が代謝系に与える影響も大きく，成長過程にある子どもへの使用に関して，長期的な観点からさらなる有効性と安全性の評価が必要であろう。

最後に本研究の限界であるが，単一施設で実施され，診療録などの記載に依拠した後方視的調査という点である。さらに児童思春期における精神症状は非特異的であり，状況や時間経過で変化しやすいなど，診断自体が変化し得るため，初診時のみの診断で検討することにも限界がある。縦断的な視点から，診断の変遷を含めた追跡調査が必要であろう。

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第105回日本精神神経学会総会

教育講演

統合失調症の進行性変化の根底にあるもの
——白質異常とオリゴデンドロサイトの動態——

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脳画像研究により，統合失調症の脳の構造的異常があきらかになっており，急性期の統合失調症の患者では脳の変化が起こっていることは証拠が積み重なっている．慢性期の脳の構造的進行性変化については，脳の構造的変化について慢性統合失調症では，発症から少なくとも20年までは持続的な脳実質の減少と脳室の拡大があり，健常者の減少は1年あたり0.2%であり，慢性統合失調症は1年あたり0.5%と健常者の2倍に上る．進行性の萎縮は前頭野側頭野（灰白質）で大きく，その程度は予後不良，陰性症状，認知症状と関係している．この脳の進行性変化の基礎にある病態生理学的プロセスを明らかにすることは重要である．なぜなら，それによって最終的にプロセスを停止させる，あるいは逆転させることも可能であると考えられるからである．オリゴデンドロサイトに関係した機能障害の可能性が示されており，これが脳組織の持続的減少の原因の一つとも考えられる．またMRI画像解析による白質容積の減少や拡散テンソル画像解析による白質における質的な異常も認めている．われわれの統合失調症の動物モデル実験でも，オリゴデンドロサイトの異常を認めており，このオリゴデンドロサイトの異常は，発症後の進行性変化にとって重要であると思われる．

1．慢性期に脳の進行性の変化は存在するか？

脳画像研究により，統合失調症の脳の構造的異常があきらかになっており，統合失調症患者の脳は，健常者に比較して灰白質で2%，白質で1%，全脳で3%と減少し，側脳室（20%）・第三脳室・髄液腔CSFの増加を伴う[1]．この構造的異常がいつ生じてきているのかはいまだ明らかでないが，臨床症状の激しくまた機能レベルの低下も著しくなりやすい初回精神病エピソードで灰白質の減少が報告されており，初回エピソードの統合失調症患者34名の1年のMRIによるフォローアップ研究では，大脳灰白質で2.9%の減少を認めている[1]．同様に初回入院の統合失調症患者13名，初回入院の気分障害患者15名，健常者22名のフォローアップ研究では発症の時点ですでに存在していた左上側頭回の萎縮は1.5年で約7%の進行性の萎縮を認めている[4]．また初回入院統合失調症患者11名，初回入院気分障害患者13名，健常者13名でのクロスセクショナルな1.5年のMRIと聴覚性MMNを測定したフォローアップ研究では，当初MMNの振幅は各群で差がなかったが，統合失調症ではMMNの振幅とヘルシュ回の容積との間に相関があり，1.5年後の聴覚性MMNの振幅（ヘルシュ回と側頭葉聴覚皮質を主な起点とするエコーイック記憶の機能的プロセスの電気生理的指標）の低下と左ヘルシュ回の容積減少とに強い相関があることが示された[8]．これは側頭の脳の構造的変化とその機能的変化が

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相関していることは脳の経時的かつ進行性の構造的変化が機能的変化の基礎となっている，またその逆も想定されることを示している．また早期つまり出生前・周生期の神経発達病変が思春期後の神経発達病変を惹起すると考えられており，発症前から前頭前野の灰白質の減少と正常でないconnectivityが存在し，発症後，海馬傍回，眼窩前頭皮質，帯状回は進行性に萎縮するとの報告もある[8]．さらに前述のとおり，発症の時点ですでに存在していた左上側頭回の萎縮は1.5年で約7％の進行性の萎縮を呈する[4]との報告もあり，急性期の統合失調症の患者では脳の変化が起こっていることは証拠が積み重なっている．

初回エピソード以降にみられるいわゆる慢性期の脳の構造的進行性変化についてはいかがであろうか．慢性期の統合失調症に見られる脳の構造的変化についてのMRIとCTの縦断的研究11論文のレビューによると，慢性統合失調症では，発症から少なくとも20年までは持続的な脳実質の減少と脳室の拡大があり，健常者の減少は1年あたり0.2％であり，慢性統合失調症は1年あたり0.5％と健常者の2倍に上る，進行性の萎縮は前頭野側頭野（灰白質）で大きく，その程度は予後不良，陰性症状，認知症状と関係していた[3]．このように慢性の統合失調症患者において脳の進行性変化が続いているという所見は，脳内で1種類以上の病態生理学的プロセスが活発に生じていることを示唆する証拠である．この脳の進行性変化の基礎にある病態生理学的プロセスを明らかにすることは重要である．なぜなら，それによって最終的にプロセスを停止させる，あるいは逆転させることも可能であると考えられるからである．

2．病態生理学的プロセスと白質異常

われわれはその病態生理学的プロセスについて推測することしかできないが，統合失調症患者の死後脳研究では，神経細胞の密度の上昇と樹状突起（シナプス結合）の減少があり，神経細胞の密度の増加は前頭前野の深部白質のmaldistributionと関係しているなど白質の異常が示唆され[2]，またマイクロアレイによるオリゴデンドロサイト関連因子の低下や電子顕微鏡解析によるミエリンの菲薄化などが報告されており[10]，ミエリン（オリゴデンドロサイト）に関係した機能障害の可能性が示されており，これが脳組織の持続的減少の原因の一つとも考えられる．またMRI画像解析による白質容積の減少や拡散テンソル画像解析による白質のfraction anisotropy（FA）値の低下，すなわち白質における質的な異常も報告されている．また発症早期と慢性期の白質異常を検討した思春期統合失調症の統合失調症患者23名と慢性期の成人統合失調症35名の拡散テンソル画像による研究では白質異常は，側頭葉では発症時から存在し，前頭葉では発症後に進行していた[9]．オリゴデンドロサイトは，軸索を覆うミエリンを形成する細胞であり，跳躍伝導により軸索の伝導速度を促進させている．オリゴデンドロサイトが障害されると神経伝導速度が低下し，その脳機能に異常をきたす．オリゴデンドロサイトが障害される代表的疾患は異染性白質ジストロフィー（metachromatic leukodystrophy：MLS）と多発性硬化症（multiple sclerosis：MS）である[2]．MLSは常染色体劣性遺伝病で，ライソソーム酵素の一つであるarylsulfatase Aの欠損により，その基質であるスルファチドが，脳・腎などに蓄積する疾患で，臨床的には白質ジストロフィー・末梢神経障害を呈する．病型は，乳幼児型，若年型，成人型に分類される．成人型は幻聴や感情鈍麻などの統合失調症様の精神症状を呈し，統合失調症と間違われることもある．MSは中枢性脱髄疾患の一つで，脳，脊髄，視神経などに病変が起こり，多彩な神経症状が再発と寛解を繰り返す疾患であり，それらの一部の患者では統合失調症様の症状が現れる．また両疾患とも統合失調症と同様に認知機能の障害を生じる（表1）．これは統合失調症症状発現とオリゴデンドロサイト異常との関連を示唆している．われわれは統合失調症とオリゴデンドロサイト異常との関連を検討すべく，統合失調症の動物モデルを用いた解析及び拡散テンソル画像を用いた統合失調症患者脳の白質の解

表1　統合失調症・異染性白質ジストロフィー・多発性硬化症の認知機能

cognitive domain	schizophrenia	MLD	MS
IQ	↓	↓	↓
遂行機能	↓	↓	↓
注意	↓	↓	↓
記銘と遅延再生	↓	↓	↓
視覚認知	−	↓	±
非言語的推論	↓	↓	↓
反応時間	↓	↓	↓
言語の流暢性	↓	−	↓
処理速度	↓	↓	↓
陳述/作業	↓	↓	↓
新規学習	↓	↓	↓
問題解決	↓	↓	↓
視覚処理	−	↓	↓
読書力	−	−	−
言語能力	−	−	−
言語の繰り返し	−	−	−
手続き記憶	−	−	−

MLD：異染性白質ジストロフィー(metachromatic leukodystrophy)
MS：多発性硬化症(multiple sclerosis)
下向きの矢印（↓）は低下を示す．
文献6）より改変

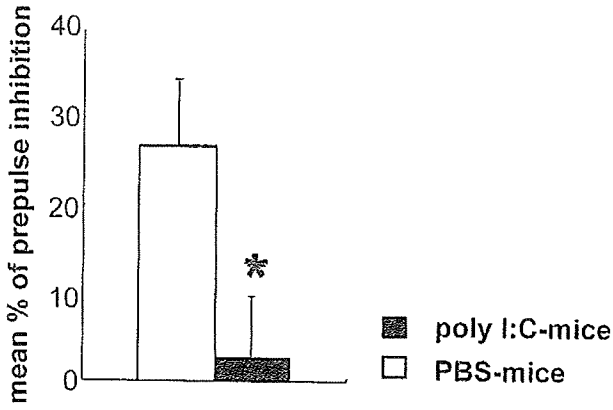

図1
インフルエンザウイルス感染と同様の免疫応答を発現させた2本鎖RNAであるpoly I:Cを胎生9.5日に母体マウスに腹腔内投与された仔マウスは，63日齢で統合失調症様の感覚ゲーティングの行動異常，驚愕刺激のプレ刺激による驚愕反応の抑制の減弱を認めている．

析を行っている．

3．オリゴデンドロサイトの動態

われわれの動物モデルを用いた解析は次のとおりである．

インフルエンザウイルス感染症など何らかの感染症に母体が罹患すると，その母体から生まれた子が統合失調症を発症しやすいということは疫学的に明らかになっているが，インフルエンザウイルス感染と同様の免疫応答を発現させる2本鎖RNAであるpolyinosinic - polycytidylic acid (poly I:C) を妊娠マウスに注入し，その母体から生まれた仔マウスを統合失調症のモデルとして作製した[5]．C57BL/6マウスの胎生9.5日に母体マウスにpoly I:Cを腹腔内投与し，63日齢で統合失調症様の感覚ゲーティングの行動異常，驚愕刺激のプレ刺激による驚愕反応の抑制の減弱を認めた（図1）．その仔マウスを用いてオリゴデンドロサイトについて解析した．poly I:Cを注入し

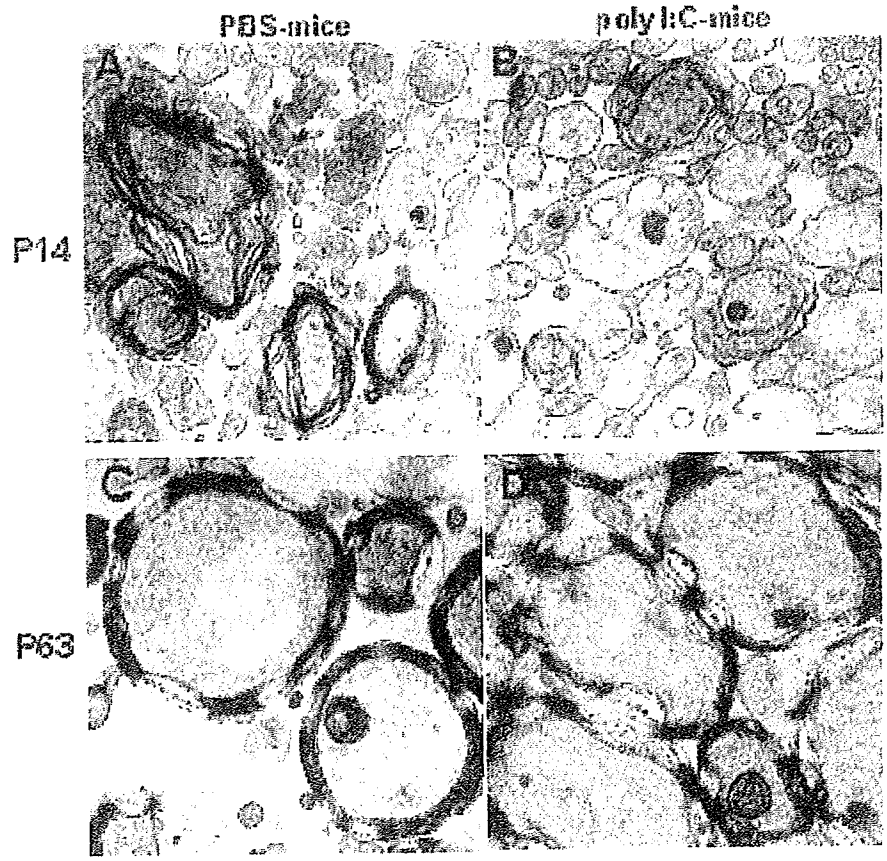

図2
poly I:Cを注入された母体から生まれた仔マウス（poly I:Cマウス）の幼若期（14日齢）の海馬では，溶剤（PBS）のみを注入されたコントロールに比較しミエリン化が遅延しており，またそれに伴い軸索径にも異常がみらる．一方，成熟期（63日齢）にはオリゴデンドロサイトの異常を確認できない．

た母体から生まれた仔マウス（poly I:Cマウス）の幼若期（14日齢）の海馬ではミエリン化が遅延しており，またそれに伴い軸索径にも異常がみられた．一方，成熟期（63日齢）にはオリゴデンドロサイトの異常を確認できなかった（図2）．poly I:Cマウスでは，幼若期に海馬のミエリンの形成不全を一過性に認めるが，成熟期ではミエリンの形成不全は消失するものの，統合失調症のモデルの指標の一つである驚愕反応のプレパルスの刺激による抑制減弱は成熟期においても持続していた．この結果は母体の感染症による統合失調症発症には幼若期海馬のミエリン化や軸索発達の障害が関与している可能性を示唆している．次にその可能性を確かめるため，ラットの幼若期海馬におけるミエリン化をlysophosphatidylcholine投与により遅延させたところ，poly I:Cマウスと同様の統合失調症様の行動異常が認められた．この結果からも，幼若期海馬のミエリン化の遅延は統合失調症症状と関連していることが考えられた[6]．

次に，ミエリンの形成不全すなわち脱髄モデルを作り検討した．脱髄を生ずるcuprizoneを食べさせたマウスは統合失調症のようになることが報告されている[11]が，その脱髄の時期がどのように行動に影響を及ぼすか検討した．マウスの思春期と成体期に相当する時期にミエリンを障害し，その後の行動を観察し，発症時期の相違が行動にどのような影響を及ぼすのかを観察するために，B57BL/6マウスに，思春期群は29日齢から56日齢までのあいだ，成体期群は57日齢から84日齢までのあいだ，それぞれcuprizoneを食事とと

もにあたえ，そののちは同剤を含まない通常の食事を与えて，126日齢で行動実験解析をおこなった（図3）．両群とも，4週間のcuprizone摂取により脳梁にて脱髄が確認され，126日齢ではその脱髄は回復した．両群で物体認識や不安については差がなかったものの，思春期にミエリンが障害されたマウスは，長期にワーキングメモリが障害され，また思春期にミエリンが障害されたマウスは，フィールドの中央に行きにくく，社会性が低下している（図4）という興味深い結果が得られた[12]．Cuprizoneは広範囲に脱髄を生じ，多発性硬化症のモデルとして認知されているので，統合失調症のモデルとするには困難があるが，成熟期の脱髄は回復すれば行動には影響を及ぼさないものの，思春期の脱髄はそののち回復しても行動異常に影響を与え，その異常が持続する．これは思春期発症の統合失調症の予後がよくないというわれわれの臨床的経験と一致するものである．

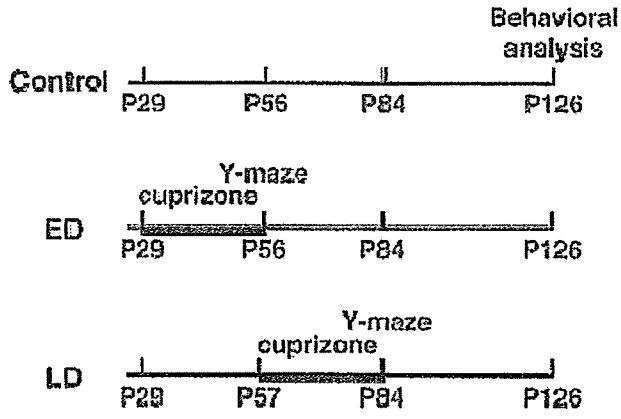

図3
マウスの思春期と成体期に相当する時期にミエリンを障害するために，B57BL/6マウスに，思春期（ED：early demyelination）群は29日齢から56日齢までのあいだ，成体期（LD：late demyelination）群は57日齢から84日齢までのあいだ，それぞれcuprizoneを食事とともにあたえ，そののちは同剤を含まない通常の食事を与えて，126日齢で行動実験解析をおこなった．

また統合失調症の患者の死後脳で認めるオリゴデンドロサイトの異常[10]は発症後の変化によるのではないかと考えた．そこでpoly I:Cマウスにさらに心理的ストレスとしての水浸拘束ストレスをかけたところ，オリゴデンドロサイトの動態に変化がみられた．またそのマウスではストレスを負荷していないpoly I:Cマウスで認めない空間認知異常が現れた．統合失調症の認知異常は発症後にも進行することがあり，これらの結果は発症後の認知機能の変化とオリゴデンドロサイトの動態との関連性を示唆するものである．動物モデルの結果を統合失調症症状と関連付けることには多くの議論があるものの，統合失調症患者死後脳のオリゴデンドロサイトの異常は，発症後の進行

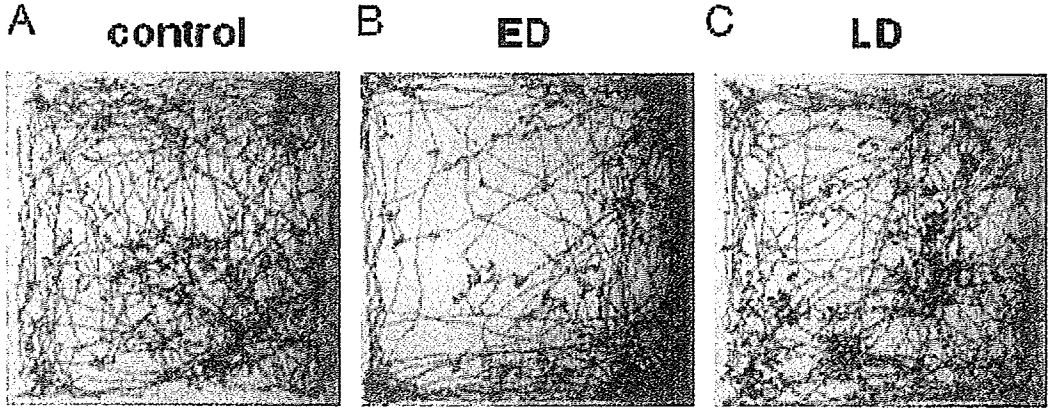

図4
オープンフィールド・テストはcuprizoneの投与が終了して，ED群は10週後，LD群は6週後におこなわれた．コントロール群に比較してLD群は社会性に差がないが，ED群，つまり思春期にミエリンが障害されたマウスは，フィールドの中央に行きにくく，社会性が低下している．

性変化にとって重要な位置を占めているのではなかろうか.

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第105回日本精神神経学会総会

シンポジウム

大学病院における地域医療への貢献
――精神科三次救急の実践から――

岸本　年史（奈良県立医科大学精神医学講座）

奈良県立医科大学は県立の医科大学であり，また設立当初に県立橿原精神病院を吸収合併して付属病院精神科病棟とした経緯があり，県立精神科病院としての機能も求められている．奈良県は人口約142万人，精神科救急医療圏1，精神科入院医療機関10病院，精神科入院ベッド数約3000床である．奈良県の精神科救急は平成12年5月より，県下国立及び民間の8精神科病院が輪番制で時間外救急患者の診療開始し，当大学は輪番制には加わらず，妊婦や透析が必要な患者などの救急患者の診療を受け持ち，また平成13年7月，精神科救急情報センターを当大学が開設しその運営にあたってきた．輪番制により，1次・2次救急ケースの対応は可能となったが，時間外の緊急措置入院の受け入れが円滑に行われていないという問題があったため，平成18年11月，当大学の精神科病棟を新たに建て直しかつ増床し，精神医療センターとし，時間外の3次救急すなわち緊急措置鑑定，緊急措置入院の受け入れを行うことになった．つまり，1次・2次救急は，すなわち夜間・休日の精神科病院受診については平日の夜間（17時から翌8時30分）及び休日（24時間）において，緊急的に精神科治療が必要でありかつ，かかりつけの医療機関で受診できない場合，必要に応じて県内の8精神科病院が，365日当番制で診療を受け付けている（精神科病院輪番制）．3次救急は奈良県立医科大学精神医療センターにおいて，精神保健福祉法の第24条の警察官通報による緊急措置入院鑑定診察と，妊婦・透析患者等の特殊な身体合併症患者の対応をしている．なお，精神科救急ではないが，平成20年度に当大学精神医療センターに身体合併症治療のために入院した患者は36名であり，そのうち外科的処置を要するものは22名（63％）であった．

精神医療センターおよび医局の診療体制の概要は入院病床数90床（本来は110床，看護師不足のため90床で運用），うち救急入院料病棟1（50床：保護室4床，PICU 3床，個室27床），一般の精神病棟1（40床：合併症，老年期，児童思春期，一般閉鎖・開放のユニットからなる）の二つの病棟からなり，医師22名（うち精神保健指定医13名），精神保健福祉士4名（2名は正規職員，2名は研究費より雇い入れ），外来患者数250～300人/日，年間入院患者数326名（平成20年度）である．

2次救急について精神科救急について，平成20年4月1日～平成20年12月31日の9月間の実績を見ると，輪番病院では，相談件数474件，診察件数435件，入院件数144件であり，1日あたりの平均診察件数1.6件，1日あたりの平均入院件数0.5件であった．

平成20年度奈良県での措置入院及び緊急措置入院の件数は，措置入院については県全体で40件，うち当大学12件（30％）であり，緊急措置入院件数は県全体36件うち当大学19件（53％）であった．そのうち当大学での緊急措置鑑定53件であったが，緊急措置鑑定結果，入院は31件（緊急措置入院19件36％，医療保護入院10件

19％，応急入院2件4％）であり，入院治療の必要がなく帰宅に至った件数22件42％であった．

精神科救急の課題と大学病院が果たす役割を当大学の経験をもとに考察すると，大学病院は総合病院であり，手術や透析など単科の精神科病院では対応困難な患者を受け入れることができ，また精神科救急では措置および緊急措置診察を行う指定医の確保が肝要だが，当大学は精神保健指定医の数が多く常駐できるので，措置，応急，医療保護入院など非自発的な入院に対応できることが大学病院として地域の精神科救急に貢献できる大きな要因であろう．

以上のとおり，1．奈良県における精神科救急のシステム及び現状を紹介した．2．奈良県の精神科医療において，大学病院が果たす役割は，医師数が少なくなる時間外の措置入院の受け入れ及び重篤な身体合併症を有する患者の受け入れであることを示した．3．当大学での緊急措置鑑定の実績及び身体合併症患者の受け入れ状況を提示した．4．またシンポジウム当日は救急入院料を算定することで収益に貢献するも併せて提示した．5．大学病院の特性を生かして，地域の精神科救急システムに参加していくことが重要であると考え，教室員の理解と協力のもと教室員とともに実践している．

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Demyelination in the juvenile period, but not in adulthood, leads to long-lasting cognitive impairment and deficient social interaction in mice

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ABSTRACT

Background: Dysmyelination is hypothesized to be one of the causes of schizophrenic symptoms. Supporting this hypothesis, demyelination induced by cuprizone was recently shown to cause schizophrenia-like symptoms in adult rodents [Xiao L, Xu H, Zhang Y, Wei Z, He J, Jiang W, et al. Quetiapine facilitates oligodendrocyte development and prevents mice from myelin breakdown and behavioral changes. Mol Psychiatry 2008;13:697–708]. The present study asked if the timing of demyelination (i.e., juvenile period or adulthood) influenced abnormal behavior.

Methods: B57BL/6 mice were fed with 0.2% cuprizone either from postnatal day 29 (P29) to P56 (early demyelination group) or from P57 to P84 (late demyelination group), and then returned to normal mouse chow until P126, when the behavioral analysis was initiated.

Results: In both groups, the intake of cuprizone for 28 days produced massive demyelination in the corpus callosum by the end of the treatment period, and subsequent normal feeding restored myelination by P126. In a Y-maze test, the spatial working memory was impaired in both groups right after the cuprizone feeding ceased, consistent with previous studies, whereas only the early demyelination group exhibited impaired working memory after remyelination took place. In an open field test, social interactions were decreased in the early demyelination group, but not in the late group. Novel cognition and anxiety-related behaviors were comparable between the two groups.

Conclusions: Our findings suggest that the timing of demyelination has substantial impacts on behaviors of adult mice.

1. Introduction

Xiao et al. (2008) reported that demyelination in the brains of mice which had been administered a copper chelator, cuprizone, led to abnormal behaviors such as spatial working memory impairment and hyperlocomotion (Xiao et al., 2008). Because the former is regarded as an endophenotype of schizophrenia, they proposed that cuprizone-fed mice may represent an animal model of schizophrenia. This study and their following study (Zhang et al., 2008) also revealed that quetiapine ameliorated both the demyelination and the abnormal behaviors, underscoring the causal relationship of the demyelination with schizophrenic symptoms. In addition, high anxiety by cuprizone was also shown in another report (Torkildsen et al., 2009). Therefore, demyelination by cuprizone could be involved in some psychiatric symptoms of mice.

In general, the prognosis of schizophrenia is related to the age at which a patient first develops symptoms: the earlier the onset of schizophrenia, the more severe and prolonged the disturbance due to negative symptoms is likely to be (Alptekin et al., 2005). Several lines of evidence have implicated abnormal myelin and broken white matter in schizophrenia (Hof et al., 2002; Tkachev et al., 2003; Byne et al., 2006) and negative symptoms and cognitive impairment of schizophrenia are thought to be associated with white matter abnormalities (Hof et al., 2003; Wexler et al., 2009). Taking altogether, we hypothesized that the timing of demyelination may influence severity of abnormal behaviors in animal models. To test the hypothesis, we took advantage of the above-mentioned cuprizone-fed mice, because we can easily change the timing of demyelination by altering cuprizone-feeding periods. We produced demyelination by administering cuprizone to mice for 4 weeks, in either the juvenile (early-onset) period or the adult (late-onset) period, and analyzed their behavior just after the cuprizone feeding ended and at the later period when remyelination took place. We found that spatial

Abbreviations: ED, early demyelination; LD, late demyelination; P, postnatal day; PBS, phosphate buffered saline; BSA, bovine serum albumin; NORT, novel object recognition test; EPMT, elevated plus-maze test.

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working memory and social activities were persistently affected in the early-onset demyelination group even at the later period, while late-onset demyelination group showed significant recovery in those functions, suggesting that the timing of demyelination has strong influence on the outcomes of psychosocial behavioral abnormalities.

2. Materials and methods

2.1. Animals and treatments

Five female C57BL/6 mice per cage were housed in a temperature- and humidity-controlled animal facility under a reversed light–dark cycle (lights on 8:00–20:00). Mice from different litters were assigned to three groups: control, early demyelination (ED) and late demyelination (LD) groups. Mice in the control group were fed with normal chow throughout, while mice in the ED group were fed with chow containing 0.2% (W/W) cuprizone from postnatal day (P) 29–P56, and those in the LD group were fed with the same chow from P57–P84. The mice in ED and LD groups were returned to normal feeding after cuprizone treatment. All animals were provided with food and water *ad libitum* throughout the experiments. All these treatments induced no seizures. Experimental protocols followed the guidelines of the Animal Care Committee of Nara Medical University, in accordance with the policies established in the NIH Guide for the Care and Use of Laboratory Animals.

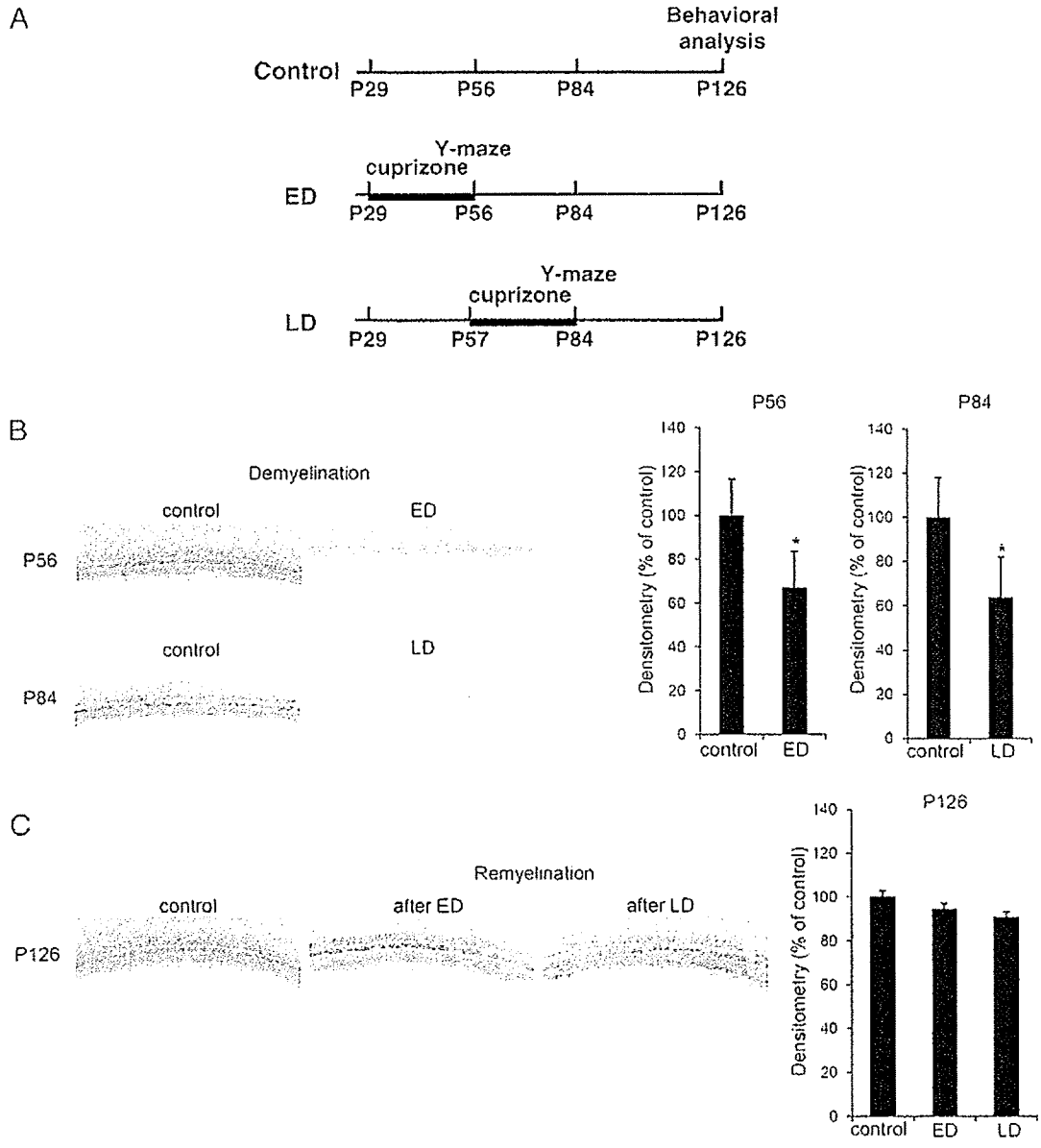

Fig. 1. The time course of this study is shown in (A). ED mice were fed chow containing cuprizone from P29–P56 and LD mice were fed the same chow from P57 to P84. Control mice were fed chow lacking cuprizone throughout the experiments. The behaviors of mice were analyzed from P125 to P126. Panels B and C show the extent of demyelination in the corpus callosum at each period: Representative pictures of MBP immunohistochemistry for control mice at P56 (B), ED mice at P56 (B), control mice at P84 (B), and LD mice at P84 (B) are shown. Control, ED, and LD mice at P126 are shown in panel C. The bar graphs on the right show the result of semi-quantitative analyses of myelination in each mouse. Panels D and E show the extent of demyelination in the prefrontal cortex at each period: Representative pictures of MBP immunohistochemistry for control mice at P56 (D), ED mice at P56 (D), control mice at P84 (D), and LD mice at P84 (D) are shown. Each semi-quantitative analysis of MBP immunoreactivity is shown on the right side. Control, ED, and LD mice at P126 are shown in panel E. The results of semi-quantitative analyses are also shown. Note that ED mice and LD mice had significant demyelination at P56 and P84, respectively (asterisks in the bar graphs), while at P126, there are no significant difference between three groups. CC: corpus callosum.

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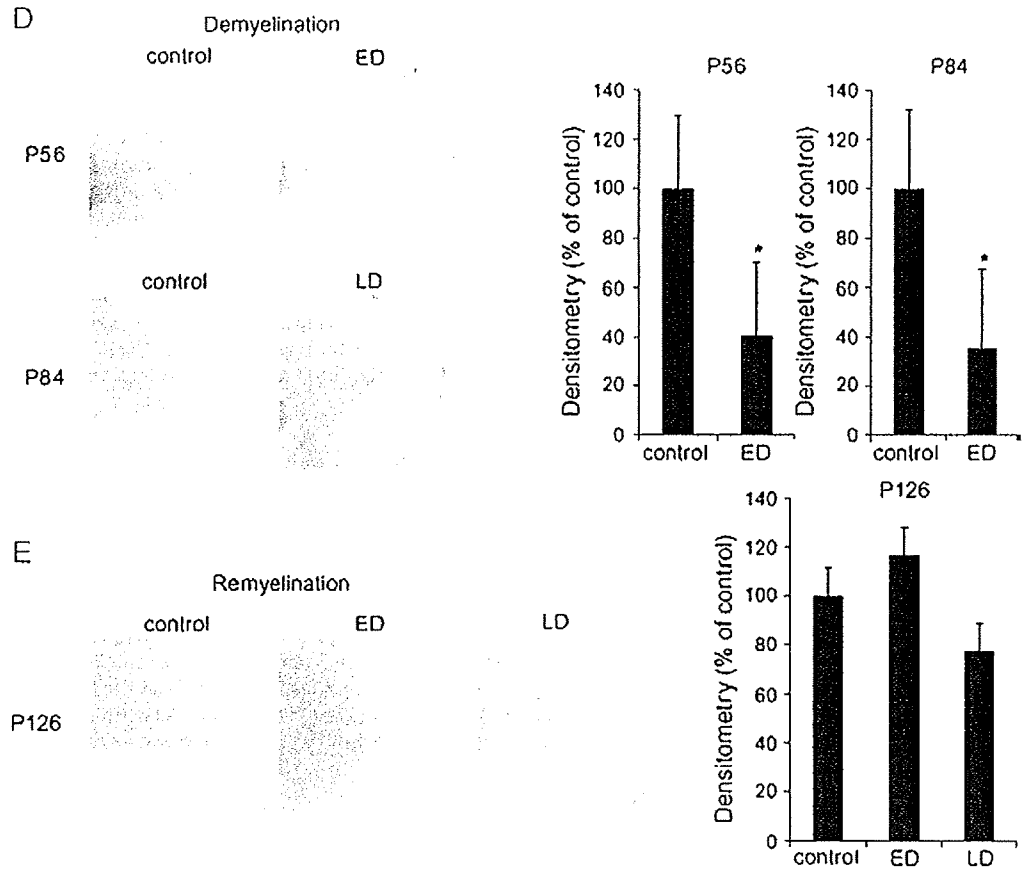

Fig. 1 (*continued*).

2.2. Histology

At P56, P84 and P126, the mice were anesthetized with pentobarbital and transcardially perfused with saline followed by 4% paraformaldehyde. The brain was removed and postfixed in 4% paraformaldehyde overnight at 4 °C, and then dehydrated in graded alcohols (70%; 1 h, 80%; 2 h, 95%; 2 h, 100%; 2 h×4) and xylene (1 h×2). Subsequently, brains were blocked and embedded in Paraffin. Sagittal sections were cut at 8 μm thickness between 1.20 and 1.32 mm for the corpus callosum and 0.72 and 0.84 mm for the prefrontal cortex relative to the bregma and mounted on APS coated slides. Following overnight de-paraffinization in fresh xylene, sections were rehydrated in graded alcohols to distilled water. After antigen-activating with citric acid buffer and blocking in 5% BSA, sections were first incubated overnight at 4 °C with anti-myelin basic protein antibody (MBP) (rabbit polyclonal IgG, 1:200, SIGMA). Sections were then washed and incubated with biotin-conjugated anti-rabbit IgG (1:200, Vector) for 1 h at 37 °C. After washing, the sections were processed for 1 h using a standard Vectastain ABC kit (Vector). Staining was visualized with diaminobenzidine (DAB, Vector) as a chromatic agent. Control slices were incubated as described above, without primary antibodies. No immunoreactivity was seen in controls (data not shown). The gross MBP-immunoreactive areas in three continuous, non-overlapping images from arbitrary brain hemispheres were measured with ImageJ software, in the corpus callosum and the prefrontal cortex by placing fixed squares in the respective region. Positive pixels of a region of interest were distinguished from negative ones by the automatic threshold process of the ImageJ program. The sum of the positive pixels (i.e., immunoreactive area) was averaged per an animal.

2.3. Behavioral testing

All behavioral tests were conducted during the dark period (22:00–6:00). Y-maze test immediately after cuprizone feeding was performed at the age of P56 (ED group and control group) and P84 (LD group and control group), and the other behavioral tests after remyelination were done at the age of P125 or P126. The group order of animals subjected to each test was random.

2.4. Y-maze test

This task is based on exploration of novelty and employed to measure spatial memory. The maze consists of three arms (34 cm long, 6 cm wide and 14.5 cm deep, labeled A, B, or C) diverging at a 120° angle from the central point. The experiments were performed in a dimly illuminated room, and the floor of the maze was cleaned with 70% ethanol-soaked paper after each mouse was tested. Each mouse was placed at the end of one arm and allowed to move freely in the maze during an 8-min session. The sequence of arm entries was recorded manually. An actual alternation was defined as entries into the three arms on consecutive occasions (e.g. the sequence, ABCBCBCA is counted two with the first consecutive ABC and the last consecutive BCA). Then, the maximum alteration was the total number of arm entries minus two and the percentage of alternation was calculated as (actual alternations / maximum alternation) × 100. The total number of arms entered during the sessions was also recorded.

2.5. Open field test

The open field consists of a square acrylic box (40×40 cm). To monitor locomotor activity in a novel whole field (40×40 cm) and a

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novel central field (20×20 cm) (novel test), mice were placed in a novel open field for 20 min and the activity was analyzed using TopScan Suite (Clever Sys) ($n=10$, control; $n=10$, ED; $n=10$, LD).

After a novel open field test, we analyzed social interaction in the familiar open field. One group consisted of 10 mice from two cages, each housing five mice. Social interaction was analyzed within each group. Two mice from different cages of one group were placed at opposite corners in the open field and their social activities were monitored for 10 min using TopScan Suite. Pairs of mice from the same group were analyzed sequentially in the same way, and, after the fifth pair was analyzed, the social activities of a pair consisting of one mouse from the first session and one from the second session were studied. Round sessions (10 sessions, collectively) were thus performed within one group.

We monitored social contact, social approach and social sniffing as aspects of social interaction. These activities were measured for two parameters, namely the action and its duration. First, an action in social contact is defined as an inter-body distance of <20 mm between the two animals and its duration is defined as a continuous time for the action of >0.5 s. Next, an action in social approach is defined as the moving direction and moving criterion. The moving direction requires that the approaching mouse moves towards the other mouse, and that the angle between the moving direction and a straight line to the other mouse should be less than the specified threshold of 30°; the moving criterion requires that the approaching mouse should move faster than the specified threshold of 10 mm/s. The duration in social approach is defined as the continuous time of the action (>2.0 s). Finally, an action in social sniffing is defined as the distance between the nose of the sniffing mouse and the body of the mouse being sniffed (<30 mm). The duration in social sniffing is defined as the continuous time of the action (>0.27 s).

2.6. Novel object recognition test

In a novel object recognition test (NORT) session, two objects, which are different in shape and color but similar in size, were placed diagonally, 18 cm from the nearest corner, in a familiar box (40×40 cm). Each mouse was allowed to explore in the box for 10 min. After this training, the mouse was returned to its home cage, and the box and objects were cleaned with ethanol to avoid any effects of odors. The mouse was then allowed to explore for 5 min in the same box, with one novel object replacing one object used in the training session, 24 h after the training session. A mouse was considered to be exploring the object when its nose was within 2 cm of the object. The time spent exploring each object was recorded using TopScan Suite, and the ratio of time spent exploring the novel object to the total time spent exploring both objects was calculated as the index of memory function.

2.7. Elevated plus-maze test

The elevated plus-maze test (EPMT) was used to evaluate anxiety-related behaviors. The apparatus for EPM consists of two opposed open arms (5×30 cm) and two opposed closed arms of the same size, at right angles to the open arms. The latter were enclosed with walls 15 cm high. The plus-maze was elevated 65 cm above the floor. Mice were placed in the center of the maze and allowed to explore the maze freely for a 5-min testing period. The time spent in open and closed arms, the number of entries into each arm, and the distance traveled during the session were recorded and analyzed using TopScan Suite.

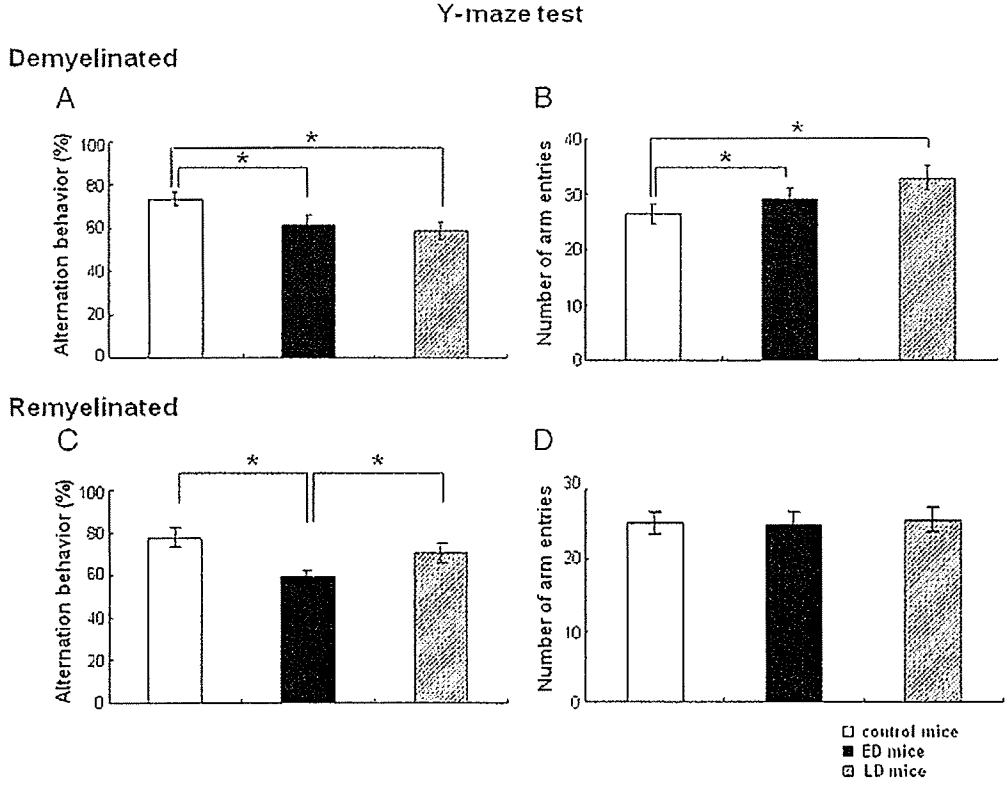

Fig. 2. Y-maze test. Spatial working memory was analyzed by Y-maze test immediately, 10 weeks (ED mice) and 6 weeks (LD mice) after cuprizone feeding. The percentage of alternation behaviors in Y-maze tests decreased immediately after cuprizone feeding in both ED mice and LD mice compared with control mice (A) ($p<0.05$). The number of arm entries in the Y-maze test increased in both ED mice and LD mice compared with control mice (B) ($p<0.05$). Although the percentage of alternation behaviors for LD mice in the Y-maze test reverted to the level of control mice, that for ED mice remained low 10 weeks after cuprizone feeding (C) (LD: $p>0.05$; ED: $p<0.05$). The number of arm entries was not different among the three groups (D) ($p>0.05$). $n=10$ per group.

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2.8. Statistical analysis

All data in the present study showed normal distribution and no different variant verified by Kolmogorov–Smirnov test and *F*-test, respectively. The differences between groups were determined with one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn test ($p<0.05$ defining significance) except for two-paired histological data with Student's *t*-test ($p<0.05$ defining significance).

3. Results

3.1. Cuprizone intake produces demyelination and its cessation induces remyelination

The corpus callosum is a representative white matter in mice and humans which could be involved in the pathophysiology of schizophrenia (Gasparotti et al., 2009) and the prefrontal cortex is a responsible region for spatial working memory and social behaviors which are pivotal behaviors for schizophrenia (Brunet-Gouet and Decety, 2006; Enomono and Floresco, 2009). We therefore focused on the extent of demyelination and remyelination in these regions after the cessation of cuprizone treatment. The intake of 0.2% cuprizone for 4 weeks resulted in distinct demyelination either in the ED group at P56 or in the LD group at P84, whereas no demyelination was observed in control mice either in the corpus callosum or in the prefrontal cortex (Fig. 1B, corpus callosum: ED, $p=0.0008$, LD, $p=0.001$; Fig. 1D, prefrontal cortex: ED, $p=0.002$, LD, $p=0.0008$). Myelination in the cuprizone-treated groups had been restored at P126, when behavioral analyses were performed (Fig. 1C, corpus callosum: $F=0.77$, $p=0.926$; Fig. 1E, prefrontal cortex: $F=0.989$, $p=0.409$).

3.2. Spatial working memory deficit is restored with concomitant remyelination in LD but not in ED mice

First, we analyzed spatial working memory by Y-maze test. The alternation behavior in this test decreased in both the ED and LD groups immediately after the termination of cuprizone feeding, which is consistent with a previous report (Xiao et al., 2008), while the control mice showed no decrement (Fig. 2A: $F=5.367$, $p=0.011$; control vs ED, $p=0.004$, control vs LD, $p=0.012$). The number of entries to each arm was higher in the ED and LD mice than in the control mice (Fig. 2B: $F=5.825$, $p=0.008$; control vs ED, $p=0.016$, control vs LD, $p=0.024$). After remyelination had occurred, the alternation behavior decreased only in the ED mice, and not in the LD mice (Fig. 2C: $F=6.793$, $p=0.004$; control vs ED, $p=0.006$, control vs LD, $p>0.05$, ED vs LD, $p=0.02$), and the number of entries to each arm was not different among the three groups (Fig. 2D: $F=0.039$, $p>0.05$).

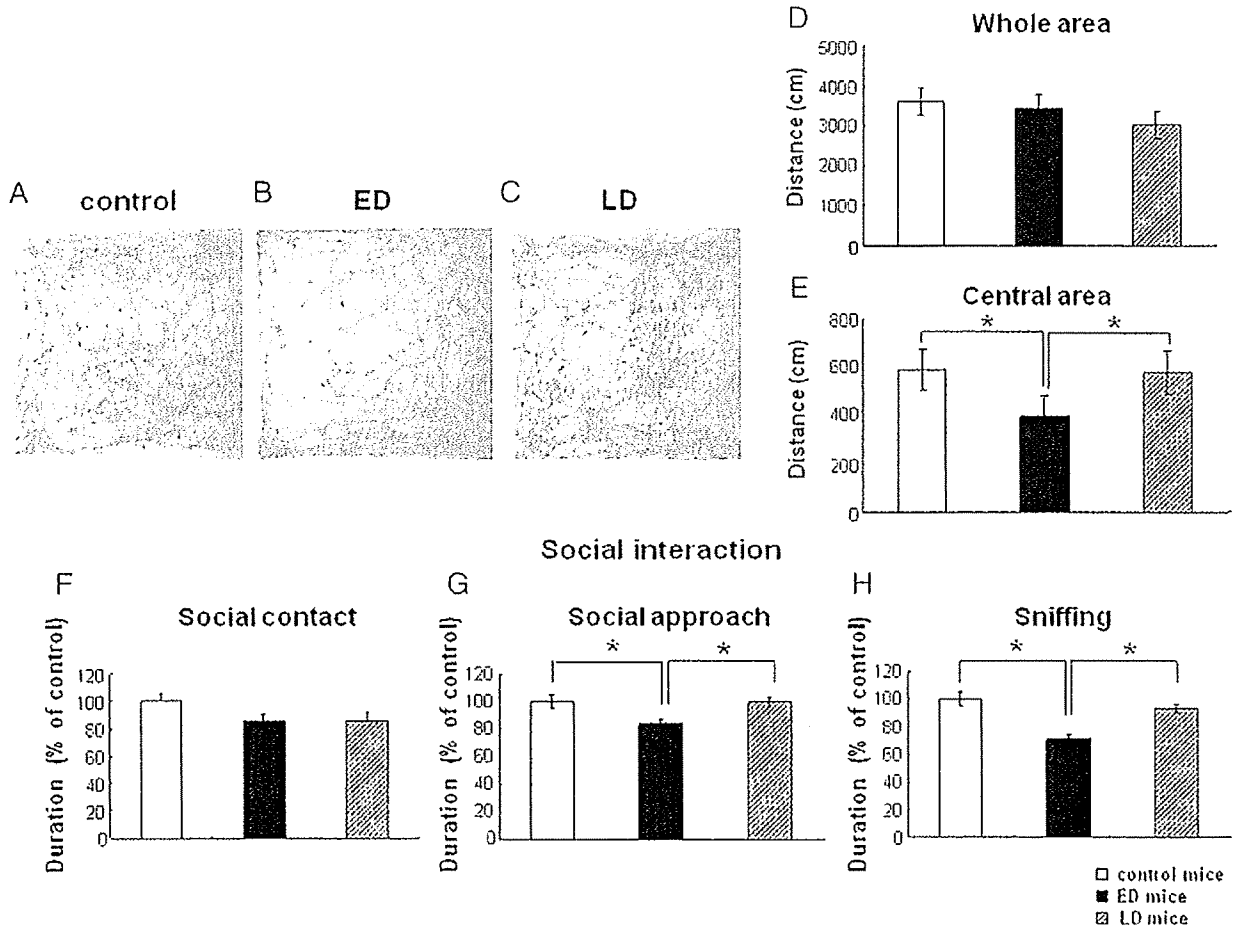

Fig. 3. Open field test. The locomotor activity and the social activity were analyzed 10 weeks (ED mice) and 6 weeks (LD mice) after cuprizone feeding. The locomotive distance of mice in a novel open field was not different among control mice, ED mice and LD mice (A, B, C, D; $p>0.05$); however, the locomotive distance in a central area of the open field was lower in ED mice than in control mice and LD mice (A, B, C, E; $p<0.05$). Social activities were measured in three ways: social contact, social approach, and social sniffing. The duration of social contact was not different among the three groups (F; $p>0.05$), but the duration of social approach and social sniffing in ED mice was lower than in control and LD mice (G, H; $p<0.05$). $n=10$ per group.

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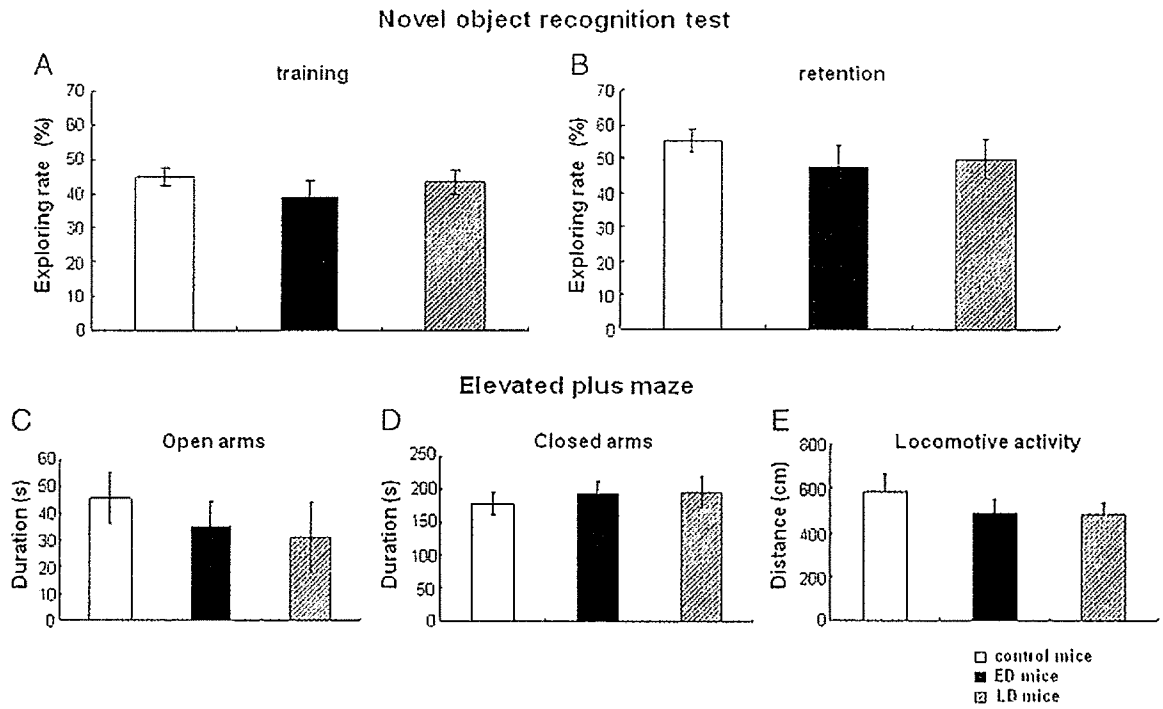

Fig. 4. NORT and EPMT. There were no differences in cognitive functions in NORT between control mice, ED mice and LD mice. The length of time spent exploring the two objects did not differ among the three groups in the training session (A). In the retention session, also, the time spent exploring the novel object was not significantly different in the three groups (B). EPMT revealed that anxiety level did not differ significantly between control mice, ED mice and LD mice. The time spent by all three groups in either the open arms or the closed arms was comparable (C, D) ($p>0.05$), as was the exploring distance in the three groups (E) ($p>0.05$). $n=10$ per group.

3.3. ED mice have high thigmotaxis and low social interaction after remyelination

The locomotive distance in a novel open field was not different in control, ED and LD mice (Fig. 3A, B, C, D: $F=0.629$, $p>0.05$). However, the moving distance in a novel central field was lower in the ED mice than in the control and LD mice (Fig. 3A, B, C, E: $F=6.344$, $p=0.006$; control vs ED, $p=0.02$, control vs LD, $p>0.05$, ED vs LD, $p=0.01$). While the duration in social contact was not different in the three groups (Fig. 3F: $F=2.190$, $p=0.12$), the duration in social approach and sniffing was shorter in ED mice (Fig. 3G, social approach: $F=6.700$, $p=0.004$; control vs ED, $p=0.01$, control vs LD, $p>0.05$, ED vs LD, $p=0.013$; Fig. 3H, sniffing: $F=7.998$, $p=0.002$; control vs ED, $p=0.005$, control vs ED, $p>0.05$, ED vs LD, $p=0.006$). These data suggest that ED mice show higher thigmotaxis and lower social interactions than the LD and control mice.

3.4. ED mice are normal in the novel object recognition test and do not show anxiety-related behaviors

The length of time spent exploring two objects in the field was not different in the three (control, ED and LD) groups in the NORT training session (Fig. 4A: $F=0.628$, $p>0.05$). In the retention session, the three groups of mice spent comparable periods exploring the novel object (Fig. 4B: $F=0.692$, $p>0.05$). In the EPMT, the duration spent in the open arms and closed arms was not different in any group of mice (Fig. 4C, D: $F=0.516$, $p>0.05$, $F=0.243$, $p>0.05$, respectively), and the locomotive distance during the session was also not different among the three groups (Fig. 4E: $F=0.825$, $p>0.05$).

4. Discussion

Increasing data have shown that schizophrenia is a neurodevelopmental disorder which is attributed to genetic and environmental factors (Weinberger, 1987, 1995; Murray et al., 1992; Pearce, 2001; Meyer et al., 2005). Imaging studies revealed not only atrophic gray matter, but also white matter deficits in frontal and temporal lobe (Janssen et al., 2008; Lui et al., 2009; Schneiderman et al., 2009) and postmortem histological examination has shown dysmyelination or oligodendrocytic apoptosis in schizophrenic patients (Uranova et al., 2001; Flynn et al., 2003; Stewart and Davis, 2004; Aberg et al., 2006) although the causality remains unclear. These data mean that myelination in tracts could have deficits in schizophrenic patients. Additionally, neuregulin1 and sphingomyelin could be involved in the pathophysiology of schizophrenia, which suggests that myelination could be affected in the brains of schizophrenic patients because either neuregulin1 or sphingomyelin is critical for myelination (Schmitt et al., 2004; Corfas et al., 2004; Roy et al., 2007).

Therefore, in the present study, we focused on the relationships between the onset of disease and the cognitive deficits of schizophrenia. To this goal, we sought to mimic early- and late-onset schizophrenia using a cuprizone-induced demyelination model (Xiao et al., 2008). This model has an important advantage that we could easily set "onset time" by starting and ending cuprizone treatment arbitrarily. However, it should be noted that cuprizone causes widespread demyelination in the brain and used to produce model mice of multiple sclerosis (Koutsoudaki et al., 2009), which is not generally observed in the brains of schizophrenic patients (Mitterauer et al., 2007). Therefore, the cuprizone-fed mouse never fully reflects the actual situation of human schizophrenia, but rather be a model for studying an aspect of the pathophysiology of schizophrenia. In this context, the present findings must be corroborated with different models, such as amphetamine-induced model and phencyclidine-induced model of schizophrenia (Featherstone et al., 2007; Enomono et al., 2007).

Generally, the peak of myelination is around P14 in the brains of mice and around P1–2 in the brains of humans, but that still continue until adulthood (Matthieu et al., 1973; Lauriat et al., 2008). On the other hand, behavioral alterations like schizophrenia are not obvious in mice until P35

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(Howland et al., 2004; Ozawa et al., 2006). Therefore, we administrated cuprizone for ED from P29 to P56, when myelination continues slowly in brains and abnormal behaviors like schizophrenia could become manifest in mice. This means that the period of ED corresponds to puberty or adolescence in humans when myelination continues slowly at least in the prefrontal cortex and the early onset of schizophrenia is. On the contrary, LD is from P57 to P84 when myelination might continue, but abnormal behaviors like schizophrenia could be recognized in most cases in mice. Then, we could compare the behaviors of ED and LD mice as early-onset schizophrenia and late-onset schizophrenia, respectively.

We analyzed spatial working memory in each mouse by a Y-maze test. The early and late demyelination (ED and LD) treatment groups approximately correspond to the periods typical for an early manifestation of psychosis from puberty to late adolescence (disorganized schizophrenia) and a late manifestation of psychosis in adulthood), respectively. Both treatments led to the impairment of spatial working memory, consistent with previous data (Xiao et al., 2008). However, when remyelination was achieved, spatial working memory was kept impaired only in the ED mice, but not in the LD mice. These findings revealed that the impairment of spatial working memory in the ED mice was irreversible or very long-lasting by unknown mechanisms as compared to the LD mice. In addition, the ED mice, but not the LD mice, showed high thigmotaxis and low social interaction with the same level of locomotion as control mice after remyelination in an open field test. These results suggest that early demyelination, but not late demyelination, in mice results in long-lasting abnormal behaviors which may be reminiscent of the adverse prognosis in early-onset schizophrenia.

Given the present results, an obvious question is rising: why were the abnormal behaviors of the ED mice long-lasting and irreversible in spite of the remyelination? The discrepancy between the ED and LD mice may be attributed to the plasticity of developing neural circuits. Myelin is indispensable for the formation of neural circuitry in the visual cortex during a critical period in which neural plasticity continues (McGee et al., 2005), and myelination in the prefrontal cortex occurs later than in other brain areas (Lalonde and Badescu, 1995). We speculate that myelination during the early period (P29 to P56) in the present study could be critical for the formation of neural circuits in certain brain areas, such as the prefrontal cortex. In the ED case, the abnormal behaviors are initially caused by demyelination, but they persist for longer, primarily because of secondary defects in neural circuits. In contrast, demyelination in the LD mice produces abnormal behaviors similar to those observed in the ED mice, but remyelination can restore the normal functions of neural circuits. Consistent with this hypothesis, the levels of novel object recognition and anxiety in the ED mice were comparable to those in the control and LD mice. Novel object recognition and anxiety are predominantly subject to the perirhinal cortex (Barker et al., 2007) and amygdala (Hannesson et al., 2008) respectively, both of which are myelinated early in the postnatal period. We speculate that the capacity for novel object recognition and anxiety may already have been determined before the ED period.

Our data reveal that early but not late demyelination induces long-lasting disturbances in cognitive function and social activity, and support a previous suggestion that pro-myelinating interventions are potential approaches to treat schizophrenic patients (Xiao et al., 2008), especially in the case of early-onset schizophrenia. However, we should be cautious enough to apply this model to schizophrenia because demyelination is one of the many pathological facets of schizophrenia. All together, these findings are highly suggestive for onset-dependent differential symptoms of schizophrenia.

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Yi-Gan San Restores Behavioral Alterations and a Decrease of Brain Glutathione Level in a Mouse Model of Schizophrenia

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Abstract: The traditional Chinese herbal medicine yi-gan san has been used to cure neuropsychological disorders. Schizophrenia can be one of the target diseases of yi-gan san. We aimed at evaluating the possible use of yi-gan san in improving the schizophrenic symptoms of an animal model. Yi-gan san or distilled water was administered to mice born from pregnant mice injected with polyinosinic-polycytidilic acid or phosphate buffered saline. The former is a model of schizophrenia based on the epidemiological data that maternal infection leads to psychotic disorders including schizophrenia in the offspring. Prepulse inhibition and sensitivity to methamphetamine in open field tests were analyzed and the total glutathione content of whole brains was measured. Yi-gan san reversed the decrease in prepulse inhibition, hypersensitivity to methamphetamine and cognitive deficits found in the model mice to the level of control mice. Total glutathione content in whole brains was reduced in the model mice but was restored to normal levels by yi-gan san treatment. These results suggest that yi-gan san may have ameliorating effects on the pathological symptoms of schizophrenia.

Keywords: Yi-gan san (yokukansan), schizophrenia, open field test, prepulse inhibition, cognitive deficits, glutathione

Introduction

Yi-gan san (YGS, yokukan-san in Japanese) has been administered to children for the treatment of restlessness and agitation. Recent studies revealed that YGS is also useful in treating neuropsychological disorders such as behavioral and psychological symptoms of dementia (BPSD) in the elderly,[1-4] a number of symptoms of borderline personality disorder,[5] tardive dyskinesia and psychotic symptoms of schizophrenia.[6,7]

In the case of schizophrenia, many effective antipsychotics have been developed and widely used, but some of them induce drowsiness and extrapyramidal symptoms (EPS). YGS has only mild sedative effects and induces no EPS, making it a promising candidate as an antipsychotic for schizophrenia. In the present study, we examined the efficacy of YGS in treating a mouse model of schizophrenia, which is based on the epidemiological data that maternal infection leads to schizophrenia in offspring.[8] Polyinosinic-polycytidilic acid (poly I:C) is commonly used to induce an immune response similar to that induced by viral infection.[9] When early pregnant mice receive intraperitoneal poly I:C injection, the behavior of their pups (poly I:C-mice) becomes schizophrenic. The prepulse inhibition is decreased, the sensitivity to dopamine release is increased, and the cognitive function is impaired in poly I:C-mice.[10-13] Several reports have shown that the abnormal behaviors were improved by antipsychotic treatments. In this model, we investigated whether YGS could restore impaired PPI, methamphetamine hypersensitivity in an open field test and cognitive deficits in a novel object recognition test (NORT). In addition, we measured total glutathione content (both reduced and oxidized species) in whole brain after oral administration of YGS to poly I:C-mice, since the cellular glutathione level is linked to the pathogenesis of schizophrenia[14] and the brain and cerebrospinal fluid glutathione contents were decreased in schizophrenic patients.[15]

Material and Methods

Animals, prenatal treatment and YGS administration

C57BL/6 mice were mated at about 3 months of age and the first day after copulation was defined as embryonic day 0 (E0). Pregnant mice received either a single i.p. injection of poly I:C (20 mg/kg,[13])

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dissolved in phosphate buffered saline (PBS) or an equivalent volume of PBS at embryonic day 12.5 (E12.5). Pups born from poly I:C-treated mice and PBS-treated mice are hereafter referred to as poly I:C-mice and PBS-mice, respectively. Pups were weaned and housed 4 to a cage according to sex and litter at postnatal day 21 (P21), and housed in a temperature- and humidity-controlled animal facility under a reversed light-dark cycle (lights on 8:00~20:00). Poly I:C-mice and PBS-mice were each divided into two groups containing twelve pups from three different litters. The three groups of pups were designated as PBS-control, poly I:C-control, and poly I:C-YGS groups. YGS suspended in distilled water (DW) (1 g/kg, 0.5 ml/day×21 days; from P56 to P76) was administrated using a syringe with a metal feeding tube to poly I:C-YGS groups and the same amount of DW was given to control pups on the same schedule. Locomotor activity in an open field test and PPI were measured at P77. For the biochemical analysis of total glutathione content (see below), three other groups of pups (four pups in each group) were treated as above (YGS group and control group) and whole brains were removed at P77. All animals were male and maintained with food and water ad lib through the duration of experiments. Experimental protocols were according to the guidelines of the Animal Care Committee of Nara Medical University and were in accordance with the policies established in the NIH Guide for the Care and Use of Laboratory Animals.

Prepulse inhibition test

The mouse was placed in a translucent acrylic cage (7 cm × 7 cm × 16.5 cm). Movements of animals were detected by a piezoelectric accelerometer (GH313A, GA245SO: Keyence, Kyoto, Japan) attached to the bottom of the cage. White noise at 115 dB for duration of 50 msec was used as the acoustic startle stimulus (pulse). A noise prepulse of 85 dB was presented for 30 msec. Background noise was kept at a relatively constant level, 70–73 dB. The test session consisted of a total of 19 trials: 10 startle trials without a prepulse (habituation), followed by 9 trials of prepulse test session. The mean inter-trial interval was 25 sec (range, 15–45 sec). In the prepulse trials, prepulse with a lead time of 50 msec was followed by the pulse. Four pulse-alone trials and five prepulse trials were presented in random order. Relative startle response (RSR) was calculated using the formula RSR = (1-PP/N) × 100, where PP was designated as the mean response with prepulse and N was designated as the mean response without a prepulse. (n = 12, PBS-mice-control; n = 12, poly I:C-mice-control; n = 12, poly I:C-mice-YGS).

Open field test

The open field consists of a square acrylic box (40 × 40 cm) and a video camera for recording locomotion of mice. For monitoring of locomotor activity in a novel environment (novel test), mice were placed in a novel open field for 10 min, and the successive activity in the same field during the following 10 min was measured as basic locomotor activity. Next, the locomotor activity was monitored for 10 min in the same open field starting 30 min after the injection of methamphetamine (1 mg/kg). We then analyzed the video-recorded locomotion using tracking software, TopScan Suite (Clever Sys Inc.) (n = 12, PBS-mice-control; n = 12, poly I:C-mice-control; n = 12, poly I:C-mice-YGS).

Novel object recognition test (NORT)

In a session, two objects, which are different in their shape and color, but similar in size, were placed diametrically opposite each other apart from the corner (18 cm) in a familiar box (40 × 40 × 40 cm). Before the session, mice were habituated in the box for 3 days. Each mouse was allowed to explore in the box for 10 min. The mice were considered to be exploring the object when the nose, not the body, was within 2 cm from the edge of the object. The time spent exploring each object was recorded using TopScan Suite (Clever Sys Inc.,). After the training, the mice were returned to their home cages, and the box and objects were cleaned with ethanol to avoid any effects of odors. The mice were allowed to explore for 5 min in the same box with one novel object instead of one object used in the training session 24 h after the termination of the training session. The time spent exploring each object was recorded as described above. The ratio of time spent exploring any one of the two objects (training session) or the novel object (retention session) to the total time spent exploring both objects was employed for the measure of memory function. (n = 11, PBS-mice-control; n = 10, poly I:C-mice-control; n = 9, poly I:C-mice-YGS).

Total glutathione assay

The whole brains of PBS-mice with DW, poly I:C-mice with DW and poly I:C-mice with YGS were removed. Brains were homogenized in 5% sulphosalicylic acid (0.5 mg/ml) and centrifuged at 8000 × g for 10 min. The supernatant was assayed using a total glutathione quantification kit (Dojin Molecular Technologies Inc., Japan) according to instructions provided by the manufacturer. Briefly, the total glutathione content was detected by measuring the optic density of samples and glutathione standard solutions. (n = 4, PBS-mice-control; n = 4, poly I:C-mice-control; n = 4, poly I:C-mice-YGS). Reduced glutathione and 2-nitrobenzoic acid in the kit react to generate 2-nitro-5-thiobenzoic acid and glutathione disulfide. Since 2-nitro-5-thiobenzoic acid is a yellow colored product, glutathione concentration in a sample solution can be determined by the measurement at 412 nm absorbance. Reduced glutathione is generated from 2-nitro-5-thiobenzoic acid by glutathione reductase, and reacts with 2-nitrobenzoic acid again to produce 2-nitro-5-thiobenzoic acid. Therefore, this recycling reaction improves the sensitivity to total glutathione detection.

Statistical analysis

Bonferroni's test was used to determine the significant differences. Values of $p < 0.05$ were considered to be statistically significant.

Results

YGS restored a decrease in prepulse inhibition of poly I:C-mice

PPI was decreased in the offspring born from poly I:C-injected mice (poly I:C-control group) compared with control mice (PBS-control group)(Fig. 1, $p < 0.05$). YGS administration (poly I:C-YGS group) significantly reversed the reduction in PPI in poly I:C-control mice (Fig. 1, $p < 0.05$) to a similar level as in PBS-control mice (Fig. 1, $p > 0.05$).

YGS had no effect on basal activity, but prevented hyperlocomotion after methamphetamine injection in an open field test

In the novel open field, locomotor activity in PBS-control, poly I:C-control and poly I:C-YGS mice

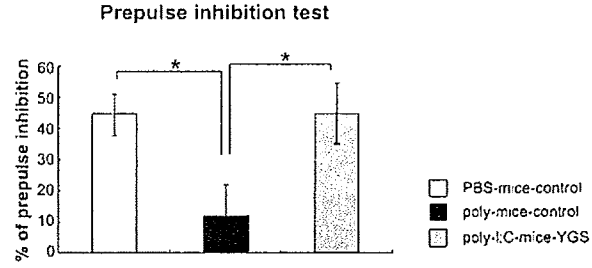

Figure 1. Prepulse inhibition test. Prepulse inhibition was decreased in poly I:C-control mice compared to PBS-control mice (*; $p < 0.05$). YGS reversed the disruption of prepulse inhibition in poly I:C-control mice to the level of PBS-control mice (*; $p < 0.05$).

was not different (Fig. 2A, $p > 0.05$). Basal locomotor activity in the familiar open field was also not different in the three groups (Fig. 2B, $p > 0.05$). The activity of poly I:C-control mice was significantly higher than that of PBS-control mice after the injection of methamphetamine (Fig. 2C, $p < 0.05$). YGS administration significantly reversed the increased activity of poly I:C-control mice (Fig. 2C, $p < 0.05$) to a similar level to that of PBS-control mice (Fig. 2C, $p > 0.05$).

YGS improved cognitive deficits of poly I:C-mice

Poly I:C-mice showed cognitive deficits in NORT as reported previously.[12] The rate of time spent exploring the two objects was not different in PBS-control, poly

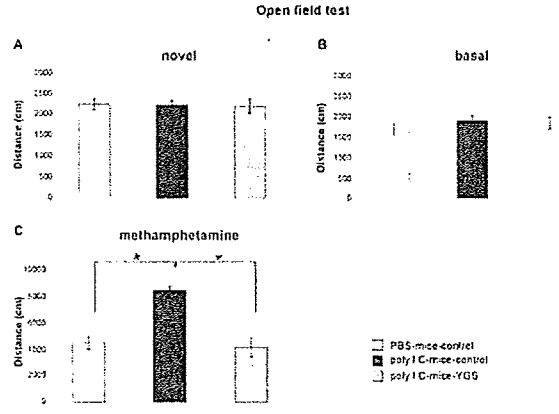

Figure 2. Open field test. Locomotor activity in the novel environment for the first 10 min was not different in PBS-control mice, poly I:C-control mice and poly I:C-YGS mice (A). In the next 10 min, the locomotor activity in the same field was also not different in the three groups (B). YGS attenuated the hyperactivity of poly I:C-control mice to the level of PBS-control mice in the same field for 10 min, 30 min after the injection of methamphetamine (C,*; $p < 0.05$).