＜精神科神経科＞

①総合病院の無床精神科におけるコンサルテーション・リエゾン活動の現況

②古庄立弥

③岩本泰行

④広島医学

⑤Vol.60：P442-446、2007

総合病院の無床精神科における コンサルテーション・リエゾン活動の現況

古庄　立弥・岩本　泰行

Ⅰ．緒　　言

マツダ㈱マツダ病院（当院）は広島市近郊の広島県安芸郡府中町に位置し，昭和13年開設の総合病院である。開設以降昭和36年と昭和60年に増床し，現在は診療科数19科，病床数300床で，地域の二次医療圏に対応する職員数約350名の臨床研修指定病院である。近隣に700床規模の大学病院および急性期病院が4施設，当院と同規模の急性期病院が3施設，精神科を主体とした病院（精神科病院）5施設が位置している。精神科神経科（当科）は総合病院内に精神保健福祉法に定められた病床を有さない無床の総合病院精神科である。常勤医2名，非常勤看護師1名，非常勤臨床心理士1名で診療をおこなっている。

総合病院精神医学の実情に関して，近年までに数多くの施設から報告されており，平成15年には日本総合病院精神医学会より施設構造や診療体制などに関する報告[1]がなされている。しかし，無床の総合病院精神科に関する報告は少なく[2)-4)]，個々の活動や診療体制，今後の方向性についての資料は多くない。

今回われわれは，当院における総合病院精神科の重要な役割の一つであるコンサルテーション・リエゾン（援助と連携：以下CLと略す）活動について調査し，その状況を当院の地域性や病院としての特色も踏まえて若干の考察を含めて報告したい。

Ⅱ．対象と方法

平成18年1月より同年12月までの1年間に，当院他科入院中に当科に診療依頼のあった患者を対象とし，患者数，性別，年齢，主訴・診療依頼理由，依頼診療科，精神科診断，転帰について，受診記録及び当科診療録より後方視的に調査した。身体疾患で複数回入院した場合があるため，患者数は診療依頼延べ数を採用した。精神科診断については，国際疾病分類（ICD-10）を用いて分類した。診断が二つ以上併存する場合は，本人の問題を構成する主だった障害の方を採用した。

各診療科で疾患特性や期間中の入院患者数が異なるため，これらについても調査し，入院患者数に対するCL対象患者の割合についても検討した。

Ⅲ．結　　果

1．患者数と性別，年齢

当科に診療依頼のあった患者数は延べ285名で男性145名，女性140名であった。平均年齢は74.4±16.7歳であり，70歳以上が全体の約68％，85歳以上が全体の約31％を占めていた。調査期間中に20歳未満の患者はいなかった。なお，調査期間中の全入院患者の平均年齢は59.3±22.5歳であった。

2．依頼元診療科別患者数とその割合，各診療科別入院患者数と依頼割合

依頼元診療科は整形外科85名（29.8％），循環器内科44名（15.4％），消化器内科41名（14.4％），その他の内科41名（14.4％），外科28名（9.8％），泌尿器科27名（9.5％），脳神経外科11名（3.9％），その他8名（2.9％）であった（図1）。

各診療科の期間中の入院患者数は整形外科494名，循環器内科369名，消化器内科463名，その他の内科227名，外科530名，泌尿器科345名，脳神経外科391名，その他482名であった。入院患者数に対する依頼割合は全入院患者の7.6％で，診療科別では整形外科17.2％，循環器内科11.9％，消化器内科8.9％，その他の内科18.1％，外科5.3％，泌尿器科7.8％，脳神経外科2.8％，その他0.9％であった。患者数・依頼割合とも整形外科からの依頼が多かった（図2）。

3．主訴・診療依頼理由

当科への診療依頼理由については，先行研究での分類を参考に図3のような分類にまとめた。院内紹

Tatsuya Furusho, Yasuyuki Iwamoto: Current status of consultation-liason service in general hospital psychiatry without a ward. Department of Psychiatry, Mazda Motor Corporation Mazda Hospital.
マツダ㈱マツダ病院精神科神経科

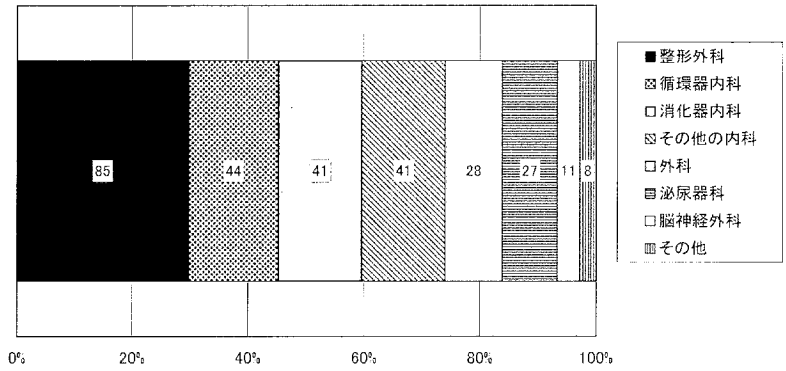

図1　紹介患者数とその割合

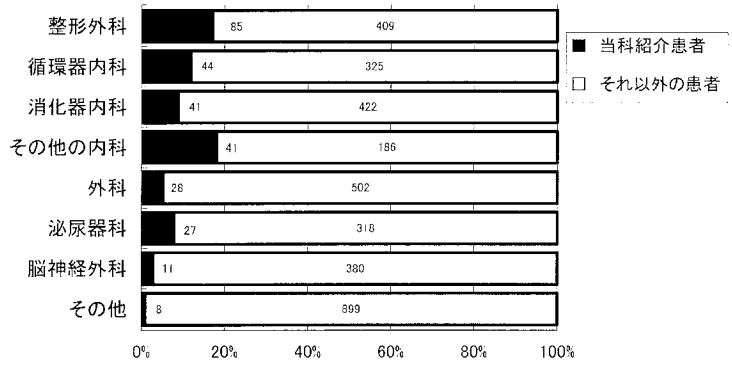

図2　入院患者数に対する依頼割合

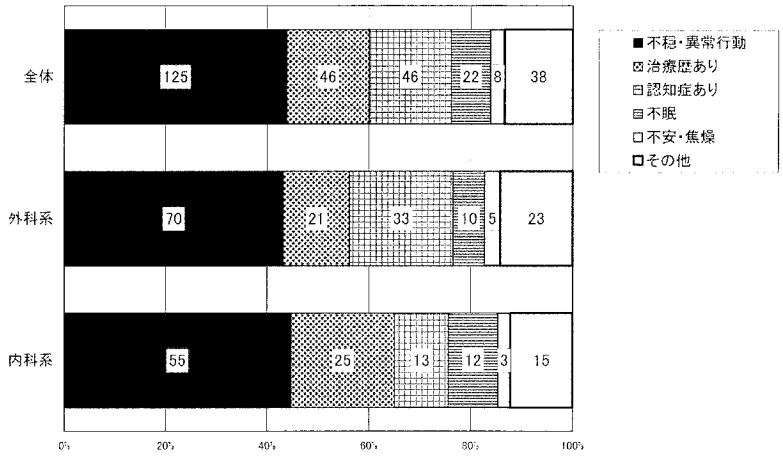

図3　主訴・依頼理由

介状に複数の依頼理由が記述されている場合は，最大の依頼理由と思われる項目に分類した。身体科主治医の依頼理由は「不穏・異常行動」が125名（43.9%）と最多で，「精神科治療歴あり」が46名（16.1%），「明らかな認知症あり」が46名（16.1%），「不眠」が22名（7.7%）と続いた。

内科系診療科患者では，「治療歴あり」の割合が多く（20.3%），「認知症あり」の割合が少なかった（10.6%）。逆に外科系診療科患者では「治療歴あり」の割合が少なく（13.0%），「認知症あり」の割合が多かった（20.4%）。

4.　精神科診断

診断分類はICD-10を用いた（図4）。診断コードは主な症状に対してのものを採用している。精神医学的診断は，F0（症状性を含む器質性精神障害，以下器質性精神障害と略す）が285名中197名（69.1%）で最も多く，F4（神経症性障害，ストレス関連障害および身体表現性障害，以下神経症性障害と略す）が34名（11.9%），F2（統合失調症，統合失調型障害および妄想性障害，以下統合失調症圏と略

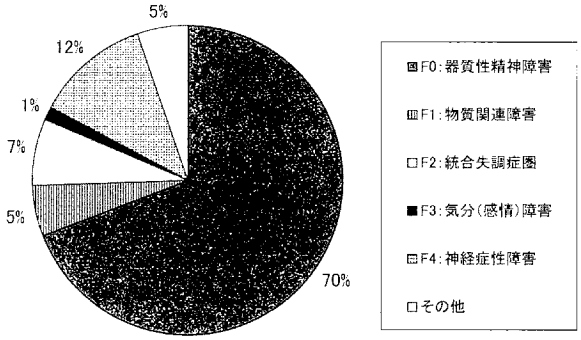

図4　精神医学的診断（ICD-10）

す）が20名（7%），F1（精神作用物質使用による精神および行動の障害，以下物質関連障害と略す）が15名（5.7%）と続いた。F3（気分（感情）障害）は4名（1.4%）であった。

5.　転　帰

当院での身体治療終了時の転帰は，「当科終診」172名（60.4%），「当科外来通院」54名（18.9%），「当科への転科入院継続」1名（0.4%），「他院精神科への紹介」23名（8.1%），「死亡退院を含めて何らかの要因で治療中断」35名（12.3%）であった（図5）。器質性精神障害（F0）に分類された197名のみの転帰では，「当科終診」142名（72.1%），「当科外来通院」24名（12.2%），「他院精神科への紹介」4名（2%），「死亡退院を含めて何らかの要因で治療中断」27名（13.7%）であった。器質性精神障害（F0）以外に分類された患者88名では，「当科終診」30名（34.1%），「当科外来通院」30名（34.1%），「他院精神科への紹介」19名（21.6%），「死亡退院を含めて何らかの要因で治療中断」8名（9.1%）であった。

Ⅳ．考　　察

日本総合病院精神医学会の報告[1]によると，無床の総合病院精神科の診療体制に関する調査項目では常勤医師数1名，精神保健指定医数1名，臨床経験7年以上，専門医・認定医数1名が最も多かった。コメディカルについては，ソーシャルワーカーが常勤，精神保健福祉士が不在，臨床心理士が常勤，精神科作業療法士不在が最も多い。治療構造については，緩和ケアチームなし，精神科救急に未対応が最も多かった。CL患者についての報告では器質性精神障害，気分（感情）障害，神経症性障害の患者が多かった。

当科の診療体制は常勤医が2名で臨床心理士が非常勤である以外は上述の調査項目で最も多い項目に属している。当科は常勤医が2名であることで，他の無床総合病院精神科と比べて診療の幅は広がると思われる。その他の項目は概ね標準的な無床総合病院精神科に属していると考える。

当院のCL患者の年齢構成は非常に特徴的である。平均年齢は74.4±16.7歳であり，調査期間中の全入院患者の平均年齢の59.3±22.5歳と比較して高い。70歳以上が全体の約68%，85歳以上が約31%を占めていた。病院規模は異なるが，他院の年齢構成[8]では60歳以上が全体の約58%，70歳以上が約35%であり，当院のCL患者の年齢は比較的に高いと考えられる。また，小児科の診療体制が常勤から非常勤に移行し，調査期間中に小児科入院患者が不在であったことが，20歳未満のCL患者がいない主な要因と思われる。

当科への紹介率は全入院患者数の7.6%であり，他の総合病院での報告[2),3),5),6)]では精神科への紹介

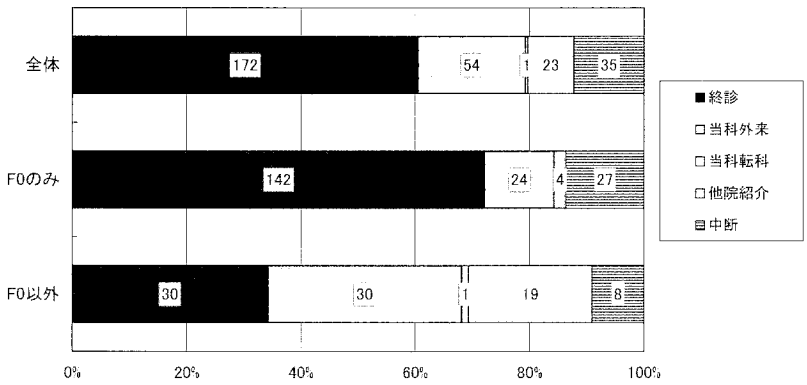

図5　身体治療終了時の転帰

率が全入院患者数の1.5％程度であったことを考慮すると，比較的に高い率といえる。患者数・紹介率とも整形外科からの依頼が多かった。この点については，認知症を有した高齢者が転倒・骨折で入院することが多く，術前の牽引などで拘禁反応やせん妄を呈しやすい状態という認識が整形外科医・病棟スタッフに浸透していることが理由として考えられた。

内科系診療科からの紹介も多く（285名中123名），紹介率も全入院患者における紹介率を上回っている。内科系診療科からの紹介においても，「認知症を有した高齢者」という患者背景の特徴はあるが，後述の依頼理由や診断面では若干異なっていた。

身体科主治医の依頼理由は全体では「不穏・異常行動」が最多で43.9％，「精神科治療歴あり」16.1％，「明らかな認知症あり」16.1％，「不眠」7.7％と続いた。「明らかな認知症あり」は多くの報告では「不穏・異常行動」に含まれていると考えられるため，広い意味での「不穏・異常行動」は60％を占めている。内科系診療科と外科系診療科で「治療歴あり」と「認知症あり」が順位逆転を生じることは，認知症を有する患者では手術など身体的侵襲が強い場合にせん妄に発展するリスクが高く，せん妄が身体治療に影響を及ぼすという認識が，身体科主治医にある程度浸透しているのではないかと考える。内科系診療科からの依頼でめだつものは「アルコール歴あり」で全体では率は低いものの，内科系診療科よりの依頼理由の5.7％を占めていた。アルコール関連疾患での入院は消化器内科に多く，長期アルコール歴のある場合は，離脱防止も含めて当科に紹介されている。

精神医学的診断では，器質性精神障害が圧倒的に多く，神経症性障害，統合失調症圏，物質関連障害と続いていた。他の調査では，器質性精神障害，気分（感情）障害，神経症性障害が多い[2),3),5)-8)]とされていたが，当科のCL患者では気分（感情）障害が少ないことと統合失調症圏，物質関連障害が多いことが特徴的である。背景としては，近隣に精神科病院が多く，当院の身体科医が積極的に身体合併症治療をおこなっていることや，内科にアルコール関連疾患での入院もあることが挙げられる。精神科病院での治療歴のある患者では，当院入院早期に当科紹介となることが多いため，統合失調症圏の患者数が比較的に多いと思われる。気分（感情）障害の患者数が少ない要因としては，依頼理由として「抑うつ・意欲低下」「食欲低下」が少ないことや，器質性精神障害との併存例も存在することが挙げられる。また，当院が平均在院日数16.1日の急性期病院であることも，気分（感情）障害患者の紹介が少ない要因の一つかもしれない。なお，気分（感情）障害は併存例を併せても，CL患者285名中7名（2.5％）であった。一般に身体疾患の患者の精神疾患合併率は10～20％といわれているが，身体疾患の30～40％に抑うつが，10～40％にせん妄が合併しているとする文献もある[9)]。今回の調査結果と照らし合わせると，せん妄についてはある程度高い率で依頼されているが，気分（感情）障害や神経症性障害に分類される潜在患者が多く存在する可能性が高い。神経症性障害に分類される患者の診療依頼理由は「治療歴あり」「不安・焦燥」「不眠」の順に多く，「抑うつ・意欲低下」「食欲不振」などの気分（感情）障害を想起させる依頼理由は少なかった。

転帰は当院退院とともに精神科治療も終診する場合が多かった。一つの要因として，上述の診断との関連もあると思われる。診断で器質性精神障害（F0）が圧倒的に多く，特にせん妄（F05）は一過性であることが多く，身体状態の回復とともに症状改善するため，終診とできる割合が高いのではないかと考える。認知症（F00～F04）では，当院入院前にかかりつけ医により抗認知症薬の処方や介護保険などの対応がなされており，退院後は引き続きかかりつけ医が対応することが多い。また，当院が急性期病院であることから，自宅退院ではなく療養型病院への転院や老人保健施設への入所も多く，当科は一旦終診となる割合が高いと考えた。他院精神科への紹介となった患者のほとんどは，紹介元の通院・入院中であった医療機関への逆紹介であったが，一般病棟で管理困難な精神症状を呈したため，精神科病院へ転院した例もあった。器質性精神障害以外に分類された患者では，身体疾患治療終了後も当科外来通院と他院精神科紹介で精神科治療を継続した割合が55.7％であった。器質性精神障害以外に分類された88名の依頼理由で「治療歴あり」が多いことや，身体疾患への罹患と入院治療自体が神経症性障害を引き起こしやすい状況であり，退院後も治療継続を要することも精神科治療を継続した割合が高い一因と思われる。今後CL活動のさらなる充実により，気分（感情）障害や神経症性障害の潜在患者への介入が進めば，精神科治療を継続する患者数が増加すること

が予想される。

現在の当科のCL活動をまとめると，比較的に高い紹介率であるが，認知症を含む器質性精神障害患者が主であり，気分（感情）障害患者や神経症性障害患者の紹介は少なく，潜在患者は多く存在すると推測された。今後のCL活動の方向性・課題としては，①気分（感情）障害や神経症性障害の紹介率を上げるべく身体科医や病棟スタッフへの啓蒙，②認知症を含む器質性精神障害患者への対応の標準化・効率化が重要と考える。

現在の当科のCL活動は個別の症例に対してのコンサルテーションの域を脱しておらず，身体科医師や病棟スタッフとの当科紹介以前の相談や身体科医師や病棟スタッフ自身のメンタルヘルス相談など積極的なリエゾン活動は十分にはおこなえていない。渡辺らは，がん患者のCL活動について，4年間の推移の中で依頼患者数の増加と「興奮・異常行動」での依頼割合の減少，「不安・抑うつ」での依頼割合の増加を報告している[10]。また，この報告[10]では，軽症例への対応や各身体疾患の特徴に配慮したCL活動や一般身体科の教育にも力を入れたCL活動の重要性が述べられている。今後はリエゾン活動の充実が，当科のCL活動の質を向上させると考える。具体的には，定期的リエゾン回診や相談の開始を計画しており，この中で高齢者や認知症患者の不眠やせん妄についての啓蒙活動，気分（感情）障害や神経症性障害についての啓蒙活動をおこなう予定である。

リエゾン活動の充実により，院内全体として精神疾患への理解が進めば，器質性精神障害のみならず，気分（感情）障害や神経症性障害への治療的な介入がより多く可能になると予想される。また，精神科病院からの身体合併症治療目的の入院もより円滑におこなわれると考える。CL活動の充実と発展によって，当該診療科として患者を支える状況から，病院として患者をより全人的に支える状況になり，患者のQOLの向上に寄与できるのではないかと考える。

今後の取り組みの結果，状況変化については，まとまり次第報告したい。

V. 結　　語

マツダ㈱マツダ病院におけるコンサルテーション・リエゾン活動の現況について報告した。当科への診療依頼率は比較的に高いが，認知症を含む器質性精神障害患者が主であり，気分（感情）障害患者や神経症性障害患者の紹介は少なく，潜在患者は多く存在すると推測された。

現状では個別の症例に対してのコンサルテーションの域を脱しておらず，積極的なリエゾン活動は十分にはおこなえていない。今後は依頼前相談や身体科医師・病棟スタッフへの啓蒙活動などリエゾン活動の充実が重要と考えた。

文　　献

1) 日本総合病院精神医学会：2002年総合病院精神科基礎調査―施設構造や診療体制などに関する報告．総合病院精神医学 15: ss2-1–ss2-12, 2003.
2) 安藤英祐，山本賢司，伊賀富栄ほか：無床総合病院精神科における外来初診患者と入院患者の動向―海老名総合病院における現状調査から．精神科治療学 20(8): 855–861, 2005.
3) 大村慶子：他科入院中の患者に対するコンサルテーション・リエゾン活動―総合病院無床精神科の現状．精神科治療学 18: 721–725, 2003.
4) 東谷慶昭：無床総合病院精神科におけるコンサルテーション・リエゾン活動に対する評価（第1報）院内医師・看護婦へのアンケート調査から．総合病院精神医学 11: 131–137, 1999.
5) 西本　光，柴崎いづみ，藤田潔史ほか：大学病院におけるコンサルテーションの動向―藤田保健衛生大学病院での活動の検討を通して―．精神誌 101: 758–759, 1999.
6) 佐藤　雄，森本修充，石倉礼二ほか：大学病院におけるコンサルテーションの動向―九州大学病院での活動の検討を通して―．臨床精神医学 25: 1535–1542, 1996.
7) 河本　勝，柏瀬宏隆，後藤健文ほか：防衛医大病院におけるコンサルテーション・リエゾン精神医学の現状と課題．心身医 36: 356, 1996.
8) 山本育代，竹内　浩，古川壽亮：名古屋市立大学病院における精神科リエゾン診療のニーズ．総合病院精神医学 16: 287–294, 2004.
9) 保坂　隆：在院日数短縮化をめざして．星和書店，東京，2002; 1–12, 23–31.
10) 渡辺清美，岩本泰行，井上真一ほか：がん患者の精神科コンサルテーション・リエゾンに関する臨床的検討．広島医学 56: 475–478, 2003.

（受付　2007－4－17）

＜精神科神経科＞

①Single prolonged stress increases contextual freezing and the expression of glycine transporter 1 and vesicle-associated membrane protein 2 mRNA in the hippocampus of rats.

②Iwamoto Y

③Morinobu S*, Takahashi T*, Yamawaki S*

④Progress in Neuro-Psychopharmacology and Biological Psychiatry

⑤2007 Apr 13;31(3):642-51

Single prolonged stress increases contextual freezing and the expression of glycine transporter 1 and vesicle-associated membrane protein 2 mRNA in the hippocampus of rats

Yasuyuki Iwamoto, Shigeru Morinobu, Terumichi Takahashi, Shigeto Yamawaki *

Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima, 734-8551, Japan

Received 16 June 2006; received in revised form 15 December 2006; accepted 16 December 2006
Available online 28 December 2006

Abstract

Rats subjected to single prolonged stress (SPS) show enhanced HPA negative feedback, exaggerated acoustic startle response, and enhanced contextual freezing 7 days after SPS, and accordingly, SPS is an animal model of PTSD. To elucidate the influence of contextual fear on gene expression in the hippocampus of SPS rats, we used cDNA microarray followed by real-time quantitative PCR analyses to compare the hippocampal gene expression profiles between rats that were or were not subjected to SPS during exposure to contextual fear. In the behavioral experiments, spontaneous locomotor activity was measured 7 days after SPS. Twenty-four hours after footshock conditioning (7 days after SPS), freezing behavior was measured during re-exposure to the chamber in which footshock was delivered. Based on the behavioral analysis, rats subjected to SPS exhibited a significant enhancement of contextual freezing compared to rats not subjected to SPS, without any changes in locomotor activity. Analyses using cDNA microarray and RT-PCR showed that the hippocampal levels of glycine transporter 1 (Gly-T1) and vesicle-associated membrane protein 2 (VAMP2) mRNA in rats subjected to SPS were significantly increased relative to sham-treated rats. Administration of SPS alone did not affect the expression of these 2 genes. These findings suggest that the upregulation of Gly-T1 and VAMP2 in the hippocampus may be, at least in part, involved in the enhanced susceptibility to contextual fear in rats subjected to SPS.

Keywords: cDNA microarray; Contextual freezing; Glycine transporter 1; Hippocampus; Posttraumatic stress disorder; Single prolonged stress; Vesicle-associated membrane protein 2

1. Introduction

Posttraumatic stress disorder (PTSD), an anxiety disorder defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994), involves intrusive recollections of a severe traumatic event, avoidance of reminders of the event, and symptoms of hyperarousal following exposure to the event (Yehuda, 2002). One of the major symptoms of PTSD is hyperarousal and hypervigilance to trauma-related and more general aversive (trauma-unrelated) stimuli (Liberzon and Martis, 2006). For example, Orr et al. (2003) reported larger heart rate responses to sudden, loud tones (trauma-unrelated) in Vietnam combat veterans with PTSD. In addition, enhanced autonomic arousal responses such as increased skin conductance were reported to be induced by stressors unrelated to trauma (Pitman et al., 1999).

Several clinical neuroendocrinological studies of PTSD have helped to elucidate the pathogenesis of PTSD. One of the core neuroendocrine abnormalities of the disorder is dysfunction of the hypothalamo–pituitary–adrenal (HPA) axis, characterized by low levels of plasma cortisol, low levels of urinary cortisol, and enhanced suppression of cortisol in response to low-dose dexamethasone administration (Yehuda, 2001; Yehuda et al.,

Abbreviations: ANOVA, analysis of variance; CF, contextual fear paradigm; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; Gly-T1, glycine transporter 1; LIMK1, LIM kinase1; NMDA, *N*-methyl-D-asparate; PCR, polymerase chain reaction; PTSD, posttraumatic stress disorder; RCK4, potassium channel RCK4; SPS, single prolonged stress; VAMP2, vesicle-associated membrane protein 2.

* Corresponding author. Tel.: +81 82 257 5205; fax: +81 82 257 5209.
E-mail address: yamawaki@hiroshima-u.ac.jp (S. Yamawaki).

1993). These neuroendocrine findings specific to PTSD have served as the basis for animal models that are useful for elucidating the pathophysiology of PTSD. For instance, rats subjected to single prolonged stress (SPS) show enhanced negative feedback through the upregulation of glucocorticoid receptor mRNA and downregulation of mineralocorticoid receptor mRNA in the hippocampus 7 days after SPS but not 1 day after (Liberzon et al., 1999). Thus, it has been proposed that SPS is a good animal model of PTSD based on the time-dependent dysregulation of the HPA axis (Liberzon et al., 1997). Furthermore, SPS rats have been shown to exhibit an exaggerated acoustic startle response (Khan and Liberzon, 2004) and enhanced contextual freezing (Imanaka et al., 2006; Takahashi et al., 2006). These findings suggest that SPS causes enhanced sensitivity to stimuli, which resembles the trauma-related and -unrelated psychophysiological responses in patients with PTSD. However, the precise mechanism by which the contextual fear response is enhanced in SPS-exposed rats remains unknown.

It has been demonstrated that the rapid development of contextual memory is one of the major functions of the hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). Rats with hippocampal lesions were reported to fail in detecting changes in spatial configuration among objects (Save et al., 1992). Based on these findings, it is hypothesized that hippocampal dysfunction may be associated with enhanced contextual freezing in SPS rats, though hippocampal-based contextual memory is regulated by the prefrontal–amygdala neuronal circuit (Sotres-Bayon et al., 2006).

In this context, we extended our previous findings regarding the ability of SPS to increase responses such as anxiety and fear in rats in a contextual fear paradigm (CF). In the present study, we first replicated previous findings that rats subjected to SPS exhibit enhanced contextual freezing without any changes in the spontaneous locomotor activity 1 week after SPS. We then compared the hippocampal gene expression profiles between the rats that were or were not subjected to SPS in the CF, using a cDNA microarray (Atras Glass Array Rat 1.0 Microarray, Clontech) followed by real-time quantitative PCR (RT-PCR) analysis.

2. Methods

2.1. Animals

Male Sprague–Dawley rats (8 weeks old) were purchased from Charles River Japan (Yokohama, Japan). The animals were group-housed (3 per cage) and maintained on a 12-h light/dark cycle with food and water freely available. After a 2-week acclimatization period, the animals were randomly assigned to the sham-treated (controls not receiving SPS) group or SPS group. Experimental procedures are shown in Fig. 1. A total of 80 rats were used in the study and a different set of rats was used for each of the methods (i.e., contextual freezing, locomotor activity, cDNA microarray, and RT-PCR). All animal procedures were in strict accordance with the Hiroshima University School of Medicine Animal Care Committee Guiding Principles on Animal Experimentations in Research Facilities for Laboratory Animal Science, School of Medicine, Hiroshima University.

2.2. Single prolonged stress paradigm (SPS)

SPS was conducted according to the method of Liberzon and colleagues (1997, 1999) as follows. Rats were restrained for 2 h. Each rat was immobilized by placing it inside a disposable clear polyethylene rodent restraint cone (Harvard Apparatus, South Natick, MA, USA) with only the tail protruding. The large end of the cone was closed with tape at the base of the tail. The bag size was adjusted according to the size of the rat in order to achieve complete immobilization. A hole in the small end of the cone allowed the rats to breathe freely. They were then

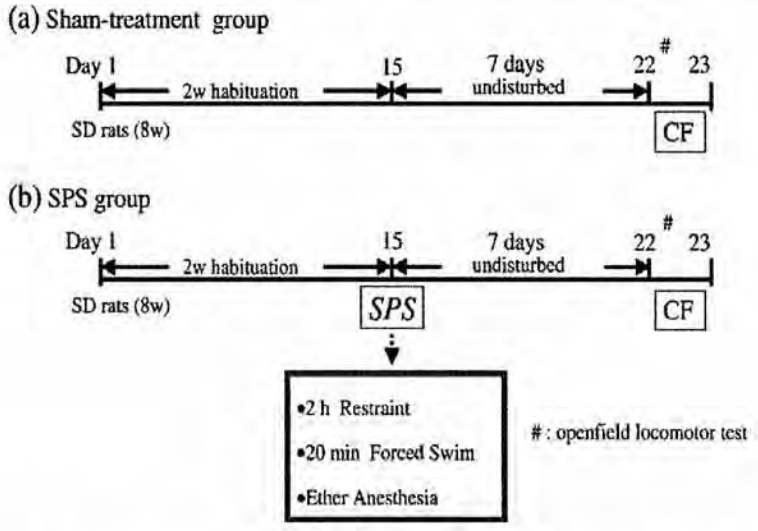

Fig. 1. Experimental paradigms. (a) Sham treatment; sham-treated rats (Sham) were undisturbed from day 16 to day 22 and were subjected to the contextual fear paradigm (CF) on days 22–23. (b) Single prolonged stress (SPS); rats were subjected to SPS on day 15 and CF on days 22–23. Both Sham and SPS rats were subjected to an open field locomotion test on day 22, 30 min before CF.

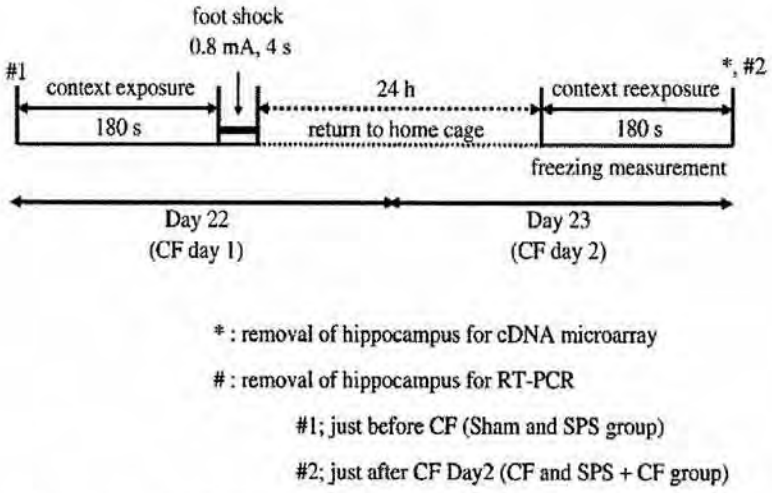

Fig. 2. Contextual fear paradigm (CF). On the first day (CF day 1), each rat was exposed to the conditioning context (180 s, in the conditioning chamber without any stimulation). Immediately after that, a footshock (0.8 mA, 4 s) was delivered through a stainless steel grid floor by a shock generator-scrambler. Twenty-four hours after the initial footshock (CF day 2), the rat was placed in the conditioning chamber where it received a footshock, and then contextual fear response was evaluated by measuring the duration of freezing behavior.

individually placed in a clear acrylic cylinder (240-mm diameter, 500-mm height), filled two-thirds from the bottom with water (24 °C) and forced to swim for 20 min. Following 15-min recuperation, animals were exposed to diethyl ether until loss of consciousness, and then left undisturbed in their home cage for 7 days. Sham-treated rats were left undisturbed in their home cage prior to the CF studies.

2.3. Contextual fear paradigm (CF)

The contextual fear paradigm (CF) was conducted 7 days after SPS. Rats were kept undisturbed for 7 days from the end of SPS to the beginning of conditioning. Experimental procedures are shown in Fig. 2. Only one subject at a time was present in the experimental room. On the first day, each rat was exposed to the conditioning context (180 s, in the conditioning chamber [325 W×280 H×500 D mm] without any stimulation). Immediately after that, a footshock (0.8 mA, 4 s) was delivered through a stainless steel grid floor by a shock generator-scrambler (SGS — 003: Muromachi, Tokyo, Japan). After footshock, the rat was immediately removed from its home cage. Twenty-four hours after the initial footshock, the rat was placed in the conditioning chamber where it had previously been footshocked, and the contextual fear response was then evaluated by measuring the duration of freezing behavior during a 180-s interval. Freezing was defined as a total absence of body or head movement except for that associated with breathing. Freezing behavior was recorded using a video recorder, and later scored blindly by the experimenter. Fear was quantified as the amount of time (in seconds) spent freezing.

2.4. Measurement of spontaneous locomotor activity

Seven days after SPS (on day 22), the level of spontaneous activity in a novel environment was measured for 5 min after each rat was placed individually in a clear cage (480 W×480 H×480 D mm) in a lighted room. The level of activity, measured by automatic actography (SCANET MV-10; Melquest, Toyama, Japan), was estimated as the number of interruptions of near infrared rays (photobeams in the animal chamber) by the animal's horizontal movements, and the counterinterface was connected to a personal computer.

2.5. cDNA microarray

For the microarray analysis, rats subjected to SPS and sham treatment were sacrificed by decapitation after completion of the measurement of CF on day 23 (Fig. 2). In this procedure, the lateral choroid plexus was carefully removed from the hippocampus. Total RNA from the hippocampus of 6 rats in each group subjected to the CF test (group subjected to SPS followed by CF [SPS+CF]; group subjected to CF alone [CF]) was isolated with an RNAqueous Phenol-free Total RNA Isolation Kit (Ambion, Austin, TX, USA) and pooled. The yield and purity of the total RNA were also assessed by A260 and by A260 to A280 ratio (CF group 1.81, SPS+CF group 1.74). After treatment with ANTI-RNAse (Ambion), 20 μg of the pooled total RNA was used for the synthesis of cDNA probes labeled by Cy3 (Amersham Pharmacia Biotech) with an Atlas Glass Fluorescent kit (Clontech, Palo Alto, CA, USA). The cDNA probes were denatured and then hybridized to Atlas Glass Array Rat 1.0 Microarray (Clontech), which contains 1081 genes. The fluorescent signal intensity of each gene was assessed using a GenePix fluorescent scanner (Fuji Film, Tokyo, Japan), and quantified with an ArrayGauge analysis system (Fuji Film). The revision among the array was normalized by global revision.

2.6. Real-time quantitative PCR (RT-PCR)

For RT-PCR to confirm cDNA microarray, 4 groups of rats (group subjected to SPS followed by CF [SPS+CF]; group

subjected to SPS alone [SPS]; group subjected to CF alone [CF]; sham treatment group [Sham]) were used. A different set of rats was used for each of the methods (i.e., cDNA microarray and RT-PCR). To examine whether the changes in hippocampal gene expression profiles, shown by microarray analysis, between the CF and SPS+CF groups were simply due to the effect of SPS, 2 groups of rats (group subjected to sham treatment [Sham]; group subjected to SPS [SPS]) on day 16 (24 h after SPS) were used. In this procedure, the lateral choroid plexus was carefully removed from the hippocampus. Total RNA was extracted from the hippocampus with an RNAqueous Phenol-free Total RNA Isolation Kit (Ambion). After treatment with RNase-free DNase I (Takara), single-stranded cDNA was synthesized using reverse transcriptase (Toyobo, Osaka, Japan). RT-PCR was performed with an ABI PRISM 7900 sequence detection system (PE Applied Biosystems, Foster City, CA, USA) to quantitate relative mRNA levels in samples. The primers and TaqMan hybridization probes were designed using Primer Express software (PE Applied Biosystems). 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 3′ end. PCR was carried out with TaqMan Universal PCR Master Mix (PE Applied Biosystems). All standards and samples were assayed in triplicate. Each plate contained the same standard. Thermal cycling was initiated with 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 the TaqMan Rodent GAPDH Control Reagents kit (PE Applied Biosystems).

It has been shown that the levels of GAPDH mRNA are not always constant, and the use of GAPDH as a valid reference for data normalization can be inappropriate for some experiments (Bustin, 2002). For this reason, we previously examined the effect of SPS+CF as well as acute restraint stress (ARS) for 2 h on the levels of GAPDH, 18s-ribosomal RNA (18s-rRNA) [TaqMan Ribosomal RNA Control Reagents kit (PE Applied Biosystems)] and β-glucuronidase (GUSB) (supplied by PE Applied Biosystems) in the rat hippocampus by RT-PCR.

The PCR assay for unknown samples was performed simultaneously with standard samples (rat hippocampus) to construct a standard curve. The relative concentrations of GAPDH and genes identified by cDNA microarray in unknown samples were calculated from this standard curve, and the ratios of the relative concentrations of these genes were calculated relative to the concentration of GAPDH.

2.7. Statistical analyses

The results of experiments containing 4 groups of rats were analyzed by two-way analysis of variance (ANOVA). One factor was SPS (non-SPS or SPS) and another factor was CF (non-CF or CF). Post hoc comparisons were performed using Tukey's test. The results of experiments examining the effect of stress on the mRNA levels of 3 housekeeping genes were analyzed by Kruskal–Wallis test. The results of experiments containing 2 groups of rats were analyzed by independent *t*-test. Significance was set at $P<0.05$.

3. Results

3.1. The effect of SPS on the contextual fear response

Exposure to SPS, followed by a 1-week undisturbed period, produced a significant increase in the contextual freezing response compared to sham treatment ($N=8$; Sham: 83.8±8.8 s, SPS: 125.1±6.2 s, $t=-3.857$; $df=14$; $P=0.0017$; Fig. 3a). There was no significant difference in the duration of freezing behavior between the Sham and SPS groups at baseline (during a 180-s interval prior to shock on day 22) ($N=8$; Sham: 1.43±0.80 s, SPS: 1.03±0.68 s, $t=0.382$; $df=14$; $P=0.708$).

3.2. Spontaneous locomotor activity

In order to exclude the possibility that SPS influenced spontaneous locomotor activity, we measured the level of spontaneous locomotor activity of the sham treatment and SPS groups. Independent *t*-test revealed that there was no significant difference between the sham treatment and SPS groups ($N=8$; Sham: 2154.1±346.5, SPS: 1979.1±467.3, $t=0.301$; $df=14$;

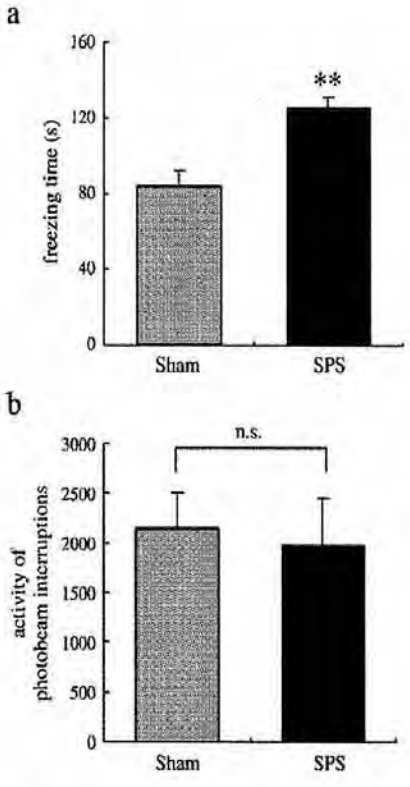

Fig. 3. Behavioral data. (a) The effect of SPS on contextual freezing between sham-treated and SPS rats. The duration of contextual freezing in the SPS rats was significantly higher than that in the sham-treated rats (**$P<0.01$, unpaired Student's *t*-test). Results are expressed as the mean of freezing when re-exposed to the context. Mean±SEM ($n=8$) is shown. (b) Spontaneous activity in sham-treated and SPS rats. There was no significant difference in the level of locomotor activity between these 2 groups. Results are expressed as the mean score of horizontal movement. Mean±SEM ($n=8$) is shown.

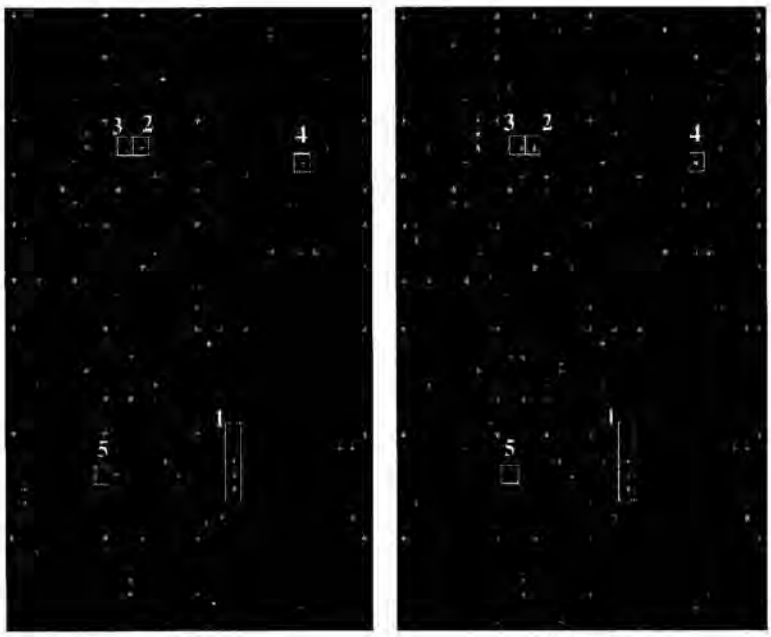

Fig. 4. Hippocampal gene expression profiles determined by cDNA microarray. Total RNA from the hippocampus of 6 rats in each group was isolated using an RNAqueous Phenol-free Total Isolation Kit (Ambion), and pooled. The cDNA probes synthesized from the pooled total RNA by reverse transcriptase and labeled by Cy3 were hybridized to an Atlas Glass Rat 1.0 Microarray (Clontech). The fluorescent signal intensity of each gene was assessed using a GenePix fluorescent scanner (Fuji Film), and quantified with an ArrayGauge analysis system (Fuji Film). Gly-T1: glycine transporter 1; RCK4: potassium channel RCK4; VAMP2: vesicle-associated membrane protein 2; LIMK1: LIM kinase 1.

P=0.768, Fig. 3b), indicating that SPS did not affect spontaneous activity.

3.3. Analysis of gene expression profiles in the hippocampus of SPS rats subjected to CF

To elucidate the influence of SPS on hippocampal gene expression profiles in response to CF, we used the Atlas Glass Array Rat 1.0 Microarray (N=6; Clontech; Fig. 4). Examination of the gene expression profiles following episodes of CF in SPS and sham-treated rats revealed 6 genes that were upregulated by at least 2.5-fold and 2 genes that were downregulated by at least 2.5-fold (Table 1). We identified 4 genes (encoding glycine transporter 1; *Gly-T1* (Tsai et al., 2004), potassium channel RCK4; *RCK4* (Pan et al., 2004), vesicle-associated membrane protein 2; *VAMP2* (Hirling and Scheller, 1996) and LIM kinase 1; *LIMK1* (Meng et al., 2002)) that are relevant to anxiety or memory formation in the central nervous system. Table 2 shows the sequences and fluorescent dyes of PCR primers and TaqMan probes for each molecule. Because of the low sensitivity of this

Table 1
Hippocampal genes whose mRNA expression was markedly changed (2.5-fold or greater change in the mean expression level) by CF in SPS rats in comparison with those in sham rats determined by cDNA microarray

Gene	Spot intensity		Fold change
	CF	SPS+CF	SPS+CF/CF
Genes upregulated in SPS rats			
Glycine transporter 1 (Gly-T1)	**56**	**199**	**3.6**
Potassium channel RCK4	**20**	**60**	**3.0**
Multispecific organic anion transporter (OAT1)	55	164	3.0
Multidrug resistance protein (MRP)	21	55	2.6
B7-1	25	65	2.6
Vesicle-associated membrane protein 2 (VAMP2)	**61**	**154**	**2.5**
Genes downregulated in SPS rats			
Gastrin	191	24	0.1
LIM kinase 1 (LIMK1)	**78**	**29**	**0.4**

The four genes marked boldface are relevant to anxiety or memory formation in the central nervous system.

Table 2
Sequences and fluorescent dye of PCR primers and TaqMan probes

Glycine transporter 1 (Gly-T1)
Forward primer 5′-TCCTGAAGATGGGTTTGAGGTT-3′
Reverse primer 5′-AGCCGTTACTGCCCACGAT-3′
TaqMan probe 5′-FAM-TGCACCCGGACAAGGCCCA-TAMRA-3′
Vesicle-associated membrane protein 2 (VAMP2)
Forward primer 5′-TGGATGATCGCGCAGATG-3′
Reverse primer 5′-CCACCAGTATTTGCGCTTGAG-3′
TaqMan probe 5′-FAM-CCTCCCAGTTTGAAACAAGTGCAGCC-TAMRA-3′
Potassium channel RCK4 (RCK4)
Forward primer 5′-TCCGAGTGTTCCGGATCTTC-3′
Reverse primer 5′-CTTAGGGTGTGGCCCAGGAT-3′
TaqMan probe 5′-FAM-TCCAGACACTCCAAGGGCCTGCA-TAMRA-3′
LIM kinase 1 (LIMK1)
Forward primer 5′-CCCGGATGTGAAGAATTCCA-3′
Reverse primer 5′-TCGTCCAGCGGCACATT-3′
TaqMan probe 5′-FAM-TTGGAAATCAACGGCACGCCCAT-TAMRA-3′

cDNA microarray, we performed RT-PCR analysis for each of the genes detected by cDNA microarray to confirm these differences in expression. Different hippocampal samples from those in the cDNA microarray analysis were used.

3.4. The levels of mRNAs encoded for by 3 housekeeping genes (GAPDH, 18s-rRNA, and GUSB) in the hippocampus of rats subjected to SPS+CF and ARS

To elucidate that GAPDH is a valid reference for data normalization, we examined the effect of SPS+CF as well as ARS on the levels of *GAPDH*, *18s-rRNA* and *GUSB* in the rat hippocampus by RT-PCR. Kruskal–Wallis test showed that there were no significant differences in the expression of these 3 genes in the hippocampus of rats subjected to either SPS+CF [N=6: percent of *GAPDH* (stress group/average of control group): *GAPDH* (99.77±5.54), *18s-rRNA* (106.33±6.36), *GUSB* (98.67±5.01); P=0.907; Fig. 5a] or ARS [N=8; *GAPDH* (100.00±6.61), *18s-rRNA* (103.63±4.91), *GUSB* (99.38±5.71); P=0.893; Fig. 5b]. Based on these findings, *GAPDH* was used for normalization in this study.

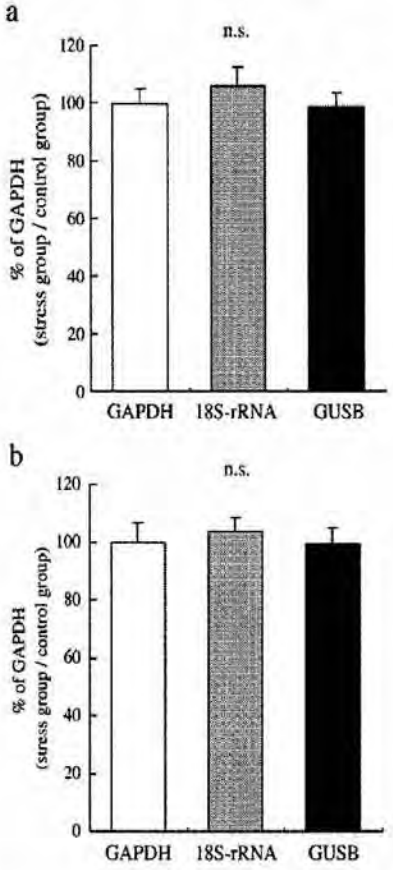

Fig. 5. Expression of GAPDH, 18s-rRNA and GUSB mRNA in the hippocampus of rats subjected to SPS+CF and acute restraint stress (ARS). The levels of these mRNAs were determined by RT-PCR as described in Methods. Results are expressed as the percentage of GAPDH. (a) SPS+CF; there was no significant difference in the ratio (stress group/control group) among these 3 genes in the hippocampus of rats. The mean±SEM (n=6) is shown. (b) ARS; there was no significant difference in the ratio (stress group/control group) among these 3 genes in the hippocampus of rats. The mean±SEM (n=8) is shown.

3.5. The influence of CF on the levels of mRNAs encoded for by 4 genes (Gly-T1, VAMP2, RCK4 and LIMK1) in the hippocampus of SPS and sham-treated rats

We measured the levels of mRNAs encoded for by 4 genes (*Gly-T1*, *VAMP2*, *RCK4* and *LIMK1*) in the hippocampus of 4 groups of rats (N=6; sham-treated [Sham], subjected to SPS alone [SPS], subjected to CF without SPS [CF], and subjected to SPS followed by CF [SPS+CF]) using RT-PCR. Statistical analysis revealed a significant effect of SPS on the expression of Gly-T1 mRNA [F(1,23)=11.180, P=0.0032], a significant effect of CF [F(1,23)=9.037, P=0.0070], and a significant interaction between SPS and CF [F(1,23)=5.153, P=0.034]. Post hoc analysis revealed that there was a significant increase in the hippocampal levels of Gly-T1 mRNA in the SPS+CF rats in comparison with any of the other groups (P<0.01; Fig. 6a). Statistical analysis revealed a significant effect of SPS on the expression of VAMP2 mRNA [F(1,23)=6.477, P=0.019], a significant CF effect [F(1,23)=8.387, P=0.0089], and a significant interaction between SPS and CF [F(1,23)=7.047, P=0.015]. Post hoc analysis revealed that there was a significant increase in the hippocampal levels of VAMP2 mRNA in the SPS+CF rats in comparison with any of the other groups (P<0.01, 0.05; Fig. 6b). Statistical analysis revealed no significant effect of SPS and CF on the expression of RCK4 mRNA [SPS; F(1,23)=0.097, P=0.759, CF; F(1,23)=0.654, P=0.428], and no significant interaction between SPS and CF [F(1,23)=0.468, P=0.502]. Post hoc analysis showed that there was no significant difference in the levels of RCK4 mRNA (Fig. 6c). Statistical analysis also revealed no significant effect of SPS and CF on the expression of LIMK1 mRNA [SPS; F(1,23)=0.278, P=0.604, CF; F(1,23)=2.292, P=0.146], and no significant interaction between SPS and CF [F(1,23)=0.049, P=0.827]. Post hoc analysis indicated that there was no significant difference in the levels of LIMK1 mRNA (Fig. 6d). These data were not consistent with the results of the cDNA microarray analysis. However, Chen et al. (2000) described that a major limitation of DNA array technology is the large number of false-positive results obtained. Further, the hippocampal samples used for the cDNA microarray were different from those used in the RT-PCR analysis. Based on our cDNA microarray analysis, it would therefore be reasonable to consider the RCK4 and LIMK1 mRNA expression results as false-positives.

3.6. The influence of SPS on the levels of mRNAs encoded for by 4 genes (Gly-T1, VAMP2, RCK4 and LIMK1) in the hippocampus 1 day after SPS

To confirm whether the differences in the levels of mRNAs encoded for by 4 genes (*Gly-T1*, *VAMP2*, *RCK4* and *LIMK1*) between the CF and SPS+CF group were simply due to the effect of SPS, we examined the levels of each gene in rats subjected to sham treatment and SPS on day 16 (1 day after SPS). We measured the levels of mRNAs encoded for by these 4 genes in the hippocampus of 2 groups of rats (N=6; sham-treated group [Sham], and group subjected to SPS followed by

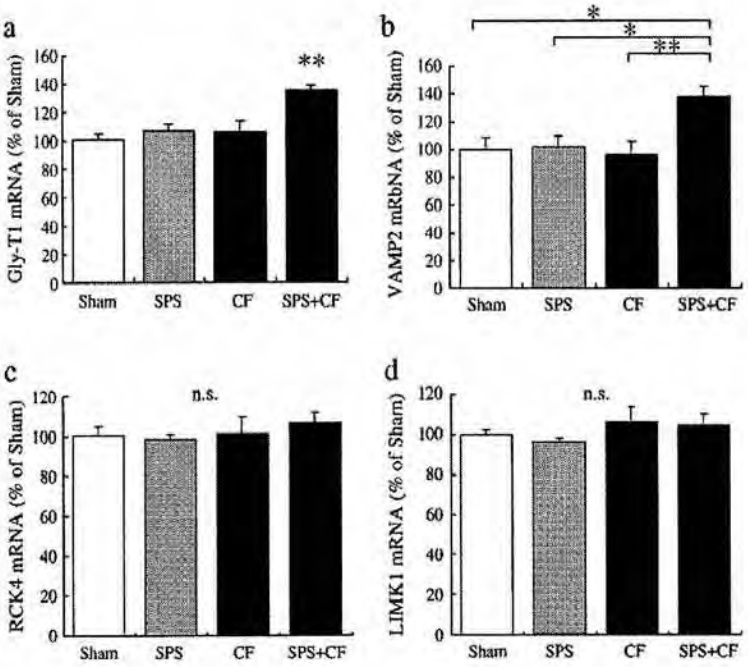

Fig. 6. Expression of Gly-T1, VAMP2, RCK4 and LIMK1 mRNA in the hippocampus of rats subjected to sham treatment (Sham), SPS on day 15 alone (SPS), CF on days 22–23 alone (CF), and SPS on day 15 followed by CF on days 22–23 (SPS+CF). The levels of these mRNAs were determined by RT-PCR as described in Methods. Results are expressed as the percentage of Sham. The mean±SEM ($n=6$) is shown. (a) Gly-T1 mRNA; $^{**}P<0.01$ compared to any other groups (two-way ANOVA followed by Tukey's test). (b) VAMP2 mRNA, $^{*}P<0.05$, $^{**}P<0.01$ compared to SPS+CF (two-way ANOVA followed by Tukey's test). (c) RCK4 mRNA; there was no significant difference in the level of RCK4 mRNA expression between the 4 groups. (d) LIMK1 mRNA; there was no significant difference in the level of LIMK1 mRNA expression between the 4 groups.

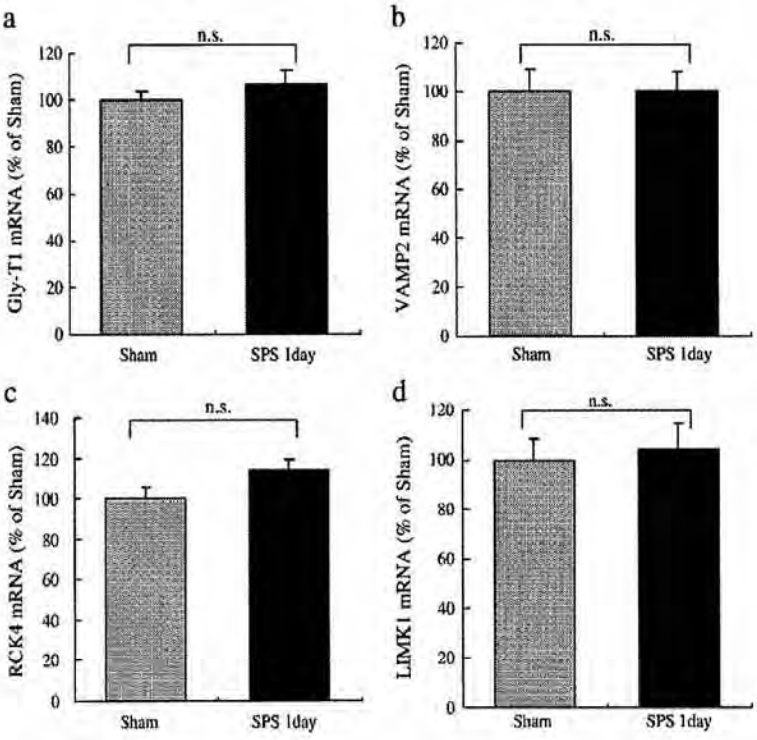

Fig. 7. Expression of Gly-T1, VAMP2, RCK4 and LIMK1 mRNA in the hippocampus of rats 1 day after SPS. Sham; rats subjected to sham treatment, SPS for 1 day; rats subjected to SPS and sacrificed 1 day after SPS. The levels of these mRNAs were determined by RT-PCR as described in Methods. Results are expressed as the percentage of Sham. The mean±SEM ($n=6$) is shown. (a) There was no significant difference in the level of Gly-T1 mRNA expression between groups. (b) There was no significant difference in the level of VAMP2 mRNA expression between groups. (c) RCK4; there was no significant difference in the level of RCK4 mRNA expression between groups. (d) LIMK1; there was no significant difference in the level of LIMK1 mRNA expression between groups.

removal of the hippocampus 1 day later [SPS 1day]). Statistical analysis revealed no significant difference in the levels of Gly-T1 (Fig. 7a), VAMP2 (Fig. 7b), RCK4 (Fig. 7c), and LIMK1 (Fig. 7d) mRNA between the sham and SPS groups.

4. Discussion

The results of the present study demonstrated that SPS significantly enhanced contextual freezing of rats in response to the context in which the footshock was delivered. This finding is in agreement with our recent studies indicating that contextual freezing is significantly enhanced in rats subjected to SPS (Imanaka et al., 2006; Takahashi et al., 2006). In addition, Rau et al. (2005) showed that prior exposure to stress significantly enhanced the subsequent learning of contextual fear in rats. It has been postulated that exposure to a severe pre-shock enhances susceptibility to the subsequent shock, and may be involved in the development of increased anxiety and fear even in response to trauma-unrelated stimuli in patients with PTSD (Orr et al., 2003).

With regard to the suitability of the animal model of PTSD, Yehuda and Antelman (1993) postulated that the stressor could not always elicit PTSD-like sequelae in all organisms. Based on epidemiological studies of PTSD, the average lifetime prevalence was approximately 1.0% to 12.3% (Fairbank et al., 1995). In this context, because the SPS paradigm induces PTSD-like behaviors in almost all animals, it may not be an appropriate animal model of PTSD. In addition, an augmentation of contextual memory in not always found in patients with PTSD, and this phenomenon is not directly indicated in the diagnostic criteria for PTSD in DSM-IV. Therefore, it is difficult to propose that SPS is an appropriate animal model of PTSD only from the aspect of enhanced contextual freezing. However, based on the present finding of enhanced contextual memory, along with previous findings in SPS rats such as the enhanced glucocorticoid negative feedback (Liberzon et al., 1997), exaggerated acoustic startle response (Khan and Liberzon, 2004), and stress-induced analgesia (Imanaka et al., 2006), it is postulated that SPS is an appropriate animal model of PTSD.

Although the molecular biological analysis demonstrated a significant increase in the levels of glucocorticoid receptor mRNA and of glucocorticoid receptor mRNA/mineralocorticoid receptor mRNA in the rat hippocampus 7 days after SPS (Liberzon et al., 1999), the precise mechanism for this sensitization to stress remains to be elucidated. In this context, we compared the hippocampal gene expression profiles between rats subjected to sham treatment or SPS in response to CF conditioning. The results derived from the microarray analysis followed by RT-PCR indicated that treatment with SPS significantly upregulated the hippocampal levels of Gly-T1 and VAMP2 mRNA in rats exhibiting enhanced contextual freezing. Furthermore, no significant change in the levels of either gene was found after exposure to SPS alone. Hence, it is conceivable that these genes may contribute to the development of the sensitization to additional environmental stimuli induced by SPS.

Interestingly, several studies demonstrated that stress increased the concentration of glycine in rat brain. For example, predator fox odor markedly increased the concentration of glycine in the rat nucleus accumbens and enhanced locomotion and burrowing (Venton et al., 2006). In adult mice, glycine release was greatly enhanced under different cell-damaging conditions (including hypoxia, hypoglycemia, ischemia, and oxidative stress). The extracellular concentration of glycine in the CA1 field of rat hippocampus 20 min after cerebral ischemia was approximately 3-fold higher than that in controls (Andine et al., 1988). It has been demonstrated that excitatory amino acids, including glycine, are released in large amounts from neural structures during hypoxia, ischemia, and other cell-damaging conditions (Saransaari and Oja, 1998), leading to eventual cell death through overstimulation of their receptors (Rothman and Olney, 1987). Despite the difference in stress severity, these findings suggest that administration of footshock in the contextual fear test may increase the synaptic concentration of glycine in the hippocampus of rats subjected to SPS. If so, it is likely that the abundant release of glycine from the presynapse may subsequently induce Gly-T1 mRNA expression. Similarly, Gly-T1 mRNA was transiently upregulated in the hippocampus of gerbils by the second day after a 3-min ischemic bout, possibly to reduce glycine concentrations in the local extracellular space (Fujita et al., 1999).

Since we did not measure the concentration of glycine by microdialysis, the possibility that exposure to footshock in the contextual fear test may directly upregulate the levels of Gly-T1 mRNA cannot be ruled out. It has been shown that activation of glycine regulatory sites on *N*-methyl-D-aspartate (NMDA) receptors by D-cycloserine facilitates signal transduction mediated by the NMDA receptor and subsequently promotes extinction (Walker et al., 2002; Ledgerwood et al., 2003). In addition, several clinical studies of PTSD have indicated that impaired extinction of fearful memory is a major symptom in PTSD (Bremner et al., 2005; Milad et al., 2006; Peri et al., 2000). Taken together, these studies show that the inactivation of glycine regulatory sites due to increased glycine uptake in the hippocampus plays a role in the development of impaired extinction. Therefore, it is highly likely that the significant upregulation of Gly-T1 mRNA in the hippocampus of rats subjected to SPS followed by footshock may be an initial event in the development of impaired extinction. However, to confirm this hypothesis, further studies are needed to determine whether extinction in rats subjected to SPS is impaired.

Another important finding in this study was that the hippocampal levels of VAMP2 mRNA in rats subjected to SPS were increased significantly in response to CF. To our knowledge, no prior studies have directly demonstrated that VAMP2 regulates contextual fear in the hippocampus. However, it has been reported that VAMP2 is, at least in part, involved in synaptic plasticity in the hippocampus through the modulation of neurotransmitter release (Hirling and Scheller, 1996; Pozzo-Miller et al., 1999). Thus, it is possible that increased expression of VAMP2 mRNA may be necessary for the massive release of neurotransmitters, which occurs as a compensatory response to CF in rats subjected to SPS.

In addition to the possibility that upregulation of Gly-T1 as well as VAMP2 mRNA facilitates contextual freezing in rats subjected to SPS, the results of the present study suggested the contribution of several other genes that are reportedly involved in the facilitation of contextual freezing. Conversely, several other genes such as the *5-HT3* receptor (Harrell and Allan, 2003), nicotinic receptor (Davis and Gould, 2006; Wehner et al., 2004), and acetylcholinesterase genes (Nijholt et al., 2004), are reported to potentiate contextual fear in rodents. Our microarray analysis demonstrated no marked difference in the levels of 5-HT3 receptors, nicotinic receptors, or acetylcholinesterase. In this context, it is unlikely that the increase in the hippocampal expression of these genes is associated with the enhanced CF observed in rats subjected to SPS.

5. Conclusions

The result of the present study demonstrated that contextual freezing in response to the context in which the footshock is delivered, was significantly enhanced in rats subjected to SPS, an animal model of PTSD. This finding is consistent with the observation that patients with PTSD show enhanced anxiety and fear in response to stimuli unrelated to trauma. To examine the mechanism for the enhanced susceptibility to subsequent contextual fear conditioning in SPS rats, we used microarray analysis and RT-PCR to compare the hippocampal gene expression profiles between SPS and control rats. Based on these molecular analyses, the levels of Gly-T1 and VAMP2 mRNA were found to be significantly upregulated in rats subjected to SPS. Since the activation of glycine binding sites by D-cycloserine alleviates the impaired extinction, the upregulation of Gly-T1 may be an initial event in the development of impaired extinction.

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, and a grant from Core Research for Evolutional Science and Technology (CREST) of Japan Science and Technology (JST).

References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders. 4th ed. Washington, DC: American Psychiatric Press; 1994.

Andine P, Jacobson I, Hagberg. Calcium uptake evoked by electrical stimulation is enhanced postischemically and precedes delayed neuronal death in CA1 of rat hippocampus: involvement of *N*-methyl-D-aspartate receptors. J Cereb Blood Flow Metab 1988;8:799–807.

Bremner JD, Vermetten E, Schmahl C, Vaccarino V, Vythilingam M, Afzal N, et al. Positron emission tomographic imaging of neural correlates of a fear acquisition and extinction paradigm in women with childhood sexual-abuse-related post-traumatic stress disorder. Psychol Med 2005;35:791–806.

Bustin SA. Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. J Mol Endocrinol 2002;29:23–39.

Chen J, Zhang Y, Kelz MB, Steffen C, Ang ES, Zeng L, et al. Induction of cyclin-dependent kinase 5 in the hippocampus by chronic electroconvulsive seizures: role of [Delta]FosB. J Neurosci 2000;20:8965–71.

Davis JA, Gould TJ. The effects of DHBE and MLA on nicotine-induced enhancement of contextual fear conditioning in C57BL/6 mice. Psychopharmacology (Berl) 2006;184:345–52.

Fairbank JA, Schlenger WE, Saigh PA, Davidson RT. An epidemiologic profile of post-traumatic stress disorder: prevalence comorbidity, and risk factors. In: Friedman MJ, Charney DS, Deutch AY, editors. Neurobiological and clinical consequences of stress: from normal adaptation to PTSD. Philadelphia: Lippincott-Raven; 1995. p. 415–27.

Fujita H, Sato K, Wen TC, Peng Y, Sakanaka M. Differential expressions of glycine transporter 1 and three glutamate transporter mRNA in the hippocampus of gerbils with transient forebrain ischemia. J Cereb Blood Flow Metab 1999;19:604–15.

Harrell AV, Allan AM. Improvements in hippocampal-dependent learning and decremental attention in 5-HT(3) receptor overexpressing mice. Learn Mem 2003;10:410–9.

Hirling H, Scheller RH. Phosphorylation of synaptic vesicle proteins: modulation of the αSNAP interaction with the core complex. Proc Natl Acad Sci U S A 1996;93:11945–9.

Imanaka A, Morinobu S, Toki S, Yamawaki S. Importance of early environment in the development of posttraumatic stress disorder-like behaviors. Behav Brain Res 2006;173:129–37.

Khan S, Liberzon I. Topiramate attenuates exaggerated acoustic startle in an animal model of PTSD. Psychopharmacology 2004;172:225–9.

Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. Science 1992;256:675–7.

Ledgerwood L, Richardson R, Cranney J. Effects of D-cycloserine on extinction of conditioned freezing. Behav Neurosci 2003;117:341–9.

Liberzon I, Martis B. Functional neuroimaging research in posttraumatic stress disorder. In: Kato N, Kawata M, Pitman RK, editors. PTSD brain mechanisms and clinical implications. Tokyo: Spinger-Verlag; 2006. p. 211–33.

Liberzon I, Krstov M, Young EA. Stress-restress: effects on ACTH and fast feedback. Neuroendocrinology 1997;22:443–53.

Liberzon I, Lopez JF, Flagel SB, Vazquez DM, Young EA. Differential regulation of hippocampal glucocorticoid receptors mRNA and fast feedback: relevance to post-traumatic stress disorder. J Neuroendocrinol 1999;11:11–7.

Meng Y, Zhang Y, Tregoubov V, Janus C, Cruz L, Jackson M, et al. Abnormal spine morphology and enhanced LTP in LIMK-1 knockout mice. Neuron 2002;35:121–33.

Milad MR, Rauch SL, Pitman RK, Quirk GJ. Fear extinction in rats: implications for human brain imaging and anxiety disorders. Biol Psychiatry 2006;73:61–71.

Nijholt I, Farchi N, Kye M, Sklan EH, Shoham S, Verbeure B, et al. Stress-induced alternative splicing of acetylcholinesterase results in enhanced fear memory and long-term potentiation. Mol Psychiatry 2004;9:174–83.

Orr SP, Metzger LJ, Lasko NB, Macklin ML, Hu FB, Shalev AY, et al. Physiologic responses to sudden, loud tones in monozygotic twins discordant for combat exposure: association with posttraumatic stress disorder. Arch Gen Psychiatry 2003;60:283–8.

Pan Y, Xu X, Tong X, Wang X. Messenger RNA and protein expression analysis of voltage-gated potassium channels in the brain of Abeta(25–35)-treated rats. J Neurosci Res 2004;77:94–9.

Peri T, Ben-Shakhar G, Orr SP, Shalev AY. Psychophysiologic assessment of aversive conditioning in posttraumatic stress disorder. Biol Psychiatry 2000;47:512–9.

Phillips RG, LeDoux JE. Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 1992;106:274–85.

Pitman RK, Orr SP, Shalev AY, Metzger LJ, Mellman TA. Psychophysiological alterations in post-traumatic stress disorder. Semin Clin Neuropsychiatry 1999;4:234–41.

Pozzo-Miller LD, Gottschalk W, Zhang L, McDermott K, Du J, Gopalakrishnan R, et al. Impairments in high-frequency transmission, synaptic vesicle docking, and synaptic protein distribution in the hippocampus of BDNF knockout mice. J Neurosci 1999;19:4972–83.

Rau V, DeCola JP, Fanselow MS. Stress-induced enhancement of fear learning: an animal model of posttraumatic stress disorder. Neurosci Biobehav Rev 2005;29:1207–23.

Rothman SM, Olney JW. Excitotoxicity and the NMDA receptor. Trends Neurosci 1987;10:299–302.

Saransaari P, Oja SS. Release of endogenous glutamate, aspartate, GABA, and taurine from hippocampal slices from adult and developing mice under cell-damaging conditions. Neurochem Res 1998;23:563–70.

Save E, Poucet B, Foreman N, Buhot MC. Object exploration and reactions to spatial and nonspatial changes in hooded rats following damage to parietal cortex or hippocampal formation. Behav Neurosci 1992;106:447–56.

Sotres-Bayon F, Cain CK, LeDoux JE. Brain mechanisms of fear extinction: historical perspectives on the contribution of prefrontal cortex. Biol Psychiatry 2006;60:329–36.

Takahashi T, Morinobu S, Iwamoto Y, Yamawaki S. Effect of paroxetine on enhanced contextual fear induced by single prolonged stress in rats. Psychopharmacology (Berl) 2006;189:165–73.

Tsai G, Ralph-Williams RJ, Martina M, Bergeron R, Berger-Sweeney J, Dunham KS, et al. Gene knockout of glycine transporter 1: characterization of the behavioral phenotype. Proc Natl Acad Sci U S A 2004;101:8485–90.

Venton BJ, Robinson TE, Kennedy RT. Transient changes in nucleus accumbens amino acid concentrations correlate with individual responsivity to the predator fox odor 2,5-dihydro-2,4,5-trimethylthiazoline. J Neurochem 2006;96:236–46.

Walker DL, Ressler KJ, Lu KT, Davis M. Facilitation of conditioned fear extinction by systemic administration or intra-amygdala infusions of D-cycloserine as assessed with fear-potentiated startle in rats. J Neurosci 2002;22:2343–51.

Wehner JM, Keller JJ, Keller AB, Picciotto MR, Paylor R, Booker TK, et al. Role of neuronal nicotinic receptors in the effects of nicotine and ethanol on contextual fear conditioning. Neuroscience 2004;129:11–24.

Yehuda R. Biology of posttraumatic stress disorder. J Clin Psychiatry 2001;62 (Suppl. 17):41–6.

Yehuda R. Post-traumatic stress disorder. N Engl J Med 2002;346:108–14.

Yehuda R, Antelman SM. Criteria for rationally evaluating animal models of posttraumatic stress disorder. Biol Psychiatry 1993;33:479–86.

Yehuda R, Southwick SM, Krystal JH, Bremner D, Charney DS, Mason JW. Enhanced suppression of cortisol following dexamethasone administration in posttraumatic stress disorder. Am J Psychiatry 1993;150:83–6.