

永田友美、 成田奈緒子	特別な支援を必要とする児童への実践的支援の試み	教育研究ジャーナル	2	19-20	2009
成田奈緒子	有害化学物質の地域分布と情動・認知行動異常発症との関連—平成 21-23 年度厚生労働省科学研究補助金(化学物質リスク研究事業) 研究より	教育研究ジャーナル 2009 (2)	2	7-8	2009
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その他

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成田奈緒子	保護者向け資料：「小さいお子さんを育てている保護者のみなさんへ 乳幼児期を大切に～心と体の基礎を育てるとき～」(分担執筆)	東京都教育委員会 乳幼児期からの子供の教育支援プロジェクト			2009 http://www.nyu-yoji-kyoiku-to-kyo.jp/download.html より全文ダウンロード可
成田奈緒子	「早寝早起き朝ごはん」指導者用資料	「早寝早起き朝ごはん」全国協議会			印刷中
成田奈緒子	子どもの生活習慣づくり支援事業「事例集」				印刷中

IV . 研究成果の刊行物・別刷

有害化学物質の地域分布と情動・認知行動異常発症との関連

平成 21 - 23 年度厚生労働科学研究費補助金（化学物質リスク研究事業）研究より

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概要：メチル水銀、ポリ塩化ビフェニル(PCB)、鉛、農薬など、環境中の有害化学物質の妊娠中のばく露と生後の情動・認知行動への影響について示唆されてきている。「情動・認知行動の異常」は臨床的には「発達障害」と捉えられるが、この発達障害の発症はこの 30 年ほどで 10 倍以上と著しく増えており、これは昨今の妊婦をめぐる環境の変化（環境有害化学物質、嗜好品、ストレスなど）と切り離しては考えられない。基礎医学実験では、妊娠動物の環境が胎仔の脳神経系の発達異常に関連することは示唆されているが、これを疫学的にヒトで調査した研究は本邦ではいまだ行われていない。そこで我々は、今年度より 3 カ年計画で、乳歯と毛髪を用いた残留元素の検索を全国規模で行い、調査票を基にした情動・認知行動異常との関連の調査研究を行うこととした。

キーワード：残留元素、発達障害、認知、脳機能

1. はじめに

メチル水銀、ポリ塩化ビフェニル(PCB)、鉛、農薬など、環境中の有害化学物質の妊娠中のばく露は、先天奇形発症の危険は言うに及ばず、最近ではこれらの物質の、生後の情動・認知行動への影響が深刻となってきた。「情動・認知行動の異常」は臨床的には「発達障害」と捉えられるが、この発達障害の発症はこの 30 年ほどで 10 倍以上と著しく増えており、基礎医学実験では、妊娠動物の環境が胎仔の脳神経系の発達異常に関連することは示唆されているが、これを疫学的にヒトで調査した研究は本邦ではいまだ行われていない。

そこで今回、三重大学医学部教授成田正明、順天堂大学医学部教授横山和仁、青山学院大学理工学部教授田代朋子、および筆者による研究組織を立ち上げ、①ヒトでの化学物質の事象（サリドマイドによる自閉症）を動物で再現したモデル動物の解析、②化学物質ばく露の有無の診断のためのバイオマーカー確定（発現遺伝子網羅的解析）、③生後の情動・認知行動異常の評価のための非侵襲的診断法の普及、④疫学的検討、即ち有害化学物質の地域分布と発達障害発症地域との関連、を解明することにより、ばく露化学物質の種類を越えて共通に存在すると考えられる、ばく露から発達障害発症への生物学的カスケードを明らかにすることを目的として研究活動を開始した。

筆者はこの 3 年間のプロジェクトのうち、「近赤外線酸素モニターを用いた非侵襲的脳機能評価」（脳機能検索）および「有害化学物質の地域分布と情動・認知行動異常発症地域との関連」（疫学調査）について分担し、研究活動を行う予定である。本稿では、現在始動している④の研究活動、すなわち疫学調査研究の概要について紹介したい。

2. 疫学調査研究の目的

妊娠中の有害化学物質ばく露は、生後の情動・認知行動異常へ影響を与えるとされている。例えば、1960 年代に多くの被害者を出したサリドマイド禍では、妊婦が安全と

信じて服用した薬物により、胎児の四肢長管骨欠損などの奇形（いわゆるサリドマイド児）を生じたことがよく知られているが、その後の調査により、耳介など微細な奇形を伴うサリドマイド児において、先天性の認知・情動の異常である自閉症の合併発症が一般人口での発症率に比して 50 倍という高値であったことが報告された¹⁾。これ以外にも、この研究と同様のコホート調査、あるいはネズミなど動物を用いた基礎研究により、薬物や化学物質、環境汚染物質、あるいは母体のウイルス感染など、さまざまな環境物質の胎児ばく露と出生児の自閉症をはじめとする情動・認知障害との関連を示唆されている²⁾⁴⁾。特に自閉症に関しては、ばく露が多い環境、あるいは排泄機能の脆弱性が原因となり、歯などの臓器に水銀や鉛などの重金属が高値に検出されることが報告されている⁵⁾。

これらの背景をもとに、今回私たちはこれまで全く行われたことのない、日本の一般児童人口におけるこれら重金属の乳歯・毛髪内濃度と認知・行動異常の程度との連関を検索する疫学的調査研究を計画した。この研究により、どのような環境有害物質が、生後の認知・行動の異常の発症と関連するかを明らかにできれば、いまだ発症機転が明らかでない自閉症を初めとする認知・行動異常を伴う脳の機能性障害についての学術的な知見が得られるだけでなく、広く国民及び行政に対して、注意や予防に関する提言を行うことができる。

3. 疫学調査研究の方法

【対象】

今回の調査研究においては、永久歯萌芽の際に脱落した乳歯と毛髪の回収を計画した。このため、乳歯脱落の頻度が高い 4~12 歳（幼稚園・保育園、および小学校）の児童を対象とした。また、調査票の記入については、個々の児童の保護者が行うこととした。対象者の居住地域については全国とし、可能であれば地域による結果の差異についても検討することを考えている。

【方法】

研究者らが関係する地域の教育委員会、保育園長会、医師会、および学校長・園長に調査研究の内容につき説明をし、承諾が得られた場合には、施設責任者を通じて、対象園児・児童に調査票一式の入った封筒を配布してもらう。

調査票一式は図1に示すように、封筒の中に説明パンフレット、調査票冊子一冊、返信用封筒3種類、乳歯収納ビニール袋、毛髪収納ビニール袋、毛髪採取の方法説明書(図解入り)、および内容説明文書(倫理委員会申請書に基づくもの)が収納されているものである。



図1. 配布する調査資料セット

説明パンフレット、質問紙、乳歯収納ビニール袋、毛髪収納ビニール袋、及び返信用封筒がセットになっている。

パンフレットは図2に示すように、平易な文章で書かれ、写真等を配して、親しみやすさを心掛けた。質問紙は52項目の質問からなり、有害物質への母体のばく露や妊娠中の薬物摂取歴、妊娠中の感染症罹患などの妊娠経過とともに、対象児の生後の発達発育歴や睡眠リズムなどの質問項目を有し、対象児童の脳機能の評価を、ある程度行える内容になっている。

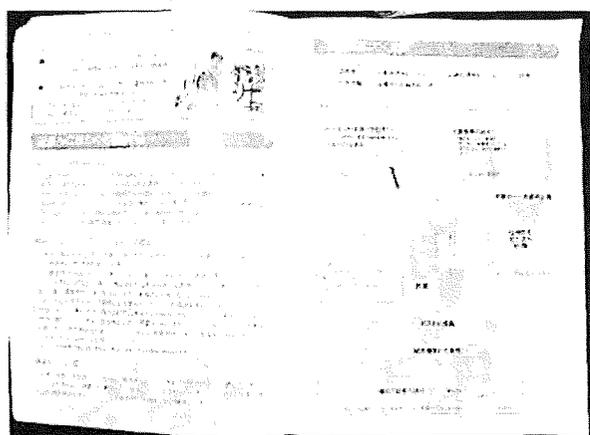


図2. パンフレットの文面

研究の目的、及び個人情報的一切収集しないこと、そして採取した検体の送付方法の図解が印刷されている。

対象児の保護者が説明パンフレットの内容を読み、同意した場合は、非侵襲的に採取できる児の生体試料(自然脱落した乳歯、毛髪)を提供していただく。また同時に、保護者が記入した質問紙も収集し、これらを統計数論的に解析する予定である。生体試料・記入済み質問紙は同封の差出人名無しの返信用封筒を用いて研究責任者あてに返送して頂く。検体や質問紙の返送をもって研究同意とみなすため、同意書は取らず調査は完全無記名で施行することとしている。質問紙の解析は三重大学及び文教大学で、生体試料(歯、毛髪)の解析は順天堂大学で行うため、質問紙(無記名)と生体試料は匿名化して通し番号で連結させておく。研究機関においては、個人情報は一切保管されない。

なお、本研究は、三重大学、順天堂大学、及び文教大学の研究倫理委員会の審査を経て承認を得て遂行されている。

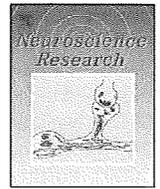
4. 現在の進行状況と今後の展望

2009年6月より本格的に始動した当該プロジェクトであるが、8月にはパンフレット、質問紙等資料も完成し、順次関係諸機関に協力の要請を研究者らが分担して行った。その結果、今年度の配布目標であった500部を大きく上回り、2009年10月現在、8都道府県の33施設(幼稚園・保育園、および小学校)と3か所の教育委員会より協力の申し出をいただき、3000部以上の配布が決定した。

今後は、順次資料の配布を行い、対象児童・保護者から返送されてくる検体と質問紙の回収を待つ、乳歯及び毛髪内の残留化学物質検出を行っていく予定である。同時に質問紙の回答を精査し、化学物質濃度と母の妊娠時の生活地域・環境、児の生活・睡眠の状態、また児の認知・行動の評価との関連性について統計学的手法を用いて細かく解析する予定である。同時に、通し番号を付けた配布資料を配布する際に記録しておいたおおまかな配布地域と、その地域での結果の状況を分析し、地域による残留化学物質の濃度差についても検索していく予定である。

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Nonexploratory movement and behavioral alterations in a thalidomide or valproic acid-induced autism model rat

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ABSTRACT

Autism is a behaviorally characterized disorder with impairments in social interactions, as well as stereotyped, repetitive patterns of behaviors and interests. Exposure of rat fetuses to thalidomide (THAL) or valproic acid (VPA) on the ninth day of gestation has been reported as a useful model for human autism. We have shown that early serotonergic neural development is disrupted in these rats. In the current study, we used a radial maze and open field experimental paradigm to investigate whether these rats present behavioral and/or learning aberrations. THAL (500 mg/kg), VPA (800 mg/kg), or vehicle was administered orally to E9 pregnant rats at 7–10 weeks of age. Although the mean number of correct and incorrect arm choices in the initial eight arm choices did not differ between control and teratogen-exposed groups, achievement of learning (seven or eight consecutive correct choices for 3 consecutive days for individual rats) seemed to be impaired in teratogen-exposed groups. Interestingly, average time to explore the maze task was shorter in the teratogen-exposed groups, indicating that correct choice might be due to mere coincidence (i.e., nonexploratory movement). Unexpectedly, no significant differences were observed in social interaction in these rats. These results indicate that prenatal exposure to THAL and VPA might alter behavior in a manner that is, in part, consistent with human autism.

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1. Introduction

Autism is a congenital neurodevelopmental behavioral disorder that presents in early childhood and is characterized by impaired socialization, communication abnormalities, as well as limited activity and curiosity (Charman and Baird, 2002; Filipek et al., 1999). Dysfunctions of the serotonergic system have been implicated in autism, including hyperserotonemia (Anderson et al., 1987), and altered PET imaging results have been obtained (Chugani et al., 1997). The prevalence of autism appears to be remarkably increasing (Muhle et al., 2004; Honda et al., 2005). This explosive increase in the prevalence of autism is hardly explained by simply widening diagnostic categories and increasing professional and public awareness. Therefore, unknown environmental factors such as chemicals, ethanol, and maternal stress during the earliest days of fetal life may be associated with autism (Lathe, 2006; Geier et al., 2009). Genetic factors have also been strongly

suggested from data of twin, family, and genetic association studies (Campbell et al., 2006; Folstein and Piven, 1991; Muhle et al., 2004).

The establishment of an animal model for autism is necessary to understand the pathophysiology and treatment of the disorder. Many studies have been conducted in primates and rodents to develop an animal model of autism, including genetically induced, lesion induced, and chemically induced animal models of autism (see review, Sadamatsu et al., 2006). However, given the complex nature of the disorder, no generally accepted model system has yet been developed. The challenge tends to be replicating the behavioral abnormalities such as hyperactivity and memory deficit.

We have established an autistic rat model through prenatal thalidomide (THAL) or valproic acid (VPA) exposure (Narita et al., 2002) based on collected pathological data of the high incidence of autism (Stromland et al., 1994; Williams et al., 2001). In particular, a study of patients in the Swedish THAL registry revealed that about 30% of children exposed to THAL on the 20–24th day of gestation became autistic, which is dozens of times more frequent than in the general population (Miller and Stromland, 1993; Stromland et al., 1994). Therefore, this animal model is quite

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unique because it faithfully replicates the embryonic time course of human THAL-induced autism. In these rats, we showed a dramatic increase in serotonin concentration in the brain that was observed only when animals were exposed to THAL on embryonic 9 (E9) (Narita et al., 2002), which is the critical period (human E20–24) for the occurrence of autism in humans (Rice and Borone, 2000). Furthermore, we also recently demonstrated that these abnormalities of the serotonergic system were due to early embryonic serotonergic development (Miyazaki et al., 2005). Similar results were also observed when using VPA exposure on E9, indicating that both THAL and VPA induce similar changes on serotonergic system, that may lead to autistic features.

Although the THAL- and VPA-induced autistic rat might replicate some of the autistic features in humans, its behavioral evaluation is essential. In this study, we examined the effect of embryonic exposure to THAL or VPA on spatial learning performance in a radial 8-arm maze task and analyzed locomotor activity, anxiety, and social behavior.

2. Materials and methods

2.1. Animals and teratogen exposure

All animal experiments were approved by the Community of Laboratory Animal Research Center at the University of Tsukuba, Japan. Details of the teratogen administration have been described elsewhere (Narita et al., 2002). In brief, female Wistar rats (SEASCO, Saitama, Japan, 7–10 weeks old) were mated overnight. The day of insemination was designated as E1. On E9 at 3:00 p.m., 500 mg/kg THAL, 800 mg/kg VPA, or vehicle was administered orally to the rats of each group without sedation using an infant feeding tube (Atom Medical, Tokyo, Japan) attached to a 2.5 ml disposable syringe. We referred to previous animal experiments using these teratogens to determine the THAL and VPA doses (Matsubara and Mikami, 1985; Ingram et al., 2000). THAL (Wako, Osaka, Japan) was prepared by dissolving it in 5% Arabic gum (Wako) in distilled water, and 5% arabic gum in distilled water was used as the vehicle treatment. VPA was prepared by dissolving 2-propylpentanoic acid (Sigma, St. Louis, MO, USA) in distilled water adjusted to pH 9.6 with sodium hydroxide. The dams were then housed individually and allowed to continue their pregnancy or to raise their own litters.

2.2. Eight-arm radial maze learning assay

The maze consisted of eight arms (nos. 1–8; 50 cm × 12 cm) extending radially from a central area (20 cm in diameter, Neuroscience, Tokyo, Japan), which was a modification of the maze originally developed by Olton and Samuelson (1976). The apparatus was surrounded by various extra-maze cues visible to the rat such as a laboratory bench, posters, and a clock. Placement of the maze and cues was consistent for the duration of the experiment. At the end of each arm, there was a cup that held a single 60-mg food pellet. The animals were kept on a restricted diet prior to performing the maze task to motivate the rats to seek the food rewards, and body weight was maintained at 85 ± 5% of their free-feeding weight over a 1-week period, with water being available *ad libitum*. The restricted diet persisted during the maze task. Rats were habituated to the maze for 30 min one day before commencing the first phase of training. Testing was performed during the dark phase.

Male Wistar rats at 7–8 weeks of age were used for this experiment. The maze test was performed once per day for 14 consecutive days. The rat was placed on the center platform that was closed off by guillotine doors. After 20 s, the guillotine doors were opened and the rat was allowed to make an arm choice to obtain food pellets until all eight pellets had been eaten or 10 min

had elapsed. The performance of the animal in each trial was assessed with the following parameters: the number of correct choices in the initial eight chosen arms, the number of errors, which was defined as choosing arms which had already been visited, and the time elapsed before the animal ate eight pellets. If the animals made seven or eight consecutive correct choices for 3 consecutive days, they were defined as having learned the maze. Their behavior was monitored by an infrared video camera and videotaped.

2.3. Open field test

Male Wistar rats at 15 weeks of age were used for this experiment. The apparatus consisted of a novel square box (80 cm × 80 cm × 60 cm), divided into 16 small squares by means of a grid of white painted lines. The box was located in a sound-attenuated room. Each rat was placed in the center of the open field and total ambulatory distance was determined over a 20 min period for 3 consecutive days. To further investigate behavioral reactivity under novel circumstances following 3 days of experimentation in the open field, rats were re-introduced to the open field on day 10 after a 7-day interval. The apparatus was illuminated by a dim red light (250 W bulb). The video image of the entire open field was displayed on a TV monitor, and peripheral, central, total ambulation (number of squares crossed in the area), and total ambulatory movement (m) were recorded. The apparatus was cleaned with 10% water–alcohol solution before each test to avoid possible bias due to odors and/or residues left by earlier tested rats.

2.4. Social interaction test

Male Wistar rats at 14 weeks of age were used for this experiment. Test rats were placed with an unfamiliar, nonexperimental rat of the same size and gender under a dim red light (250 W bulb) in a square box (50 cm × 50 cm × 30 cm). Each rat was videotaped for a 10-min period. The tapes were scored for incidence of sniffing, dominant mounts, social grooming, chasing, pinning, biting, boxing, and avoidance.

2.5. Statistical analysis

All data are presented as mean ± SEM. Differences between means were analyzed by a one-way or two-way repeated measures analysis of variance (ANOVA) or Student's *t*-test with a significance level of $P < 0.05$.

3. Results

3.1. Radial maze test

Fig. 1A shows the mean number of correct arm choices in the initial eight chosen arms. The number of correct choices obviously improved over the initial 6-day acquisition period in both VPA- and THAL-exposed and control groups as indicated by a one-way ANOVA (VPA vs. control, $P < 0.01$; THAL vs. control, $P < 0.01$). Task improvement did not differ between control and teratogen-exposed groups as evaluated by two-way repeated measures ANOVA. There also was a clear decrease in the number of incorrect choices in both teratogen-exposed and control groups as indicated by a one-way ANOVA (VPA vs. control, $P < 0.01$; THAL vs. control, $P < 0.01$) (Fig. 1B), with no significant difference between teratogen and controls either by two-way repeated measures ANOVA.

However, regarding total time spent consuming all eight pellets, both VPA- and THAL-exposed rats spent significantly shorter time as revealed by two-way repeated measures ANOVA

(VPA vs. control, $P < 0.01$; THAL vs. control, $P < 0.05$) (Fig. 1C). Subsequent post hoc analysis (Tukey–Kramer's procedure as a multiple comparison procedure) revealed that THAL-rats showed significantly shorter time to spend consuming all eight pellets compared to control rats ($P < 0.05$) on the first day of the trial. These findings indicate that it took longer time for controls to finish tasks compared to THAL- and VPA-rats. This longer time in control rats could be explained by "careful investigation". In other words, the shorter time in teratogen rats might be due to their "nonexploratory movement", which is frequently observed in human autistic patients.

To further characterize the acquisition performance of the spatial learning task among groups, we defined "achievement of learning" as the time when the animals made seven or eight consecutive correct choices for 3 consecutive days. The percentage of the cumulative number of rats that attained this criterion was less in the teratogen groups (Fig. 2) than that in the control group, suggesting that the teratogen rats performed poorly during acquisition and had difficulty learning this task despite making the same number of correct choices as control rats.

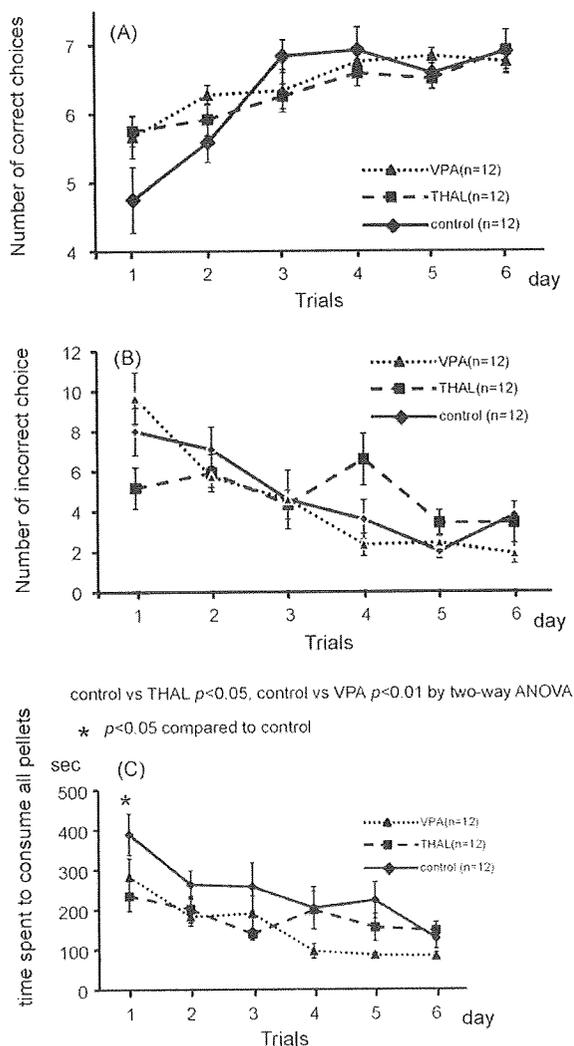


Fig. 1. Radial maze tests. Number of correct choices during the first eight choices (A), incorrect choices defined as choosing arms which had already been visited (B), the time elapsed before the animal ate eight pellets (C) on the radial maze in offspring exposed prenatally to THAL or VPA compared to control. Each point and its vertical line indicate the mean and SEM, respectively, based on 12 rats in each group.

3.2. Open field test

The open field test was performed to evaluate the movement of rats in a free environment. As shown in Fig. 3, THAL- and VPA-rats moved farther on the first trial compared to control rats ($P < 0.05$). This hypermovement in teratogen-exposed rats disappeared in the third trial, indicating that the THAL-exposed rats were only hyperactive in an unfamiliar environment. To further investigate behavioral reactivity under novel circumstances following 3 days of experiments in the open field, rats were subjected to another experiment on day 10 after a 7-day interval. Following the 7-day interval, THAL-exposed rats tended to show hypermovement again, although it was statistically not significant, suggesting that the THAL-exposed rats became hyperactive again in the unfamiliar environment, which mimics human autistic patients.

Arm preference of the hyperactive rats was examined in the open field to exclude the possibility that hyperactivity in teratogen-exposed rats was due to high anxiety. For this experiment, the floor of the open field was divided into 16 small squares by means of a grid of white painted lines (Fig. 4A). In an open field test, animals with higher anxiety prefer corners to the center. As shown in Fig. 4B, THAL and VPA had no significant effect

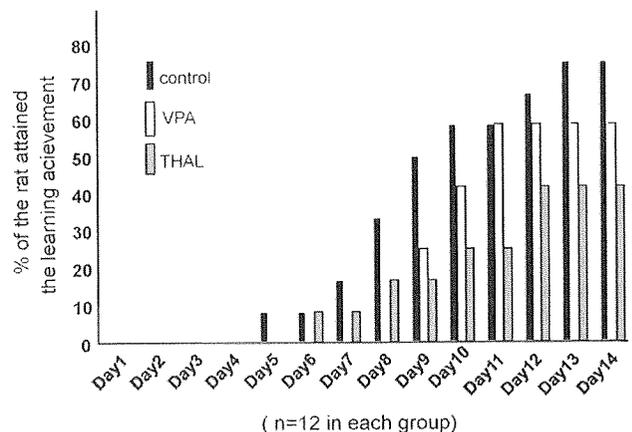


Fig. 2. Percentage of the accumulated number of rats to reach the criterion on the radial maze. When the animals made seven or eight consecutive correct choices for 3 consecutive days, they were defined as an achievement of learning.

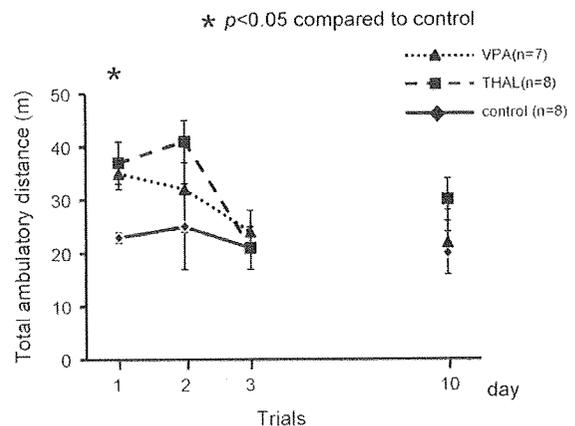


Fig. 3. Evaluation of the movement of rats in the free environment (open field tests). Each rat was placed in the center of the open field consisted of a square box (80 cm \times 80 cm \times 60 cm) and total ambulatory distance was determined over a 20-min period for 3 consecutive days. Rats were re-introduced in the open field on day 10 after 7-day interval for further investigation of behavioral reactivity under novelty circumstances following 3 days of experiment in open field.

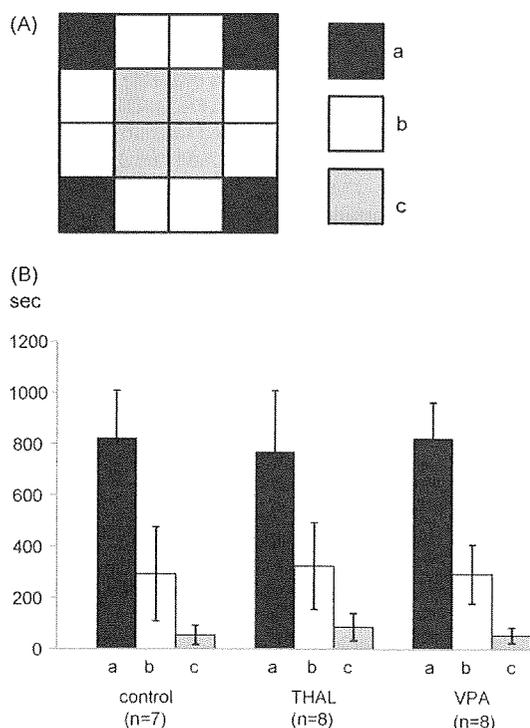


Fig. 4. Occupancy in preferential area in the open field test for the analysis of anxiogenic behavior. The floor of the open field was divided into 16 small squares by means of a grid of white painted lines (A). THAL and VPA had no significant effect on either occupancy of the center squares or corners of the apparatus (B).

on either occupancy of the center squares or corners of the apparatus, indicating that hyperactivity in the teratogen-exposed rats was not due to their high anxiety.

3.3. Social interaction test

Human autistic patients often show impairments in social interaction such as deficits in eye contact and difficulty being friendly. We analyzed and quantified markers for social interaction including sniffing, dominant mounts, social grooming, chasing, pinning, biting, boxing, and avoidance for 10 min to determine whether THAL- or VPA-exposed rats show impaired social interaction. None of the factors were statistically different between the control and teratogen rats (data not shown).

4. Discussion

The results of these experiments indicate that prenatal exposure to VPA or THAL on day 9 of gestation exerts a long-term influence on learning performance and behavior after birth. There was obvious improvement in the radial maze performance even in the teratogen groups, which did not statistically differ from that of control rats. The longer time spent in control rats is thought to be due to careful exploratory behavior such as door investigation, which decreased through training. In contrast, because there seemed to be no careful door investigation in VPA- and THAL-exposed rats, apparent memory acquisition in the number of correct and incorrect choices in these rats might have been accidental. A decrease in careful investigation, in other words "nonexploratory movement" in VPA- and THAL-exposed rats is known in human autism and is suspected to be linked to cerebellar anomalies (Pierce and Courchesne, 2001).

Maze performance in correct choices and incorrect choices in teratogen-exposed rats did not differ from that of controls.

However, judging from the results of "achievement of learning", we still believe that the THAL-rats as well as VPA-rats showed learning difficulty because "achievement of learning" was impaired in THAL- and VPA-rats. Achievement of learning was defined as seven or eight consecutive correct choices for 3 consecutive days for individual rats. Therefore, achievement of learning is thought to reflect individual improvement in evaluating learning performance compared to correct choices and/or incorrect choices (only evaluating by average number), although hard to evaluate in statistics.

The total ambulatory distance in the teratogen groups was significantly longer on the first trial of the open field test than the control group. Because this hyperactivity was not due to hyperanxiety as evaluated by preferential occupancy, longer ambulatory distance in the teratogen groups might be explained by hyperactivity in an unfamiliar environment, which is often observed in human autism. The fact that hyperactivity was gradually corrected to the control level through trial is consistent with the above interpretation.

It was unexpected to find no significant differences in the social interaction tests. It is plausible that the number of animals tested ($n = 7-8$ /group) was not enough to detect differences among the groups. Alternatively, it is possible that impaired social interaction could not be detected by the methods we chose. Therefore, further research including methodology for choosing behavioral instruments will be required to evaluate social interaction behavior in these rats.

The detailed molecular linked histological findings previously reported to be the basis of the behavioral abnormalities observed in the current study, such as nonexploratory movement and hyperactivity in an unfamiliar environment, are unknown. However, caudal displacement of 5-hydroxytryptamine neurons in the dorsal raphe nucleus of the hindbrain elicited by THAL and VPA (Miyazaki et al., 2005) is thought to result in alterations of the serotonergic pathway, which causes behavioral alterations based on the serotonergic neuron. In fact, McNamara et al. (2008) recently reported behavioral changes caused by the serotonergic agonist 5-methoxytryptamine, indicating that serotonergic alteration might cause behavioral change. However, further studies are necessary to elucidate this issue.

Although autism induced by THAL or VPA does not account for the vast majority of human autism cases, THAL or VPA autism model rats exposed especially on E9 are still important because the strategy we took is the same form as that seen in humans. Although other VPA autistic rat models have been reported to date, most studies were designed to expose teratogens later in pregnancy (E12.5 or E15) (Schneider and Przewtcki, 2005), which does not duplicate what happens in humans. Therefore, we are investigating the molecular basis of behavioral alteration in an ongoing study.

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