研究成果の刊行物・別刷

特集
臨床開発の最近の動向
臨床精神薬理　13：289-296, 2010

MATRICSコンセンサス認知機能評価バッテリーの開発

——統合失調症治療への導入を目指して——

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抄録：認知機能障害は統合失調症の中核症状の1つとされ，その改善に向けた治療法の開発が望まれている。これまで，統合失調症患者の認知機能を包括的に評価し，かつ国際標準となりうるテストバッテリーが存在せず，認知機能障害の改善を目的とした治療薬などの開発を妨げる要因の1つとなっていた。こうした中，米国立精神保健研究所（NIMH）主導のもと，MATRICSコンセンサス認知機能評価バッテリー（MCCB）が近年開発され，統合失調症の認知機能を評価する標準的なテストバッテリーとして米国食品医薬品局（FDA）にも認められている。本論文ではMCCB最終版の開発に至るまでの過程ならびに同バッテリーの特徴を紹介した。また，本邦へのMCCB導入に関して，グローバル治験への参加の必要性などの背景を踏まえ，その過程と意義について論じた。

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Key words： *cognition function, schizophrenia, MATRICS Consensus Cognitive Battery (MCCB), functional outcome*

Ⅰ．はじめに

統合失調症は，幻覚・妄想・思考障害などの陽性症状，感情的引きこもり・自閉などの陰性症状，記憶，遂行機能，注意，語流暢性などの認知機能領域の障害を伴う[18]。このうち，認知機能障害は，精神病初回エピソード[3]や顕在発症前[16,36]の時点ですでに認められ，発症後も安定して存在することが知られている[35]。また，社会適応や就労状況といった機能的転帰に影響し[11,12]，長期的予後の予測因子にもなっている[13]。以上から，認知機能障害は統合失調症の中核症状の1つとして捉えられ，認知機能改善に向けた治療法の開発が望まれている[14,17,29]。

認知機能の改善を目指した薬物の開発には，国際標準となり得る認知機能評価バッテリーを用いることが望まれる。従来は，認知機能の各領域を反映する複数の検査を個別に組み合わせた神経心理学的テストバッテリー（NTB）が用いられることが多かった[19]。しかしこの方法では実施に時間を要する上，使用する検査項目や成績にばらつきが生じ，得られた結果の施設間での比較は困難となる[18]。そのため，認知機能障害の改善薬の治

Development of the MATRICS Consensus Cognitive Battery: towards effective treatment of schizophrenia.

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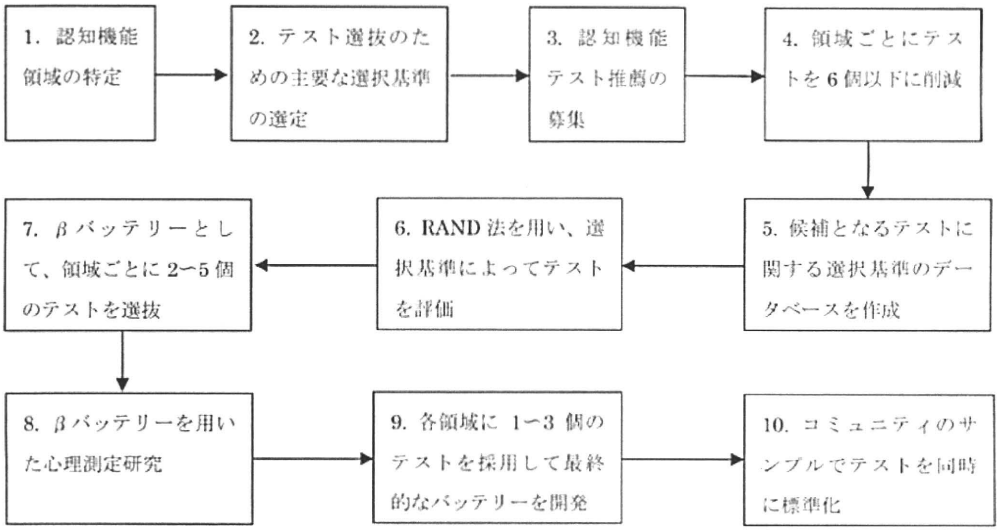

図1　MATRICS コンセンサス認知機能評価バッテリーの作成手順
MATRICS Consensus Cognitive Battery manual[31]より改編

験等においては，特定の企業や研究者により恣意的に選択された評価バッテリーでは限界があった。そこで，信頼性や妥当性，ならびに他の臨床的指標との関連性などに基づく，客観的かつ多数の専門家のコンセンサスを反映した評価バッテリーの使用が必要となった[29]。

Ⅱ．MATRICS コンセンサス認知機能評価バッテリー（MCCB）の開発

米国では，国立精神保健研究所（NIMH）主導のもと，米国食品医薬品局（FDA），学界，製薬企業が連携して"統合失調症における認知機能の改善のための測定と治療研究"（Measurement and Treatment Research to Improve Cognition in Schizophrenia：MATRICS）と呼ばれるプロジェクトを企画した[31]。このプロジェクトでは，統合失調症患者の認知機能を高める薬物の治験に使用可能な，複数の専門家による検討を経た認知機能評価バッテリー（MATRICS Consensus Cognition Battery：MCCB）を開発することが目標とされ[32]，下記の手続きで進められた（図1）。

1．認知機能領域の決定

MCCB の開発には，まず評価する認知機能領域を決定する必要があった。MATRICS コンセンサス会議に先立ち，MATRICS 神経認知委員会の小委員会は，統合失調症と関連する精神疾患を対象とした神経心理学的テストの因子分析研究を中心にレビューを行い，分離可能な7つの認知機能領域を同定した[32]。そのうちの言語理解は，統合失調症で障害が顕著ではない領域であるため[28]，バッテリーからは除かれることになった。最終的に，小委員会によって6つの領域（処理速度，注意 覚醒，ワーキングメモリ，言語学習，視覚学習，および推論と問題解決）が推薦された。

小委員会から推薦されたこれらの認知機能領域は，より大規模な研究者集団（学界，産業界，政府からの100を超える代表）からの情報提供を要請するため，2003年4月に第1回 MATRICS コンセンサス会議で公表された[31]。そこでの議論をもとに，近年になって統合失調症の認知機能障害の領域として注目され始めた社会認知が加えられ[21]，最終的に7つの認知機能領域が MCCB 作成のために採択された[32]。

表1　MCCBテストの選択基準[15]

1．再テスト信頼性
2．繰り返し用いる評価尺度としての有用性
3．機能的転帰との関連性
4．薬物に対する反応可能性
5．忍容性と実用性

2．テストの選択基準の決定

MATRICS神経認知委員会は，様々なバックグラウンドを持つ専門家からの意見聴取や，コンセンサス会議等での議論の結果をもとに，バッテリーに加えるテストの選択基準として，表1に示される5つの基準を選定した[15]。

1つ目は，再テスト信頼性である。この基準は専門家の間で最も重要な判断基準であった[15]。再テスト信頼性が低くなると，同じ量の変化を検知するのにより大きなサンプルが必要になる[27]。そのため，治験のような短期間の治療効果による変化を鋭敏に検出するには再テスト信頼性が高いことが必須となる。

2つ目は，繰り返し用いる評価尺度としての有用性である。繰り返しによるパフォーマンスの向上（いわゆる"練習効果"）が存在すると，フォローアップ時の遂行成績が上限に近づくことでその変動が小さくなり，治療効果を検知することが困難になる可能性がある[31]。そのため，練習効果のほとんどない評価尺度を選択することが望ましい。テストによっては代替フォームを用いることで練習効果を低減することは可能である。ただし，代替フォームは必ずしもオリジナルのフォームと等質とは言えず，再テスト信頼性を低下させる可能性が指摘されている[15]。

3つ目は機能的転帰との関連性である。FDAは，ある薬剤の認知機能改善効果への適応を承認するには，その投与による日常的機能を反映する評価尺度の成績向上が，認知機能の改善と相関する結果が必要という見解を示している[15]。そのため，候補となる認知機能テストの選択には，機能的転帰のいくつかの側面（たとえば，仕事や自立生活など）との関連性が実証されているものが優先された[31]。

4つ目は薬物治療に対する反応性である。上記の基準を満たしたテストであっても，薬物によって変化しない機能を評価するものである可能性が残る。今のところ，統合失調症の認知機能障害を完全に正常化する薬剤は存在しないが，新しい世代の抗精神病薬に対する反応性を示す認知機能テストが優先された[15]。

最後の選択基準は，忍容性と実用性である。忍容性とは，回答者が感じる課題の取り組みやすさのことである[31]。検査課題が難しすぎたり，課題数が多すぎたりすれば，忍容性は低下する。一方，実用性とは，実施者が感じるテスト実施のしやすさである[31]。これは，テストの準備，スタッフへのトレーニング，施行，採点の容易さによって決まる。忍容性や実用性に問題があると，回答者の反応の質は低下し，脱落率が高まることが懸念される。

3．テストの推薦とβ版バッテリーの決定

MCCBの候補となるテストは，第1回MATRICSコンセンサス会議の前に行われた調査に参加した専門家，および第1回MATRICSコンセンサス会議の参加者によって推薦された。その結果，90以上のテストが候補に挙げられた[24]。MATRICS神経認知委員会は，推薦された各々のテストを7つの認知機能領域に分類し，上記の選択基準により，1領域のテストの数を6つ以下に削減した[15]。その結果，7つの領域で36個のテストに絞られた。

同委員会は，テスト候補をさらに削減したβ版バッテリーを開発するためRANDパネル法[9]を用いた[24]。まず，MATRICSスタッフは，36の候補テストに関する学術論文やマニュアルなどのレビューを通じ，各テストの特徴について包括的で客観的なデータベースを作成した。次に，このデータベースを様々な分野から選出された14人の専門家（パネリスト）に提示し，各テストの特性を評定させた。各評定の不一致については，2003年9月に開催されたRANDパネル会議での議論によって調整された。会議に参加したパネリストはテストの特性を再度評定し，その結果をもとにMATRICS神経認知委員会が最終的なテストの順位付けを行った[31]。この順位に基づき，20個のテ

ストがβ版バッテリーに採択された。

4．最終版バッテリーの決定と共通標準化の実施

β版からMCCB最終版を決定するため，カリフォルニア大学などの5つのサイトで“心理測定および標準化研究”（Psychometrics and Standardization Study：MATRICS-PASS）が実施された[33]。MATRICS-PASSの第1段階では，176人の統合失調症患者を対象に，β版バッテリーの各テストの再テスト信頼性，練習効果，機能的転帰との関連性，実用性・忍容性に関する直接的な比較が行われた。この研究で得られたデータをもとに，MATRICS神経認知委員会が前述の判断基準を鑑み，β版におけるテストに対し領域ごとに順位付けを行った[24]。その結果，MCCB最終版には，7つの認知機能領域を評価する10個のテストが採択された（表2）。

採択された10個のテストの多くに対しては，MCCBに採用される以前に標準データがすでに報告されていたが，それぞれ異なるサンプルで標準化されていたため，結果をテスト間で比較することが困難であった[24]。そのため，MATRICS-PASSの第2段階として，上記の5つのサイトで，コミュニティから募った健常者300人が調査に参加し，共通の規準データが収集された[1]。

MCCB最終版の構成や，治験デザインのための使用法やその他の提言について，その最終決定事項が2005年5月にNIMH国内精神保健審議会に提出された[31]。これらの提言は満場一致で承認され，FDAによって認められた。

Ⅲ．MCCBの特徴

上記の手続きを経て開発されたMCCBは，7つの認知機能領域を測定する10のテストによって構成されている。表2にMCCBの認知機能領域と各テスト名，課題内容，および平均実施時間を示した。各認知機能領域を測定するテストは基本的には1つであるが，実施の容易な「処理速度」には信頼性を高めるために3つの検査（トレイルメイキングテスト：パートA[1]，統合失調症認知機能簡易評価尺度（The Brief Assessment of Cognition in Schizophrenia：BACS）：符号課題[22]，カテゴリー流暢性：動物の名前[37]）が組み込まれた[31]。また，ワーキングメモリに対しては，非言語的な評価尺度としてウェクスラー記憶検査　第3版（Wechsler Memory Scale-Ⅲ：WMS-III）の視覚性記憶範囲[39]が，言語的な評価尺度として語音整列[16]が組み込まれた。

統合失調症患者を対象としたMATRICS-PASS[33]では，MCCBの各テストの平均実施時間は2.0～13.4分，全体で63.6分であり[24]，認知機能を包括的に評価するテストバッテリーの実施時間として，専門家が最適と考える60分[26]とほぼ同値であった。また，忍容性や実用性にも問題はなかった[33]。

認知機能向上を目的とした治療法の評価に最も問題となる再テスト信頼性は，ほとんどのテストで治験に適用可能とみなされる規準を満たしていた[33]。また，練習効果に関しては，4週間後の再施行でいくつかの検査に有意な成績の向上が見られたが，天井効果等は見られず，問題はないとされた。さらに，いくつかの問題点はあったものの，機能的転帰との関連性もある程度は支持された[33]。以上のことからMCCBは，治療効果を複数回評価する治験等での使用に耐えうるバッテリーであることが示された。

Ⅳ．MCCBとBACSの比較

統合失調症の認知機能を測定するテストバッテリーとして有効であり，本邦にもすでに導入されているBACS[20,23]とMCCBを比較すると，いくつかの類似点と相違点が見られる。

まず類似点として，個々のテストが同時に標準化されたことが挙げられる。目的に応じていくつかのテストを組み合わせたNTBの場合，用いるテストの多くは出版社やテスト開発者が公表した規準データを利用することは可能である。ただし，それぞれのテストの規準データは，サンプルサイズも参加者の構成も異なるため，テスト間での比較が困難になる。そのため，テストバッテリーの規準データは同一のサンプルである必要がある[25]。MCCBとBACSは，それぞれ個々のテ

表2　MCCBテストにより測定される7つの認知機能領域

認知機能領域	テスト	検査の解説（平均実施時間[23]）
処理速度	統合失調症認知機能簡易評価尺度（BACS）：符号課題[22]	見本を見ながら無意味記号に対応した数字を書き込む制限時間ありの筆記テスト（3.0分）
	カテゴリー流暢性：動物の名前（流暢性）[37]	1分間に動物の名前をできるだけ多く述べる口頭テスト（2.0分）
	トレイルメイキングテスト：パートA（TMT）[37]	紙面に不規則に配置された番号のついた丸を，順番につなげるように線を引く筆記テスト（2.1分）
注意・覚醒	持続的注意集中検査―同一ペア（CPT-IP）[7]	同じ数字が続けて呈示された場合にボタンを押す，持続的注意を測定するコンピュータを用いたテスト（13.4分）
ワーキングメモリ（非言語）	ウェクスラー記憶検査―第3版（WMS-III）：視覚性記憶範囲[39]	10個の立方体が不規則に置かれたボードを用いて，実施者と同じ（もしくは逆の）順番で立方体を触るテスト（5.1分）
（言語）	語音整列（LNS）[10]	検査者から呈示された数字と文字の系列を頭の中で順番を直し，暗唱する口頭テスト（5.9分）
言語学習	ホプキンス言語学習テスト改訂版（HVLT-R）[4]	3種類に分類できるカテゴリーから12個の単語リストが呈示され，それぞれ3回の学習試行の後にできるだけ多くの単語を再生する口頭テスト（4.1分）
視覚学習	簡易視空間記憶テスト改訂版（BVMT-R）[2]	6個の幾何学図形を記憶によって再生する検査（4.7分）
推論と問題解決	神経心理学評価バッテリー（NAB）：迷路[8]	洞察力と計画性を測定するための筆記型の迷路検査。7つの迷路は次第に困難になり，かつ時間制限がある（11.2分）
社会認知	マイヤー・サロヴェイ・カルーソー感情知能テスト（MSCEIT）：感情の管理[30]	回答者がどのように自分の感情を管理するかを評価する多項目選択式筆記検査（12.0分）

注）MATRICS Consensus Cognitive Battery manual[31]より改編

ストを同じ基準集団を用いて標準化しているため，テスト間の比較を行うことが可能である。そのため，既存のテストを単に組み合わせたNTBに比べると，MCCBとBACSはテスト結果の解釈可能性の点で優れていると考えられる。

次に，両テストバッテリーにより測定される認知機能領域の類似点と相違点について述べる。表3に示すように，7領域中4つの認知機能領域（処理速度，ワーキングメモリ，言語学習，推論および問題解決）は同じ領域を測定する検査と考えられるが，残りの3領域はBACSには取り入れられていない領域であった。1つは視覚学習の領域であり，この領域を測定するために簡易視空間記憶テスト改訂版（Belief Visuospatial Memory Test-Revised：BVMT-R）[2]が導入されている。2つ目は注意・覚醒の領域であり，この領域を測定する持続的注意集中検査-同一ペア（Continuous Performance Test-Identical Pair Version：CPT-IP）[7]が取り入れられた。さらに，MCCBには，基礎的な認知機能領域だけではなく，社会認知[30]という領域が取り入れられている。

社会認知とは，社会的な相互作用の基礎となる心的操作から構成されており，他者の意図や内的状態を知覚するための能力，および潜在能力を含むものである[3]。MCCBでは，社会認知の領域を測定するために，マイヤー・サロヴェイ・カルーソー感情知能テスト（Mayer-Solvey-Caruso Emotional Intelligent Test：MSCEIT）[30]の部門4「感情の管理」を組み込んでいる。この下位検査は，MATRICS-PASSの第一段階において，全体的な機能的転帰と最も関連性が高い検査であった[33]。また，他の研究でも，MSCEITと機能的転帰との

表3　各認知機能領域に含まれるMCCBとBACSの下位テスト

領域	MCCB下位テスト	BACS下位テスト
処理速度	BACS符号課題	符号課題
	カテゴリー流暢性：動物の名前	カテゴリー＆文字流暢
	TMTパートA	トークン運動課題
注意/覚醒	CPT-IP	—
ワーキングメモリ	WMS-III視覚性記憶範囲	数字順列課題
	語音整列	
言語学習	HVLT-R	言語性記憶課題
視覚学習	BVMT-R	—
推論と問題解決	NAB迷路	ロンドン塔検査
社会認知	MSCEIT感情の管理	—

関連性を示す結果が報告されている[8,21]。さらに，MSCEIT以外の検査を用いた研究ではあるが，いくつかの研究で，社会認知は基礎的な認知機能領域と機能的転帰を媒介する変数となる可能性が示唆されている[6,38]。以上より，社会認知を測定するテストを下位検査に含めた意味は大きいと考えられる。

なお，MCCBとBACSでは実施時間が異なることを指摘しておきたい。前述のようにMCCBの実施時間は約60分であり，BACSの約35分[23]に比べると長い。もちろん，測定する認知機能領域の数とのトレードオフになるが，日常診療での応用という見地からは，実施時間が短いBACSの方が用いやすいと思われる。一方，治験のような薬剤の効果を評価する場合は，認知機能領域によりポジティブあるいはネガティブな効果が検出される可能性も考えられる。そのような際には，認知機能をより精緻に測定しうるMCCBの方が適していると思われる。つまり，目的によってテストバッテリーを使い分けることが望ましいと考えられる。

V．MCCB日本語版の開発

昨今，世界の有力市場における新薬の同時承認を目指すグローバル治験が盛んに行われるようになってきている。現在のところ，MCCBは英語版のみが利用可能であるが，グローバル治験の必要性からMCCBを多言語に翻訳するMATRICS for co-primary and translation（MATRICS-CT）計画がスタートしている（http://www.matrics.ucla.edu/matrics-ct/home.html）。MATRICS-CT計画では，MCCBの出版を唯一許可されているMATRICS Assessment，Inc.（MAI）が主体となって，中国語，ドイツ語，ヒンディ語，ロシア語，スペイン語（中南米の3方言を含む）への翻訳が進められている。また，MCCBの出版を目的としない研究目的での翻訳も8ヵ国で行われている。

本邦では，著者らの研究グループがMAIと各テストの原著者の許可を得た上で，研究用のMATRICSコンセンサス認知機能評価尺度日本語版（MCCB-J）の開発を行った。研究用MCCB-Jの開発では，まず仮日本語訳を作成し，その後，原文を知らない英語話者が，日本語訳の英語へのback-translationを行った。原則として原文に忠実に翻訳を行ったが，必要に応じて言語的・文化的調整を行った。

Ⅵ．おわりに

統合失調症の諸症状の中で認知機能障害は，特に機能的転帰との関連において重要と考えられ，その改善に向けた治療法の開発が望まれている。

米国では，認知機能障害の治療法の開発を妨げていた認知機能評価尺度のばらつきを解消するため，信頼性・妥当性を有した認知機能評価尺度であるMCCBを用いることが可能になった。本邦でも諸外国と比較可能なMCCB-Jを用いることは，統合失調症の認知機能障害に関する研究の推進に貢献すると思われる。

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Research report

Progesterone reduces hyperactivity of female and male dopamine transporter knockout mice

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ABSTRACT

There are gender differences in prevalence, course, and/or prognosis of schizophrenia. Yet, neurobiological factors that may account for the more favorable outcomes of women with schizophrenia are not well understood. Evidence that the steroid hormone, progesterone (P_4), may influence mood and/or arousal among some people with schizophrenia led us to examine the effects of P_4 on dopamine transporter knockout (DATKO) mice, an animal model of schizophrenia. Our hypothesis was that P_4 would have greater effects than vehicle to improve the behavioral phenotype of DATKO, more so than wildtype, mice. Young adult, male and female DATKO mice and their wildtype counterparts were subcutaneously administered P_4 (10 mg/kg) or vehicle 1 h prior to testing in pre-pulse inhibition (PPI), activity monitor, or open field. DATKO mice had impaired PPI compared to their wildtype counterparts, but there was no effect of P_4. In the activity monitor, DATKO mice showed significantly greater distance traveled during the 60 min test compared to wildtype controls. In the open field, DATKO mice made a significantly greater number of total, but fewer central, entries than did wildtype mice. Administration of P_4 decreased the hyperactivity of DATKO mice in the activity monitor and open field, but did not alter motor behavior of wildtype mice. P_4 increased the number of central entries made by DATKO and wildtype mice. Thus, P_4 administration to DATKO female or male mice partially attenuated their hyperactive phenotype.

1. Introduction

Schizophrenia is characterized by deficits in social, affective and cognitive functioning. Gender differences in schizophrenia include that some women have a lower incidence, later age of onset, fewer hospitalizations, and better response to antipsychotics than do some men [24,27,31,58]. As with other gender/sex differences that are influenced by hormonal status, there is evidence for a role of hormones to influence expression of symptoms in schizophrenia. In support, some women have a greater occurrence of negative symptoms and/or may require higher dosages of antipsychotics, when endogenous progesterone (P_4) levels are low, during the follicular phase or post-menopause [21,24,58]. Thus, there is clinical evidence for P_4 to influence the pathophysiology of schizophrenia.

In animal models, there is evidence that P_4 can modulate the expression of some behaviors that are altered in schizophrenia. First, P_4 facilities sexual and/or social behaviors of female rodents, in part through its actions in the Ventral Tegmental Area (VTA), which is notable for the many dopamine (DA) cell bodies located there. Some of P_4's actions in this region may be through DA signaling. Activation and/or attenuation of P_4's actions via DA type 1 receptors in the VTA, respectively, facilitates and inhibits, sexual responses of rodents [61]. Second, P_4 also has effects on arousal and affective measures. In support, when administered to aged mice, P_4 (10 mg/kg, subcutaneously, SC), compared to vehicle, increased: the number of central entries in the open field, open quadrant time in the elevated zero maze, time spent in the mirror-chamber, time spent in light chamber in the dark/light transition task, and increased punished drinking in the Vogel conflict task [14]. Interestingly, similar effects of P_4 to enhance sexual receptivity and arousal were observed in older C57BL/6 mice and also P_4 receptor knockout mice (PRKOs) [15]. Third, P_4 can enhance cognitive function in tasks mediated by the nucleus accumbens (conditioned place preference), cortex (object recognition; T maze), and/or hippocampus (object recognition, water maze, conditioned fear), areas which are DA sensitive and that the VTA projects to [8,10,12,65]. Moreover, P_4 can have these effects in wildtype and PRKO mice. Together, these data suggest that P_4 has effects on some normative functional process that are atypical in schizophrenia and that some of these effects may be at least/in part, independent of action at PRs.

Dopaminergic inputs from the VTA play a key role in arousal and affective function in animals. The DA and serotonin (5-HT)

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systems have been implicated in striatal dysfunction associated with schizophrenia and may also play a role in reward and/or susceptibility to drug abuse [4,26]. As such, a greater understanding of hormones' effects through these systems is important.

The DA transporter (DAT) is a plasma membrane transport protein that controls extracellular DA concentrations and is an important target for a variety of therapeutic agents [51,53,59,61,62]. DAT knockout mice (DATKOs) exhibit elevated interstitial levels of dopamine and a range of behavioral alterations, including poor cognitive function [40], hyperactivity, and some stereotyped and/or perseverative behavior [61,62]. DATKO mice also have impaired pre-pulse inhibition (PPI), a model of sensorimotor gating in schizophrenia [67]. To date, the effects of P_4 on behavior of DATKO mice and their wildtype counterparts have not been reported. Evidence from our laboratory suggests that P_4 may influence sexual, social, cognitive and/or affective behaviors in part through its actions on the DA systems. As such, we wished to test the hypothesis that P_4 may reduce hyperactivity of DATKO, but not wildtype, mice.

2. Methods

These methods were pre-approved by The Institutional Animal Care and Use Committees at the University at Albany, State University of New York and The Tohoku University Graduate School of Medicine (Sendai, Japan).

2.1. Subjects

DATKO mice are used as a model for schizophrenia [50,59]. Individual DATKO strains used in this report have been described previously [61,62]. Intact male and female wildtype and DATKO mice ($N = 120$; $n = 15$/group) were bred and obtained from the colony at Tohoku University Graduate School of Medicine. DATKO and wildtype littermates were obtained by heterozygote crosses that had been previously generated on 129Sv-C57BL/6J mixed genetic background [60].

2.2. Housing

Offspring were weaned at postnatal day 28 and group housed, segregated by sex, in a temperature- and light-controlled colony (lights on at 0800 h, lights off at 2000 h), with food and water available *ad libitum*.

2.3. Genotype

Mice were genotyped using multiplex polymerase chain reaction methods on DNA extracted from tails, as previously described [14–16].

2.4. Subcutaneous P_4-priming

All mice were randomly assigned to either P_4 (10 mg/kg) or vehicle (sesame oil) condition. P_4 was obtained from Sigma Chemical Co. (St. Louis, MO) and dissolved in sesame oil to a concentration of 10 mg/ml. Mice received SC P_4 or vehicle injections 1 h prior to behavioral testing. This P_4 regimen was utilized because it increases plasma and central progestogen levels akin to that seen during behavioral estrus of rodents, without producing gross alterations in motor behavior and/or coordination [14,16]. After mice were injected with P_4 or vehicle, they were returned to their home cages for 1 h until behavioral testing.

2.5. Behavioral testing

Limited numbers of DATKO mice were available. Given this, a repeated-measures design was utilized. Therefore, all mice were once tested in either the P_4 or vehicle condition, then they were re-tested in the opposite condition five days later. Whether P_4 or vehicle was received initially was counterbalanced across subjects [63,65]. All animals were tested for PPI and for behavior in the activity monitor and open field following vehicle and P_4 conditions.

2.5.1. Handling and habituation

To minimize the effects of handling associated with the repeated testing, on day 1 mice were picked up by the tail and placed back in the home cage. On day 2, mice were transferred to another clean home cage. On day 3, mice were picked up, weighed, and transferred on a cart to another room and then the mouse was moved to another clean home cage. On day 4, the experimenter put the mouse in their home cage on a cart, and mice were transported to another room and were placed in novel environment for 2 min. On day 5, mice cages were put in their home cages on a cart, the cart and cage were moved to another room, mice were SC injected with sesame oil, then mice were placed in a novel environment, and then returned to their home cage.

2.5.2. Pre-pulse inhibition

Following the handling period, arousal behavior was assessed in the PPI task. Experiments were conducted based upon previously reported methods [7,17,67]. Startle chambers (SR-LAB, San Diego Instruments, San Diego, CA) were used to measure the startle response. Each chamber consisted of a non-restrictive Plexiglas cylinder mounted on a frame inside a lighted, ventilated box (35 cm × 35 cm × 47.5 cm). Movement within the cylinder was detected by piezoelectric accelerometers attached to the cylinder's bottom. Force detected by the accelerometer was converted into analog signals that were digitized and stored electronically. In all experiments, 65 readings were recorded at 1 ms intervals beginning at stimulus onset; the average amplitude was used to describe the acoustic startle response. A high-frequency loudspeaker inside the chamber, mounted above the cylinder, generated broadband background noise and acoustic stimuli, which were controlled by the SR-LAB software system and interface. Sound levels (dB (A) scale) and accelerometer sensitivity were calibrated routinely, as described previously [7,17]. Mice were tested initially for baseline PPI and pseudo-randomly assigned to hormone treatment groups based on these measurements. Mice were treated with vehicle or P_4 60 min before testing. Experimental sessions consisted of a 5 min acclimatization period with 65 dB broadband background noise followed by PPI sessions. Sessions consisted of five different trial types: no stimulus trials (nostim); startle pulse alone, 40 ms duration at 120 dB (p120); and three pre-pulse + pulse trials, 20 ms duration pre-pulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms delay. The nostim trial consisted of only background broadband noise. All test sessions started and concluded with six presentations of the p120 trial, while the remainder of the session consisted of 12 presentations of the p120 trial type, 10 presentations of the nostim, the pp3, pp6, and pp12 trial types, in a pseudorandom order, with varying inter-trial intervals (mean 15 s, range 8–23 s). Each animal was tested on a PPI session for 21 min with a total of 64 trials.

2.5.3. Activity monitor

Locomotion was assessed in an activity monitor. Mice were first habituated to the apparatus (40 cm × 30 cm × 26 cm clear plastic chamber) for 180 min and then subcutaneously injected with P_4 or vehicle. After 1 h, mice were placed back in the apparatus and locomotor activity was measured in 5-min increments using digital counters with passive infrared sensors (Supermex System, Tokyo, Japan).

2.5.4. Open field

Behavior was assessed in the open field, which can be used to determine total motor activity as well as anxiety behavior. Rodents typically avoid open, bright areas so propensity to move in the center of the open field indicates a reduced anxiety-like response. Mice were placed in the open field arena (39 cm × 39 cm × 30 cm) that had a 16-square grid floor and an overhead light illuminating the central squares [14,16]. Total numbers of entries and central entries were recorded for 5 min. The total number of entries made in the open field is used as an index of general motor activity, whereas the number of central entries made is an indicator of anti-anxiety-like behavior.

2.6. Statistical analyses

Analyses of variance (ANOVAs) were used to evaluate the effects of the two between-subjects variables (genotype-wildtype or DATKO; sex-male or female), and one-within subject (P_4 or vehicle) variables. Where appropriate, one-way analyses and Fisher's *post hoc* tests were utilized to evaluate groups that were different. The α level for statistical significance was $p < 0.05$.

3. Results

3.1. PPI

There was a main effect of genotype, but neither an effect of sex, nor P_4, on the magnitude of the startle responses. As has previously been demonstrated, DATKO mice had a diminished startle response following 20 ms duration pre-pulse at 68 dB (pp3) ($F(1,112) = 4.55$, $p \leq 0.01$), 71 dB (pp6) ($F(1,112) = 14.07$, $p \leq 0.01$), or 77 dB (pp12) ($F(1,112) = 24.29$, $p \leq 0.01$), followed by a 40 ms duration startle stimulus at 120 dB after a 100 ms delay. See Fig. 1.

3.2. Activity monitor

There were main effects of genotype ($F(1,112) = 72.15$, $p \leq 0.01$) and P_4 ($F(1,112) = 3.64$, $p \leq 0.05$), but not sex, on the total distance moved in the activity monitor. The interaction between genotype

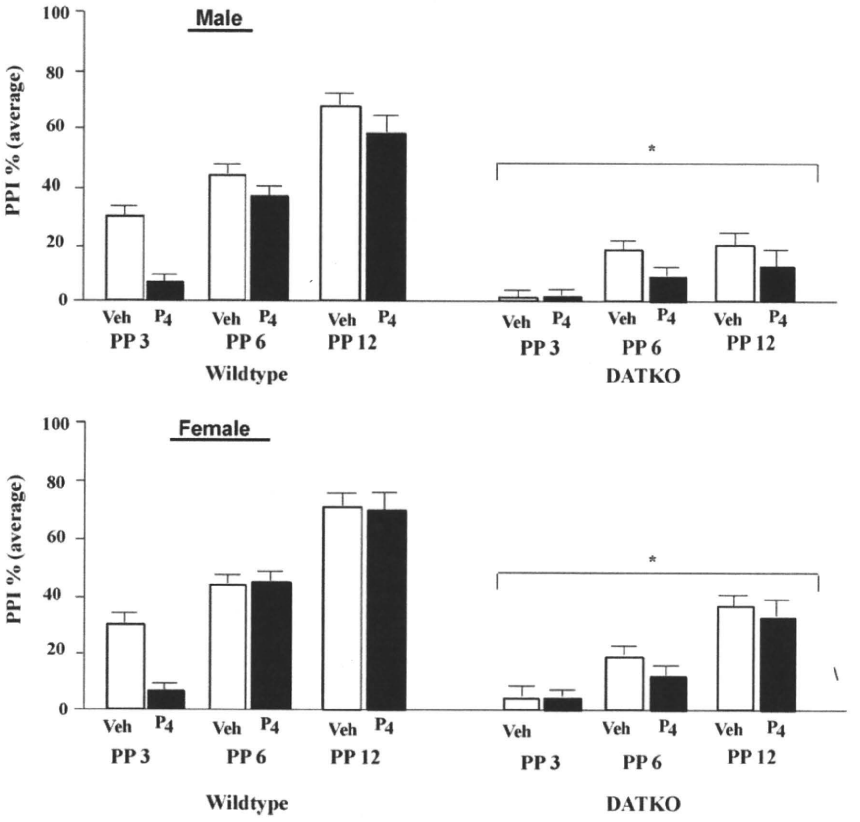

Fig. 1. The mean (+sem) startle magnitude of male (top panel) and female (bottom panel) mice administered vehicle (open bars) or P_4 (closed bars) before testing at pre-pulse at 68 dB (pp3), 71 dB (pp6), or 77 dB (pp12). * Above bar compared to wildtype (left) and DATKO (right) mice ($p < 0.05$; $n = 15$/group).

and hormonal status ($F(1,112) = 9.71$, $p \leq 0.01$) was attributed to P_4 decreasing the distances DATKO mice traveled, but also tending to increase the distances traveled by the wildtype mice. See Fig. 2.

3.3. Open field

There were main effects of genotype ($F(1,112) = 43.56$, $p \leq 0.01$) and P_4 ($F(1,112) = 30.12$, $p \leq 0.01$), but not sex, on the total number of entries in the open field. Total entries were lower in wildtype compared to DATKO mice. The interaction between genotype and hormonal status ($F(1,112) = 22.95$, $p \leq 0.01$) was due to P_4 decreasing the number of entries of DATKO, but not wildtype, mice. See Fig. 3.

Genotype ($F(1,112) = 56.58$, $p \leq 0.01$) and P_4 ($F(1,112) = 36.26$, $p \leq 0.01$), but not sex, influenced the number of central entries in the open field. The interaction between genotype and hormonal status ($F(1,112) = 13.57$, $p \leq 0.01$) was attributed to P_4 having a much greater effect to increase the number of central entries made by wildtype compared to DATKO mice. See Fig. 4.

4. Discussion

Findings from this study partially supported our hypothesis that progesterone would normalize hyperactivity of DATKO mice. Locomotion in the activity monitor and open field was significantly greater among DATKO, compared to wildtype, mice and P_4 dampened the hyperactivity of DATKO mice. Central entries in the open field were greater among wildtype, compared to DATKO, mice and P_4 significantly increased central entries of wildtype mice. DATKO, compared to wildtype, mice showed a dampened PPI response, but P_4 did not alter this effect. There were no sex differences in these effects. Thus, P_4 had circumspect effects to reduce hyperactivity in the activity monitor and open field of female and male DATKO mice.

The present findings are relevant for previous research that has shown that a prohormone for P_4, pregnenolone, is lower among schizophrenics, compared to healthy controls and is associated with trait-anxiety scores independent of acute anxiety symptoms [48,56]. Pregnenolone is synthesized during early developmental [20,28,41,49] and, when administered neonatally, influences DA turnover in the striatum [41]. Neonatal pregnenolone-treated rats are considered an animal model of cortical/subcortical dysfunction. Indeed, DA metabolites in the fronto-parietal cortex were similarly increased in pregnenolone-treated female and male rats and resulted in hyperactivity in the open field [42]. These findings and others, in conjunction with the present results that acute P_4 can normalize hyperactivity in DATKO mice, suggest that progestogens may play a role in the pathophysiology of schizophrenia.

One explanation for the effects of progestogens may be due to their effects on stress responses. Schizophrenia is characterized by dysregulation in stress responses. Although diagnosis of schizophrenia is based upon both positive (hallucinations, delusions) and negative symptoms (avolition, alogia) [44,45,57,58,66], there has been a recent emphasis on negative symptoms, which correlate with loss of social function [32], and plasma levels of the stress hormone, cortisol, albeit not P_4 [41–45,58,64,66]. How dysfunction of the hypothalamic-pituitary adrenal (HPA) axis contributes to the pathophysiology of schizophrenia needs to be better understood. Of interest is whether there are differences in HPA responses of DATKO mice compared to their wildtype counterparts in the present study.

One possible mechanism that may underlie some of the effects of progestogens to normalize behavior of DATKO mice are their effects on steroid biosynthesis. This may be particularly important

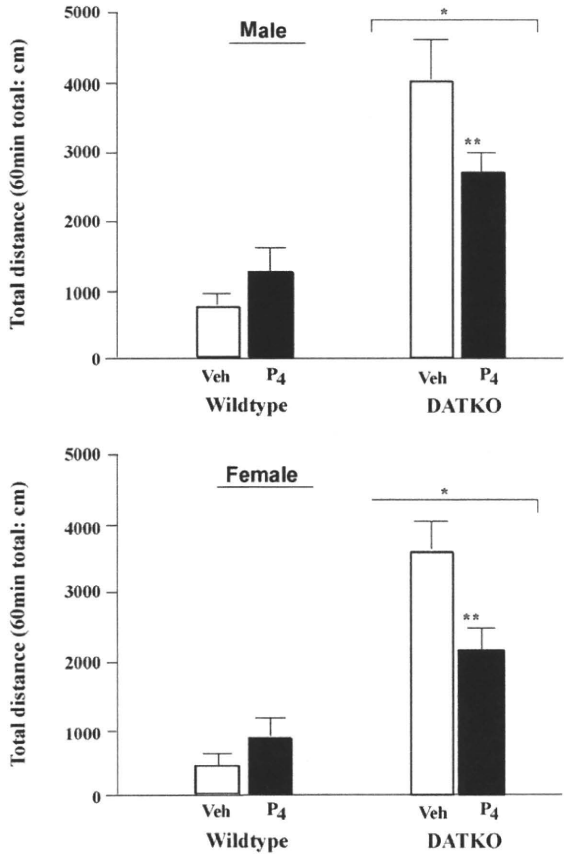

Fig. 2. The mean (+sem) total distance moved in the Super Mex activity chamber of male (top) and female (bottom) mice administered vehicle (open) or P_4 (closed bars). * Above bar compared to wildtype (left) versus DATKO (right) mice ($p < 0.05$). ** Above bar difference compared to vehicle ($p < 0.05$; $n = 15$/grp).

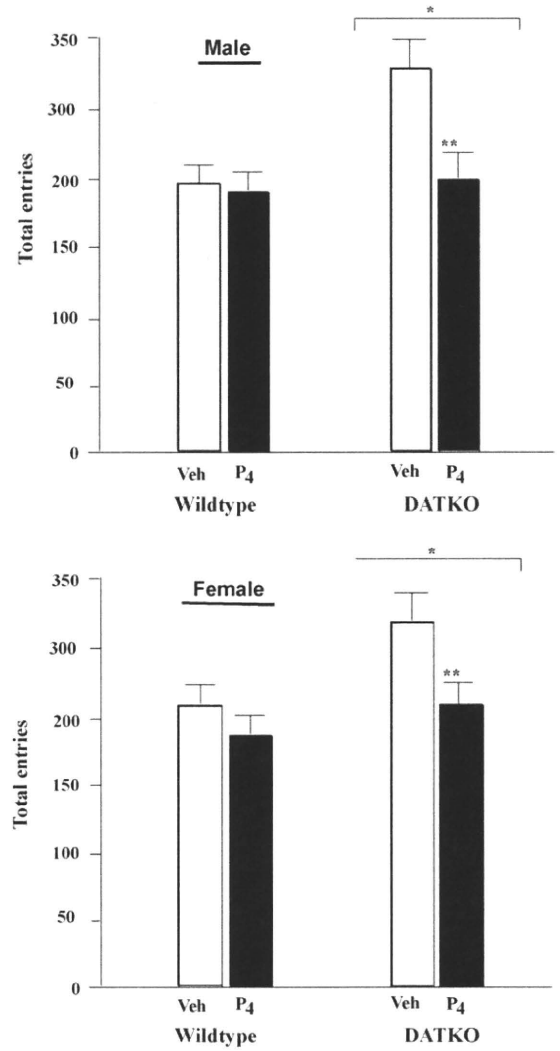

Fig. 3. The mean (+sem) total squares entered in the open field of male (top) and female (bottom) mice administered vehicle (open) or P_4 (filled bars). * Above bar compared to wildtype (left) and DATKO (right) mice ($p < 0.05$). ** Above bar difference compared to vehicle ($p < 0.05$; $n = 15$/grp).

because no sex differences were observed in the present study, suggesting that there may be a greater role of brain-derived versus ovary/gonad-derived steroids. Neurosteroids, steroid hormones produced in the brain, such as 3α-hydroxy-5α-pregnan-20-one (3α,5α-THP), are important endogenous modulators of the HPA that may serve to buffer stress responses. In response to stress, plasma and brain concentrations of 3α,5α-THP are elevated [46,47]. Stress-induced elevations in 3α,5α-THP dampen hyperactivity of the HPA axis [34,52]. 3α,5α-THP has potent agonist-like actions at $GABA_A$ receptors in the brain [52], which underlie its effects to reverse sympathetic activity. 3α,5α-THP from central metabolism of ovarian and/or adrenal P_4 [9] also mitigates stress responses. Although basal levels of 3α,5α-THP are similar for females in the follicular phase and males, during the luteal phase and pregnancy, plasma and hippocampal 3α,5α-THP levels are higher for females than males [25]. Further evidence that 3α,5α-THP mediates HPA responses include that 3α,5α-THP administration to female or male rats attenuates the elevation of plasma adrenocorticotropin (ACTH) and serum corticosterone secretion produced by emotional stress [46]. These data suggest that 3α,5α-THP, produced by glia in response to stress, and/or metabolized in neurons from peripheral sources, may serve as an important mediator of stress responses. It would be of interest to investigate how 3α,5α-THP may be mediating the reduction in hyperactivity that was observed in the present study among DATKO mice administered P_4.

Evidence suggests that 3α,5α-THP may be important in the pathophysiology of schizophrenia. First, neonatal 3α,5α-THP administration to rats disrupts the normal development of the prefrontal cortex and medial dorsal thalamus [18,20], implying that there may be a critical window of vulnerability to neurosteroid insult across development [41,42]. Second, stress-reactivity may underlie the etiology and/or manifestation of schizophrenia. Among people with schizophrenia, dysregulation of the HPA axis is common [33,35,43–45] and stress can precipitate psychiatric episodes related to schizophrenia [45]. Third, stress-induced 3α,5α-THP production can be disrupted in schizophrenia. A novel polymorphism and genetic mutation in the sequence encoding the gene for the mitochondrial benzodiazepine receptor (MBR), which is necessary for 3α,5α-THP biosynthesis in glial cells, has been demonstrated among some schizophrenics, and may create a predisposition to over-sensitivity to stress [34,44]. As well, social isolation (an animal model of schizophrenia) decreases 3α,5α-THP biosynthesis, in the frontal cortex of male Swiss-Webster mice, compared to group-housed controls [6]. Third, there is evidence that 3α,5α-THP metabolized in the brain from peripheral prohormones may reduce the incidence and/or expression of schizophrenia. Women, compared to men, typically have higher levels of 3α,5α-THP, are more likely to have schizophrenia with later onset, better prognosis, and therapeutic response to lower dosages of antipsychotics [23]. When 3α,5α-THP levels are low perimenstrually, first onset, or recurrence of psychotic episodes are

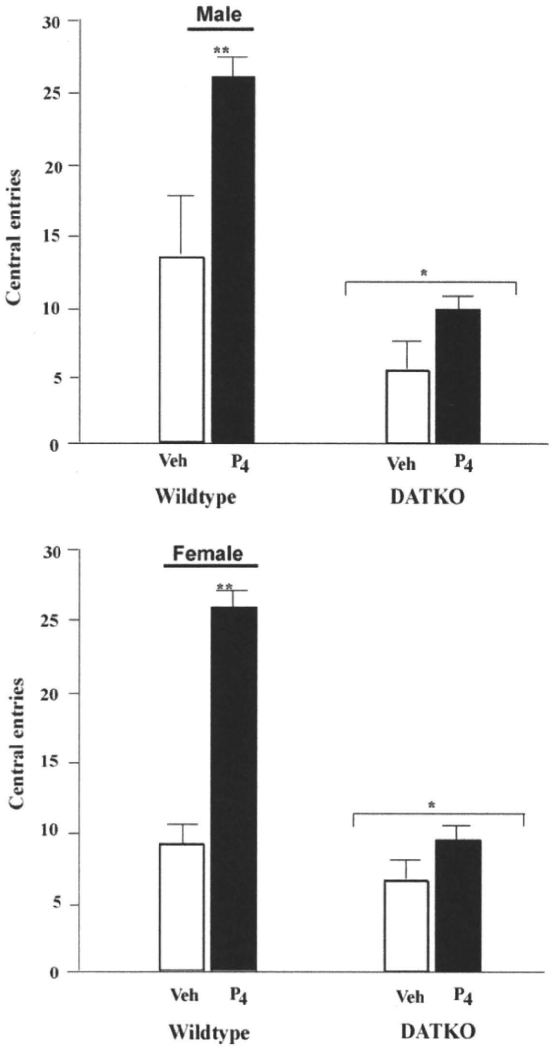

Fig. 4. The mean (+sem) central squares entered in the open field of male (top) and female (bottom) mice administered vehicle (open) or P_4 (filled). * Above bar compared to wildtype (left) and DATKO mice ($p < 0.05$). ** Above bar compared to vehicle ($p < 0.05$; $n = 15$/grp).

more likely and more negative symptoms are reported [21,24,27]. After menopause, when 3α,5α-THP levels are lower, there is a greater recurrence of psychiatric episodes than pre-menopause [8]. Fourth, effective pharmacotherapies for schizophrenia can alter 3α,5α-THP levels. The atypical antipsychotic drug, olanzapine, enhances social functioning and increases 3α,5α-THP levels [13,36]. In an animal model, clozapine can have similar neurosteroidogenic effects; however, it has not been demonstrated to alter circulating neurosteroids levels concomitant with therapeutic effects [41,42]. Together, these data suggest that schizophrenia may involve a reduced capacity to synthesize 3α,5α-THP in the brain, which may increase sensitivity to stress. Although 3α,5α-THP may underlie etiology and/or expression of schizophrenia, the present findings suggest that progestogen-based therapeutics may have a role to normalize the schizophrenic-like behavioral phenotypes [3].

3α,5α-THP may influence the function of the prefrontal cortex (PFC) to mitigate negative symptoms of schizophrenia [30]. Schizophrenia involves PFC hypofunction, poor social function, and disrupted working memory [32]. The PFC is integral for decisions related to social interactions [12] and working memory, a key component of human reasoning and judgment [32]. Notably, the PFC is sensitive to progestogens. Systemic administration of precursors of 3α,5α-THP enhance working memory [5,12] and 3α,5α-THP enhances dopamine release in the PFC in response to stress [5]. A question is why P_4 did not improve PPI among DATKO mice. It is not that DATKO mice are unresponsive to pharmacotherapies as psychostimulants; NET and SERT inhibitors improve PPI of DATKO mice, and impair PPI of wildtype mice [67]. Given the effects of progestogens for dopamine release in the PFC, it may be that P_4 administration elevated DA in the PFC without the DAT, contributing to a lack of effect of P_4 on PPI among DATKO mice. This may also underlie the apparent (but not significant) effect of P_4 to reduce PPI particularly in the pp3 trial. Further investigation of the potential of hyperdopaminergic action underlying these effects is necessary. Indeed, whether these effects are due to direct actions of progestogens on the PFC or indirect actions of progestogens on the hippocampus and/or VTA, which projects to the PFC, has not been established.

Schizophrenia is characterized by deficits in social functioning and progestogens mediate social behavior in part through actions in the VTA and its projection areas. For example, administration of 3α,5α-THP to the VTA increases time spent in interaction with a conspecific and blocking 3α,5α-THP's formation in the VTA attenuates social behavior. We have also shown that the neurosteroidogenic effects of mating can increase 3α,5α-THP and DA in the midbrain, hippocampus, striatum, and prefrontal cortex. Thus, 3α,5α-THP-enhanced social interactions may involve the VTA and its projections to the mesolimbic dopamine system. A question for future studies is the role of 3α,5α-THP in DATKO and wildtype mice for their social responding.

This is a particularly important area of research. There are differences in plasma levels of pregnenolone, a precursor for 3α,5α-THP, between those with schizophrenia and healthy controls [56]. In a recent study of 21 patients with schizophrenia or schizoaffective disorder, those with the lowest natural levels of pregnenolone, reported the best memory and concentration. In this study, patients took placebo for two weeks and were then randomly assigned to take pregnenolone, as a health supplement, or placebo for eight weeks in conjunction with an antipsychotic. Those taking pregnenolone had about a ~20% reduction in their negative symptoms than did the placebo group [37]. Other studies have also shown that plasma levels of neurosteroids correlate with the severity of negative symptoms among some men with schizophrenia [60]. Thus, it is important to understand further the role of progestogens, such as pregnenolone, P_4, or 3α,5α-THP, given the emerging evidence of their role in the etiology, expression, or treatment of schizophrenia and/or schizoaffective disorders.

Affective, and cognitive, processes are also disrupted in schizophrenia and progestins can influence these behaviors through actions in the hippocampus. In the present study, there was a clear anti-anxiety effect of P_4 among wildtype mice, as demonstrated by an increase in central entries in the open field, independent of an increase in total entries. This same effect was not observed in the DATKO mice, suggesting that there may be some involvement of the DAT for progestogens to increase anti-anxiety responding. However, it may also be that no effects of P_4 were found for central entries of DATKO mice because of the robust effect of P_4 to reduce their motor behavior. A question for follow-up studies would be the effects of P_4 to DATKO and wildtype mice in other typical measures of anxiety behavior of mice (e.g. elevated plus maze, light–dark transition, etc.). As well, whether this effect was due to 3α,5α-THP is of interest. 3α,5α-THP is increased in the hippocampus concomitant with reduced anxiety behavior and enhanced cognitive performance [54,55,65]. Blocking the formation of 3α,5α-THP in the hippocampus increases anxiety behaviors and impairs cognitive performance [5,11,53]. Given that the hippocampus projects to the PFC, an important question is whether 3α,5α-THP has direct actions in the PFC to mitigate stress and/or

behavioral responses. Another possibility is that these effects occur indirectly through connections of the PFC with the VTA and/or striatum. These questions are the topics of ongoing investigation in our laboratory.

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Behavioural Pharmacology

Impaired spatial working memory and decreased frontal cortex BDNF protein level in dopamine transporter knockout mice

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ABSTRACT

Brain-derived neurotrophic factor (BDNF), one of the key brain neurotrophins, has been implicated in neuronal plasticity and memory. Recent studies document the importance of BDNF for normal long-term memory functions. However, there are few studies of the roles of BDNF in short-term memory. Dopamine is likely to play important roles in BDNF gene expression in specific brain regions, including frontal cortical regions that are implicated in short-term working memory processes that include spontaneous alternation. We have thus tested spatial working memory in dopamine transporter knockout (DAT KO) and wild-type mice. Spontaneous alternation in the Y-maze, an index of short-term spatial working memory in mice, was significantly decreased in DAT KO mice compared to wild-type mice. BDNF protein was significantly decreased in frontal cortex, though not in striatum or hippocampus, of the DAT KO mice. The data support the hypothesis that impaired spatial working memory in DAT KO mice may be related to decreased frontal cortical BDNF in these animals, and document apparent roles for BDNF in a short-term memory process.

1. Introduction

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein that is widely expressed in the adult mammalian brain (**Murer et al., 2001**). There is abundant evidence that BDNF is involved in synaptic plasticity and memory processes (**Poo, 2001**; **Tyler et al., 2002**), particularly as BDNF relates to hippocampal dependent memory. BDNF has been suggested to be essential for normal persistence of long-term memory storage (**Bekinschtein et al., 2008**) and endogenous BDNF is required for long-term memory formation in the rat parietal cortex (**Alonso et al., 2005**). Lower levels of frontal cortical BDNF have been associated with impaired working memory performance in Ts65Dn mice, which are considered to be an animal model of Down's syndrome (**Bimonte-Nelson et al., 2003**). Reducing BDNF expression through intracerebroventricular infusion of BDNF antisense impairs performance in radial arm maze tests (**Mizuno et al., 2000**). BDNF has been implicated in long-term potentiation, an electrophysiological concomitant memory acquisition (**Korte et al., 1998**; **Lessmann, 1998**).

There is less data concerning the effects of BDNF on spatial working memory, although BDNF has been closely related to dopamine pathways and implicated in dopaminergic function (**Berton et al., 2006**; **Fumagalli et al., 2003**; **Li et al., 2006**; **Li et al., 2007a,b**). Lesions and other manipulations of mesocortical dopamine pathways can change performance in spontaneous alternation paradigms (**Pioli et al., 2008**) and alter BDNF gene expression in frontal cortical regions (**Fumagalli et al., 2003**). Dopamine has also been shown to directly regulate BDNF expression in striatal cells *in vitro* (**Küppers and Beyer, 2001**). These data therefore collectively implicate a dopamine mediated regulation of BDNF in prefrontal cortex dependent memory function.

We have produced and extensively characterized a line of DAT KO mice that display hyperlocomotion (**Sora et al., 1998, 2001, 2009**) as well as increased extracellular dopamine levels (**Shen et al., 2004**). These and other lines of DAT KO mice display altered performance in 8-arm radial maze testing, reduced prepulse inhibition and increased prefrontal cortical BDNF levels (**Gainetdinov et al., 1999**; **Yamashita et al., 2006**; **Fumagalli et al., 2003**). Each of these results suggests that DAT KO mice might also display alterations in spatial working memory due to increased dopaminergic tone, perhaps mediated by alterations in BDNF function. We thus now report results of spatial working memory Y-maze testing and evaluation of BDNF expression in DAT KO mice. We discuss ways in which this data, taken together, is consistent with the idea that direct and indirect effects of this knockout, including the altered BDNF expression, could contribute to

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the altered performance in this index of short-term memory function (**Krejcova et al., 2004**).

2. Materials and methods

2.1. Animals

DAT KO mice were produced as described (**Sora et al, 2001**), bred at the Animal Laboratory Institute of Tohoku University Graduate School of Medicine and maintained on a mixed genetic background combining C57BL/6 and 129Sv/J mouse strains. Offspring from heterozygote crosses were weaned at 28 days postnatal and housed in groups of two to five (segregated by sex), in an animal room maintained under a 12 h/12 h light/dark cycle with lights on from 8:00. Food and water were available *ad libitum*. Mice were genotyped using multiplex polymerase chain reaction methods on DNA extracted from tail biopsies, as previously described (**Shen et al, 2004**). Behavioral testing was conducted in 8–11 week old mice. All animal experiments were performed in accordance with the Guidelines for the Care of Laboratory Animals of Tohoku University Graduate School of Medicine.

2.2. Y-maze test

The Y-maze consisted of 3 arms (14×4.5×40 cm). Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The total number of arm entries (locomotor activity) and alternation behavior were recorded using a video camera. The percentage of alternation was calculated as (total of alternation/total arm entries − 2). This measure is considered to reflect short-term memory in mice (**Mamiya and Ukai, 2001**). Additionally, the number of total arm entries was calculated as an index of locomotor activity (**Ma et al., 2007**).

2.3. ELISA for measuring BDNF protein concentration

Animals were sacrificed by decapitation. The brains were quickly removed and dissected on ice. Samples taken from the frontal cortex, caudate putamen and hippocampus were frozen at −80 °C before homogenization. Brain samples were diluted (hippocampus, 1:30; frontal cortex and striatum, 1:20) and homogenized in a lysis buffer (137 mM NaCl, 20 mM TRIS, 1% NP40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml aprotinin, 1 μg/ml leupeptin, and 0.5 mM sodium vanadate. The homogenates were centrifuged at 10,000 g for 20 min, and the supernatants were collected and processed for quantification of BDNF by ELISA using a BDNF Emax Immuno Assay kit (Promega, Madison, Wis., USA) according to the manufacturer's instructions (**Schaaf et al., 1998**) and carried out as described previously (**Li et al., 2006, 2007a; Amano et al., 2007**). Nunc Maxisorp 96-well immunoplates were coated with 100 μl/well of anti-BDNF monoclonal antibody (mAb) and incubated overnight at 4 °C. The plates were incubated in a block and sample buffer at room temperature for 1 h. Then, the samples were added to the coated wells (100 μl) and shaken for 2 h at room temperature. Following this the plates were incubated with an anti-human BDNF polyclonal antibody (pAb) for 2 h at room temperature with shaking and then incubated with an anti-IgY antibody conjugated to horseradish peroxidase for 1 h at room temperature. The plates were then incubated with tetramethylbenzidine solution for 15 min and 1 M hydrochloric acid was added to the wells. The colorimetric reaction product was measured at 450 nm. BDNF standards ranging from 7.8 to 500 pg/ml were used for quantification. Standard curves were plotted for each plate (correlation coefficient; $r = 0.99$). Detection limit was 15.6 pg/ml, and cross-reactivity with other related neurotrophic factors was less than 3%.

2.4. Statistical analysis

The significance of the data was analyzed using unpaired *T*-tests. $P<0.05$ was considered significant.

3. Results

3.1. Behavioral results in the Y-maze test

Fig. 1 shows spontaneous alternation in Y-maze testing of homozygous DAT KO and wild-type littermate mice. Spontaneous alternation was decreased in DAT KO mice compared to wild-type mice ($P<0.05$). Despite these differences, there were no significant differences in the number of total arm entries between DAT KO mice and wild-type mice.

3.2. Changes of BDNF protein

Fig. 2 shows changes of BDNF protein in DAT KO mice compared to wild-type littermates. The concentration of BDNF protein was significantly decreased, by approximately 50%, in the frontal cortex of DAT KO mice compared to wild-type mice ($P<0.05$). However, there were no significant changes in BDNF level in the caudate putamen or hippocampus of DAT KO mice.

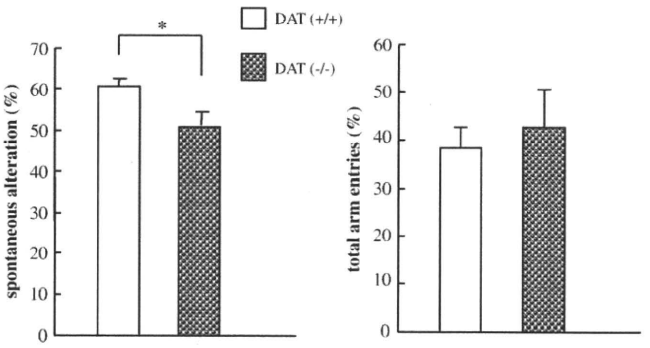

Fig. 1. Comparison of spontaneous alternation between DAT (+/+) and DAT (−/−) mice in Y-maze test. Each mouse was placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Spontaneous alternation but not number of total arm entries was decreased in DAT (−/−) mice compared to DAT (+/+) mice ($P<0.05$). Columns represent the mean ± S.E.M., $n = 10–11$, *$P<0.05$, unpaired *T*-test.

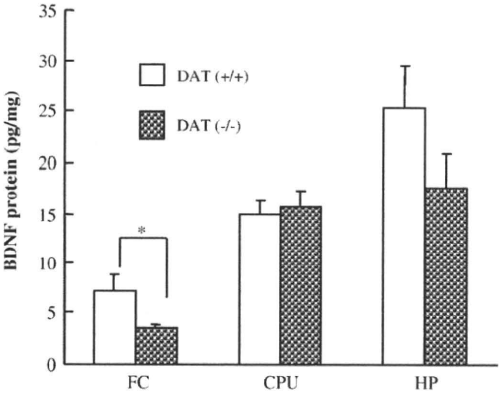

Fig. 2. Changes of BDNF protein in DAT (+/+) and DAT (−/−) mice. BDNF level was significantly decreased in frontal cortex of DAT (−/−) mice compared to DAT (+/+) mice ($P<0.05$). However, there were no changes in caudate putamen and hippocampus in DAT (−/−) mice. FC, frontal cortex; CPU, caudate putamen; HP, hippocampus. Columns represent the mean ± S.E.M., $n = 8$, *$P<0.05$, unpaired *T*-test.

4. Discussion

Observation of decreased Y-maze spontaneous alternation in DAT KO mice is potentially consistent with the idea that these animals have impaired working memory function. Since the number of total arm entries was not significantly different between the two genotypes, confounding influences of locomotor hyperactivity in DAT KO mice seems unlikely. Documentation of significantly decreased frontal cortical BDNF in the DAT KO mice provides a plausible potential mechanism for impairments in spontaneous alternation.

However, although it would seem most parsimonious to attribute differences in performance in DAT KO mice to differences in working memory function, it is possible that other behavioral changes primarily underlie these differences. Although prefrontal cortex lesions impair spontaneous alternation (**Mogensen and Divac, 1993**), manipulations of several other brain regions also affect spontaneous alternation (**Lalonde, 2002**), and performance in spontaneous alternation tests is open to attentional and motivational confounds (**Hughes, 2004**). Attentional impairments have already been noted in DAT KO mice (**Yamashita et al., 2006**) as well as differences in motivational function (**Hironaka et al., 2004**). In particular the latter study demonstrated normal operant responses for food under many conditions, but impaired extinction behavior. One way to interpret such changes is as a perseverative response. Similar perseveration of dominant or initial response tendencies has been suggested to underlie alterations in DAT KO behavior in the forced swim test (**Perona et al., 2008**), and delayed acquisition of the Morris Water Maze (Hall, Sora, and Uhl, unpublished observations). Perseverative behavior has been associated with dopamine function and is enhanced by amphetamine in a manner that is dependent on the baseline probability of a particular response (**Evenden and Robbins, 1983**). Furthermore, locomotor sensitization induced by repeated DA agonist administration is also associated with reduced spontaneous alternation (**Einat and Szechtman, 1995**). This circumstance might be considered to apply also to DAT KO mice that have enhanced extracellular dopamine function in the striatum and nucleus accumbens (**Shen et al., 2004**), although it must be considered to what extent these differences are not just mediated by differences in striatal dopamine function, but altered balance of dopamine function in the nucleus accumbens and prefrontal cortex.

There is substantial support for the idea that intact prefrontal dopamine function is important for certain mnemonic functions. Blocking dopaminergic transmission in rat the mediofrontal cortex degrades spatial choice performance in Y-maze testing (**Kozlov et al., 2001**), and the prefrontal cortex has been postulated to play key roles in short-term memory (**Goldman-Rakic, 1996; Kesner and Rogers, 2004**). Dopamine agonists can improve short-term spatial memory in human volunteers (**Mehta et al., 2001**), in ways that are postulated to involve frontal cortex (**Egan et al, 2002**). These postulated prefrontal mnemonic roles thus add to traditional roles for dopamine transmission in the prefrontal cortex that include influences on higher motor functions, motivation, and cognition (**Egan and Weinberger, 1997; Lewis et al., 1998; Yang et al., 1999**).

At least some of these dopaminergic effects may involve D_1 receptors. In the prefrontal cortex of rodents and monkeys, both the amount of receptor mRNA and the number of receptor-binding sites are significantly greater for the dopamine D_1 receptor than for the other dopamine receptor subtypes (**Lidow et al., 1991; Gaspar et al., 1995; Goldman-Rakic et al., 1992**). Disrupting dopamine transmission in the prefrontal cortex caused by infusions of dopamine D_1 receptor antagonists or by excitotoxic lesions impairs working memory in nonhuman primates (**Sawaguchi and Goldman-Rakic, 1991, 1994**). Although extracellular dopamine levels in the prefrontal cortex are not affected by DAT knockout (**Shen et al., 2004**), further studies are needed to determine whether there are postsynaptic differences in dopaminergic function, and in particular whether differences in D_1 receptor function might contribute to differences in spontaneous alternation in DAT knockout mice that are reported here.

There is also substantial support for dopamine effects on BDNF. Dopaminergic agonists can regulate BDNF mRNA and protein levels (**Küppers and Beyer, 2001**). BDNF mRNA expression is also reduced in the frontal cortex of another line of DAT KO mice (**Fumagalli et al., 2003**). Influences in the opposite direction may be more modest; **Chourbaji et al. (2004)** reported that tissue content of dopamine was unchanged in the frontal cortex of BDNF heterozygous mice. These data, combined with our current results, suggest that dopamine could contribute to reduced synaptic formation and impaired spatial working memory, in part, through reductions in neurotrophin expression. Associations between lower frontal cortical BDNF protein levels and impaired working memory in Ts65Dn Down's syndrome mice are also consistent with this idea (**Bimonte-Nelson et al., 2003**). However, this study also showed that the effect of BDNF on working memory is maybe related to cholinergic degeneration. However, whether impaired spatial working memory in DAT KO mice is related to cholinergic degeneration needs further study. Another study revealed that performance in the complex maze was better in wild-type than APP23 animals. This difference is maybe related to decreased hippocampal BDNF levels on training in APP23 animals (**Hellweg et al., 2006**). This brain region differs from ours (frontal cortex). However it still confirms that a change of BDNF level plays a role in maze behavior like in our study.

Our current data found that BDNF levels were reduced by approximately 50% in the frontal cortex of our DAT KO mice, extending results obtained in other strains of DAT KO mice (**Fumagalli et al., 2003**). While extracellular dopamine levels in the frontal cortex of DAT KO mice are similar to those of wild-type mice (**Shen et al., 2004**), their frontal cortical dopamine content is approximately 50% of wild-type levels (**Sora et al., 2001**) which may indicate changes in synaptic content that may relate directly to both differences in BDNF levels and spontaneous alteration.

While the findings that spatial working memory deficits and frontal cortical BDNF deficits in DAT KO mice are consistent with the possibility that these two observations are linked, there is no direct evidence for such a linkage. The hippocampus, for example, expresses abundant BDNF (**Li et al., 2006**) and is closely tied to spatial working memory (**Luine et al., 1994**). Conceivably, the trend toward decreased BDNF in this region might contribute to the behavioral observations made here. It is possible that the changes in dopamine alone, by affecting working memory or some other function as discussed above, may make large contributions to the behavioral phenotype in ways that make the BDNF findings coincidental. However, it seems unlikely that the robust changes in BDNF levels that are observed here would be without behavioral consequences.

In conclusion, spontaneous alternation in the Y-maze was impaired in DAT KO mice compared to wild-type mice. Concomitant changes in the expression of BDNF protein were observed in the frontal cortex but not in the caudate putamen or hippocampus of DAT KO mice. Taken together, these observations are at least consistent with the hypothesis that impaired working memory in the Y-maze in these mice may receive contributions from the decreased frontal cortex BDNF found in DAT KO mice.

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