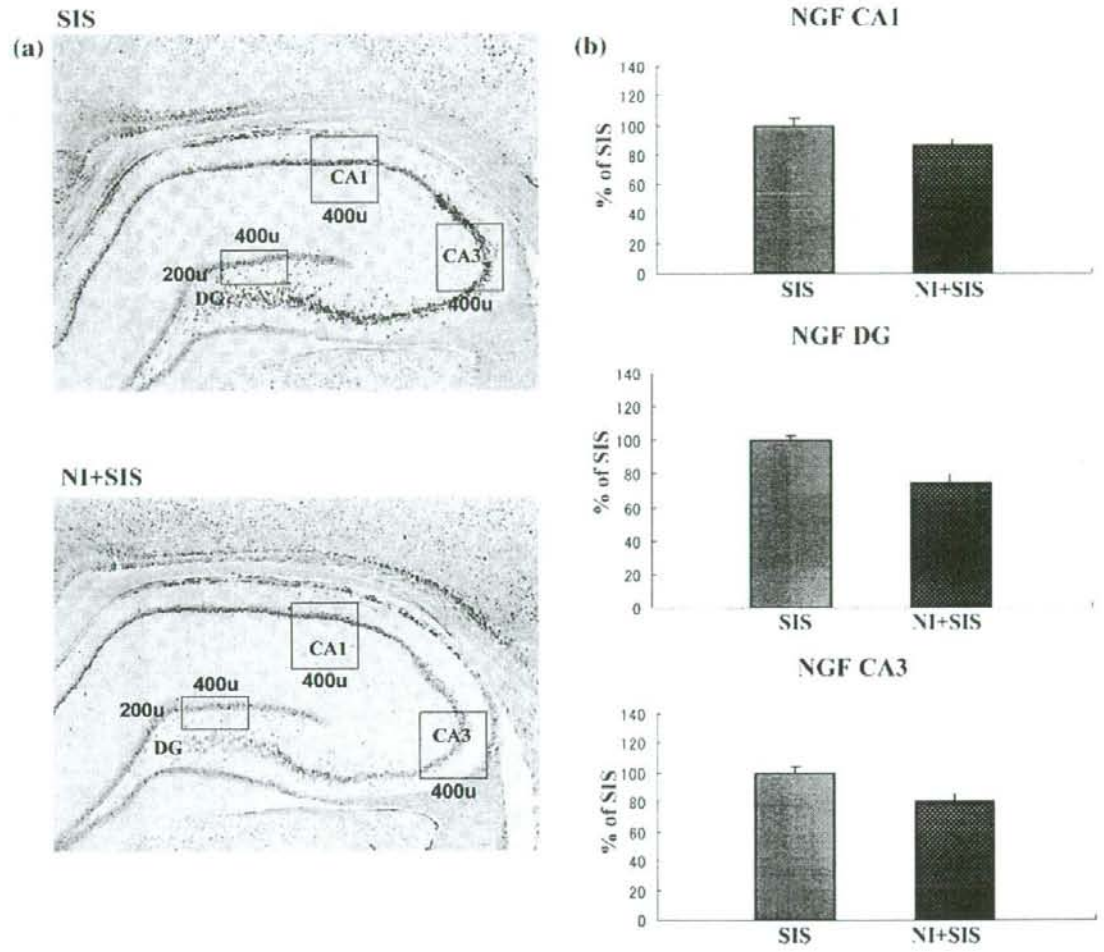

Fig. 3. Analysis of NGF mRNA expression by *in situ* hybridization in the adult rat hippocampus. (**a**) NGF mRNA expression in the rat hippocampus. SIS, single immobilization stress, NI + SIS, neonatal isolation followed by a single immobilization stress. (**b**) Mean density of NGF mRNA in the CA1 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

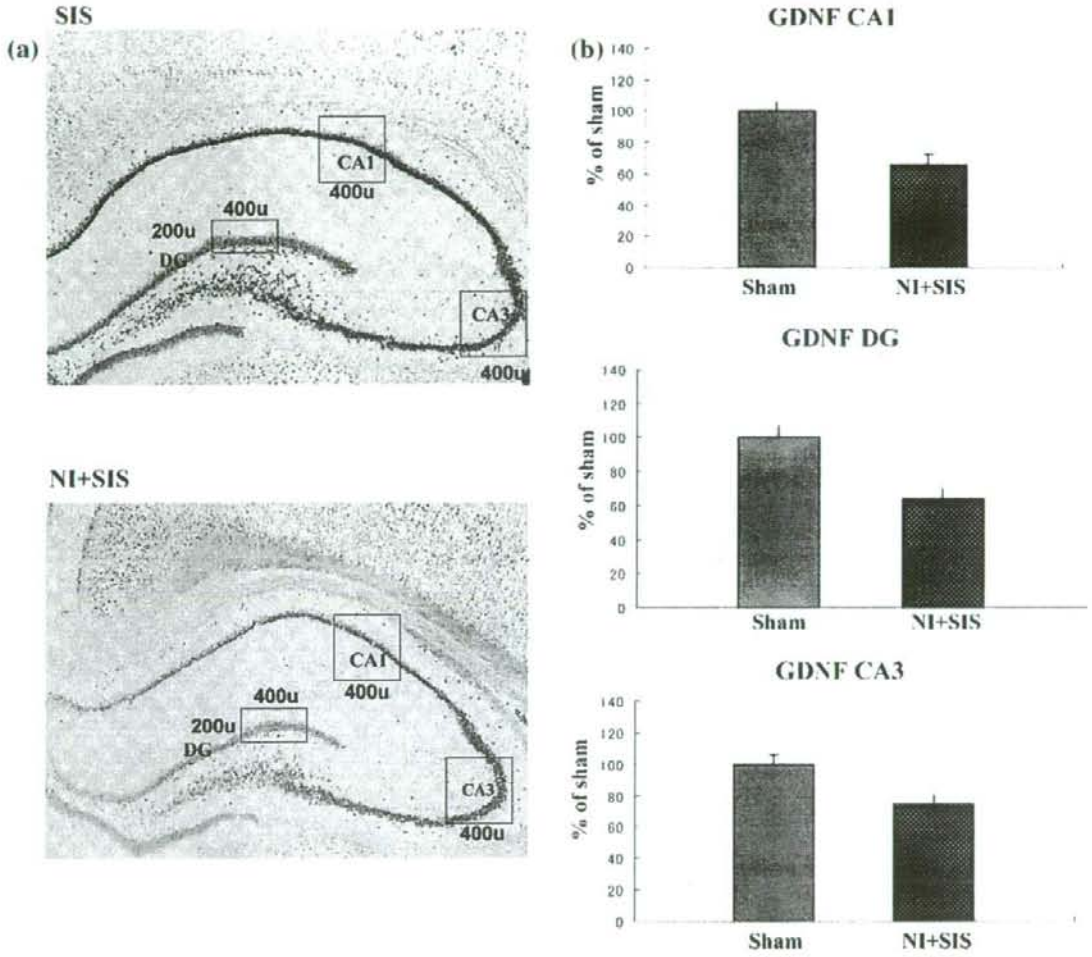

Fig. 4. Analysis of GDNF mRNA expression by in situ hybridization in the adult rat hippocampus. (**a**) GDNF mRNA expression in the rat hippocampus. NI, neonatal isolation; NI + SIS, neonatal isolation followed by a single immobilization stress. (**b**) Mean density of GDNF mRNA in the CA1 and CA3 pyramidal 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.

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Regular Article

'Insistence on recovery' as a positive prognostic factor in Japanese stroke patients

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Aim: The present study used two-step analyses to examine the effect of acceptance of disability or 'insistence on recovery' in Japanese stroke patients: first on their functional improvement and second, on their psychological symptoms.

Methods: Disability was assessed using functional independence measurements (FIM), examining the stage of acceptance of disability by observation using Fink's theory (from shock to defensive retreat, acknowledgement, and acceptance/change stage), and estimation of insistence on recovery (on a scale of 1–4) by observation. The differences over time and the effects on the improvement in their FIM were then assessed. Depression was measured using the Zung Self-rating Depression Scale (SDS); apathy was measured using the Apathy Scale (AS), and the correlation with the acceptance stage or insistence on recovery was analyzed.

Results: The acceptance stage and functional improvement progressed significantly, but insistence on recovery did not change significantly during hospitalization. Multiple regression indicated that the insistence on recovery score (but not the acceptance stage) was a good predictor of the degree of improvement in FIM (FIM gain per week) in the elderly group. Post-hoc testing showed that the SDS or AS score decreased from the first stage to the fourth stage (but increased at the third stage) of acceptance; whereas for insistence on recovery score, the SDS and AS scores decreased as insistence on recovery score changed from 1 to 3, and then increased as insistence on recovery score changed from 3 to 4.

Conclusions: The appropriate level of insistence on recovery reduced depression and apathy, resulting in enhanced improvement of disability after a stroke in elderly stroke patients.

Key words: acceptance of disability, elderly, functional independence measurement, insistence on recovery, stroke.

STROKE HAS BEEN described as a condition with a unique epidemiological profile, with high incidence and mortality rates, and in which a large proportion of survivors have significant but varying degrees of residual disability.[1,2] There are two types of improvement that occur after a stroke: neurological improvement and improvement in functional abilities or performance.[1] Neurological recovery depends upon the mechanism of the stroke and the location of the lesion. By contrast, the improvement of functional abilities, such as activities of daily living (ADL), depends upon the environment in which the stroke patient is placed and how much training and motivation there is for the patient to learn to become independent again in terms of self-care and mobility. The ability to perform ADL can improve through

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acceptance and training in the presence or absence of natural neurological recovery.[1–4] Stroke rehabilitation must therefore restore optimal physical, psychological, social, and vocational function to enable the patient to become a productive participant in the community.

Emotional responses to stroke have traditionally been thought to follow a natural course of evolution: an initial state of significant distress or depression that, over time and as a result of some active process of 'working through', resolves to a condition of acceptance and relative emotional harmony.[5,6] This process was thought to be a mourning process or acceptance of disability. Disabled stroke patients who refuse to accept their impairment, eventually realize they are sick but continue to think that they will soon get well.[5,6] They manifested this attitude through direct verbal report or inferred from behavior continuously and repeatedly (this attitude was similar to whining, and we termed this emotional status as 'insistence on recovery'). Insistence on recovery is thought to be a sign of denial and thus is regarded as an irrational belief. Denial is generally found in psychiatric illness, but it is also found in the mourning process among disabled stroke patients.[6] It was traditionally thought that patients with insistence on recovery, whose only goal is recovery, can be motivated to do any work perceived as aiding recovery; but are not motivated to learn to function as a disabled person, and so fail to gain the maximum benefit from rehabilitation services.[5–8] Thus insistence on recovery is considered a maladaptive status and is thought to be a target for psychotherapeutic intervention.[5–8] We have, however, experienced many stroke disabled patients who say repeatedly that they will recover someday, and who participate in rehabilitation and gain independence in ADL in the rehabilitation service. The question arises whether insistence on recovery is an irrational or rational belief. Although there is much popular and professional literature attesting to the veracity of stages of acceptance of disability, the empirical data do not support such a contention.[5] Moreover, insistence on recovery is regarded as counterproductive to maximizing functional abilities and enhancing quality of life,[5–8] and no previous paper has examined the nature of 'insistence on recovery'.

The aim of the present study was to evaluate the effect of the stage of acceptance of disability or insistence on recovery on functional independence in stroke patients. Aging is associated with a high incidence of physical impairment, functional disability and depression.[9,10] Therefore, we divided stroke inpatients into two age groups: middle-aged and elderly. Moreover, depression and apathy are common neuropsychiatric consequences of a stroke, and can be examined using self-reported tests: the Zung Self-rating Depression Scale (SDS) and the Apathy Scale (AS). To clarify the psychopathological aspects of insistence on recovery after a stroke, we also examined the correlation between insistence on recovery score and the SDS or AS score.

METHODS

Study design

This study consisted of two parts: first, the changes in 'acceptance of disability' and 'insistence on recovery' over time were estimated, and we examined the effect of these factors on functional recovery after a stroke. Second, we examined the psychopathological symptoms (depression and apathy) using self-reporting scales, and estimated the association between depression and 'acceptance of disability' or 'insistence on recovery' after a stroke.

Patients

The approval of institutional ethics committees was obtained for this prospective study. Informed consent for functional or psychological measurements, including acceptance of disability, was obtained on admission from all patients or from those authorized to give consent on their behalf. The subjects for the first study were 231 patients with hemorrhagic and occlusive stroke without subarachnoid hemorrhage, diagnosed on computed tomography (CT) and/or magnetic resonance imaging (MRI), who were admitted to the Nishi-Hiroshima Rehabilitation Hospital <3 months after suffering a stroke (range, 10–109 days; mean, 44 ± 21 days), and who were hospitalized for more than 1 month. The patients were divided into two age groups at admission: the middle-aged group (95 patients, age 40–65 years); and the elderly group (136 patients, aged ≥66 years).

For the second study the subjects, who completed the SDS and AS scales, included 237 patients after excluding patients with (i) a history of major psychiatric illness, such as major depression, bipolar disorder, schizophrenia, or schizoaffective disorder; (ii) subarachnoid hemorrhage; (iii) emergency discharge