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博士論文の要旨

A large part of wild animal life depends on the ability to move from one place to another. This type of behavior is referred to as spontaneous locomotive activity. The activity plays a crucial role in many situations, e.g. avoiding predators, finding food and mating partners, territorial defense, and migration. Hence, the spontaneous locomotive activity is greatly involved in the strategy for survival in the wild life.

In the laboratory, mice move spontaneously in the habituated home-cage according to a light/dark rhythm and most mice are active during the dark period. This kind of activity is described as spontaneous home-cage activity. The activity is quite different quantitatively and qualitatively, depending on the genetic background. Previous genetic studies have clarified that the activity is controlled by multiple genetic factors, quantitative trait loci (QTLs). In a variety of strains, mice of C57BL/6J are relatively hypoactive, in contrast to mice of KJR that are especially hyperactive in the habituated home-cage. The spontaneous home-cage activity is considered as a behavior, which is driven by both the motor function in the central nervous system and the physical ability. Thus, the possible genetic factors influence the spontaneous home-cage activity through regulations of the central nervous system and the physical ability. I worked for elucidating the genetic basis and biological mechanisms that determine the difference in spontaneous home-cage activity between C57BL/6J and KJR.

In order to study the genetic basis responsible for the difference in spontaneous home-cage activity between KJR and C57BL/6J, we tried to detect QTLs associated with the difference. F2 progeny (BKF2) that were made between C57BL/6J and KJR strains were analyzed for their total home-cage activity (THA) in a three-day measurement. Furthermore, I divided THA into two ethological components, active time (AT) and average activity (AA), and used them for the QTL analysis. AT indicates the total time of movement and is considered as the temporal element of THA. AA indicates the average amount of movement during the active time and is considered as the quantitative element of THA. Pearson correlation analyses showed moderate correlation (r= 0.401) between the measurement values of AA and AT. The correlation value suggested that AA and AT were almost independent measurement, and were expected to be associated with independent genetic factors. The QTL analyses identified three significant QTLs involved in the spontaneous home-cage activity. These QTLs were designated as *hyperlocomotive activity related QTL1 (Hylaq1), Hylaq2 and Hylaq3*. All *Hylaq* loci were associated with THA. *Hylaq1* was located in a middle region of chromosome 2 (chr2), and mainly associated with AT. *Hylaq1* contributed to 31 %, 214 % and 5 % of the overall differences in THA, AT and AA, respectively, between KJR and C57BL/6J. *Hylaq2* was located on the distal side of *Hylaq1* on chr2, and controlled both AT and AA. *Hylaq2* contributed to 35 %, 118 % and 21 % of the overall differences in THA, AT and AA, respectively, between KJR and C57BL/6J. *Hylaq3* was located near the telomeric region on chr10, and associated with mainly AA. *Hylaq3* contributed to 39 %, 81 % and 29 % of the overall differences in THA, AT and AA, respectively, between KJR and C57BL/6J. Thus, the QTL analyses identified three novel QTLs involved in the spontaneous home-cage activity.

In contrast to the home-cage activity, open-field activity is considered to reflect the psychological status of the subject. The open-field test is used to assess the reactivity to a novel environment including animal's exploratory activity and anxiety. Interestingly, C57BL/6J exhibits a hyperactive phenotype in the open-field test for the first one-minute period while KJR is hypoactive. I investigated the open-field activity and compared it with the spontaneous home-cage activity. A Pearson correlation analysis between the open-field activity and the spontaneous home-cage activity showed little association in BKF2. This result suggests that these traits are independent and controlled by independent genetic factors. In fact, a QTL analysis showed a suggestive QTL related to the open-filed activity on chr11 and that no or only few QTLs are common to the spontaneous home-cage activity and the open-field activity. These results suggest that the genetic mechanism involved in the spontaneous home-cage activity is different from the mechanism associated with the open-field activity.

It has been reported that the dopamine (DA) system in basal ganglia of the brain is associated with movement and spontaneous locomotive activity. To search for candidate genes in the above QTL regions, and to understand the biological mechanisms that are associated with the spontaneous home-cage activity, phenotypic analyses on the DA system have been conducted with pharmacological and biochemical methods. As a first step, the effects of dopamine-related drugs on home-cage activity were investigated. A D1-like receptor selective agonist (SKF38393), a D2-like selective agonist (quinpirole), and a DA transporter (DAT) blocker (methylphenidate-hydrochloride, MPH) were administered to mice, and the behavioral alternations were observed in C57BL/6J and KJR. As a second step, the expression levels of tyrosine hydroxylase (Th) and DAT, which are involved in the synthesis of DA and up-take of the released DA in the synapses, respectively, were analyzed. As a third step, I tried to determine the basal level of extracellular (extraneuronal) DA released from the terminals of dopaminergic neurons in the striatal synapses by a microdialysis method. Finally, the released DA levels were measured by the microdialysis method following acute administration of MPH, which prevents DA re-uptake and induces extracellular accumulation of DA proportional to the amount of release from the nerve terminals. The expression analysis and microdialysis analysis suggested that there was no difference in DA synthesis, release or uptake in the presynapse of the dopaminergic neurons between KJR and C57BL/6J. The results of the pharmacological analyses indicate that the differences in the spontaneous home-cage activity between these strains might be due to the pharmacological differences in the downstream of the DA system following activation of the DA receptors. In particular, function of D1 DA pathway that regulates the spontaneous activity negatively is possibly reduced in KJR but not in C57BL/6J.

In the *Hylaq* regions, there are many genes expressed in the central nervous system. However, none of them seems directly involved in the DA pathway. Further functional analysis of the genes in the region should reveal the genetic basis of the regulation of spontaneous locomotive activity.

論文審査結果要旨

動物の生存戦略はその運動性に大きく依存している。自発運動性は動物の系統間、個体間で差があるが、ヒトでは極端な例として多動症が学習障害の一種として問題になっている。梅森くんは、自発運動性の遺伝的な基礎を解明するため、マウスの系統間で自発運動性に大きな違いがあることに着目し、表現型の詳しい解析と遺伝子座の探索を行った。

マウスの自発運動性は飼育ケージ内での活動性として捉えることができ、複数の量的形質遺伝子座（QTL）によって調節されることが分かっている。梅森くんは、まず高活動系統であるKJR、低活動系統であるC57BL/6（以下B6と略す）、およびそれらのF1、F2世代について、総活動量（THA）、活動時間（AT）、活動時間あたりの平均活動量（AA）を測定した。F2世代では、THAとAT、THAとAAの間に強い相関が、ATとAAの間には弱い相関があり、共通の遺伝因子の存在が示唆された。一方、これら自発運動性の値と、新奇な環境への反応性を示すオープンフィールド活動性との相関はなかった。次に、自発運動性に関わる遺伝子座を同定するため、マイクロサテライトマーカーを用いたQTL解析を行い、3つのQTLを同定し、*Hylaq1-3*（*hyper-locomotive activity related QTL 1-3*）と命名した。このうち*Hylaq1*は2番染色体中央付近にあり、主にATの系統間の違いに効果があり、*Hylaq3*は10番染色体末端部にあり、主にAAに効果があった。また、*Hylaq2*は2番染色体の遠位部にあり、AAとATの両方の系統間の違いに効果があった。*Hylaq*の効果はいずれも半優性で、また互いに相加的であり、エピスタティックな相互作用はなかった。

次に梅森くんは、*Hylaq*の候補遺伝子を絞り込むには、KJRとB6についての薬理学的な比較解析が役立つと考えた。大脳基底核のドーパミン経路は自発運動性に大きく関わることが知られている。そこで、D1受容体アゴニスト、D2受容体アゴニスト、ドーパミントランスポーター（DAT）ブロッカー投与下での自発運動量の測定、チロシン水酸化酵素とDATの発現量の測定、DATブロッカー投与前・後の線状体のドーパミン量の測定などを行った。その結果、KJRとB6の自発運動性の差はドーパミン受容体より下流の経路の違いに起因すると推測された。とくに、自発運動性を負に制御するD1経路の活性が、KJRではB6より低いことが示唆された。*Hylaq*がマップされた染色体領域にはこれらの経路に関わる既知の遺伝子はなかったが、今後このような知見が蓄積されれば候補遺伝子の絞り込みに有用であると考えられた。

審査委員全員でこの論文を審査し、(1) 遺伝研で開発されたマウスリソースを活用した独自性の高い研究であること、(2) 膨大な表現型解析やマイクロサテライトマーカーの解析などの仕事を一貫して主体的に進めてきたこと、(3)自発運動性に関わる3つのQTLを検出した学問的価値の高い研究であることを評価し、当大学院の水準を満たしていると判断して合格とした。