Temporal alterations in monoamine metabolism during early development induce emotional behavior disturbances in adult C57BL/6J mice

(発達期の一時的なモノアミン代謝異常はマウスの成長後の情動性を変化させる)

Kazuyuki Yamada
(山田一之)

【日本語要約】

モノアミン酸化酵素 (monoamine oxidase: MAO) は脳内のセロトニン、ノルアドレナリン（ノルエピネフリン）およびドーパミン代謝の主要な酵素である。MAOシステム、中でもモノアミン酸化酵素A (MAO-A) の障害は動物や人において認知的、情動的な異常の原因と成り得ることが知られている。モノアミン酸化酵素A阻害剤は脳内のセロトニンとノルエピネフリンの分解を阻害するため、鬱や不安障害、および他の精神障害の治療薬として利用されてきた。先行研究において、発達期における抗うつ薬や他の向精神薬の暴露が成長後の行動に影響を与え得ることが報告されてきたが、モノアミン酸化酵素A阻害剤の影響については不明である。そこで本研究では、発達初期におけるMAO-Aシステムの一時的な障害が行動に及ぼす影響について、実験動物モデルを用いて検討を行った。出生後14日目のC57BL/6J系統の仔マウスにトラニルシプロミン (TRP, 10mk/kg) あるいは生理食塩水を経口投与し、生後10週時に社会行動および攻撃行動の評価を、また11週目から行動テストバッテリーを用いた総合的な行動評価を行った。その結果、TRP処置群のマウスでは聴覚性驚愕反応試験とホールボード試験において行動変化が認められた。一方、社会行動および攻撃行動には変化が認められなかった。これらの結果は発達期における単回のTRP投与でも、成長後の情動行動に影響を及ぼし得ることを示唆するものである。

Temporal alterations in monoamine metabolism during early development induce emotional behavior disturbances in adult C57BL/6J mice

Kazuyuki Yamada

School of Management, Shizuoka Sangyo University

Abstract

Monoamine oxidases (MAO-A and MAO-B) are key enzymes involved in the degradation of brain serotonin (5-HT), norepinephrine (NE), and dopamine (DA). MAO system impairments, especially MAO-A, can lead to cognitive and emotional abnormalities in animals and humans. As MAO inhibitors (MAOIs) inhibit the degradation of 5-HT and NE in the brain, they have long been used for the treatment of depression, anxiety disorders, and other mental disorders. Although previous studies reported that exposure to antidepressants and other psychoactive drugs during development can affect behavior in adulthood, the effect of MAOIs remains unknown. In the present study, the behavioral effects of temporal disturbances in the MAO-A system during early development were investigated using a laboratory animal model. Tranylcypromine (TRP: 10 mg/kg) or saline were administered orally to pre-weaned male C57BL/6J mouse pups on postnatal day 14. The mice were assessed from 11 weeks of age using a battery of behavioral tests. Mice treated with TRP exhibited altered behaviors in the auditory startle response and hole-board test paradigms, though they did not exhibit any observable social deficiencies or aggressive behaviors. These results suggest that even a single administration of TRP can affect the emotional behaviors of mice in adulthood.

Key Words: monoamine oxidase (MAO), monoamine oxidase inhibitor (MAOI), tranylcypromine (TRP), emotional behavior, development, mouse

1. Introduction

Monoamine oxidase (MAO) plays a crucial role in brain monoamine metabolism, and is highly associated with cognition and the emotional behaviors of animals, including humans [1, 2]. MAO has two subtypes; monoamine oxidase A (MAO-A) mainly degrades serotonin (5-HT) and norepinephrine (NE), while monoamine oxidase B (MAO-B) degrades dopamine (DA) [1, 2]. Mutations in *MAOA/B* genes can cause serious cognitive and emotional abnormalities in humans, known as Brunner syndrome [3, 4]. Patients with Brunner syndrome exhibit antisocial behaviors, such as aggression [5], impaired impulse control [6], developmental disorders [7], and stress sensitivity [8]. When combined with traumatic life events and/or maltreatment in early life, low MAO-A activity can increase aggressive behaviors [9–12]. These studies suggest that MAOs, especially MAO-A, play a significant role in emotional control in humans.

The role of MAO-A in behavior has also been studied using laboratory animals. MAO-A-deficient mice exhibit behavioral abnormalities, such as anxiety [13], perseverative behaviors [14], autism spectrum disorder (ASD)-like behaviors [15], defensive behaviors [16], and altered emotional memory function [17, 18]. Inhibition of the MAO-A system at early developmental stages leads to depressive-like behaviors in mice during adulthood [19]. These behavioral changes are analogous to Brunner syndrome in humans [3, 4, 19]. The behavioral changes observed in both animals and humans may be caused by increased brain 5-HT concentrations during the early developmental period [14, 15, 20]. Although MAO-A-deficient mouse pups show severe behavioral abnormalities, chronic administration of para-chlorophenylalanine (pCPA), which inhibits tryptophan hydroxylase and blocks 5-HT synthesis, can rescue the behavioral abnormalities in these mice [20]. In addition, inhibition of the MAO-A system at early stages of development can affect neuronal development in MAO-A-deficient mice, i.e., barrel formation in the somatosensory cortex [21], locomotor network maturation [22], and the segregation of axons in the retinogeniculate and thalamocortical systems [23]. As an excess of 5-HT in the embryonic brain affects neural development [24, 25], and 5-HT itself can act as a growth factor in the immature brain [26], impaired neuronal development in MAO-A-deficient mice may be induced by disturbances in the metabolism of 5-HT.

MAO inhibitors (MAOIs), such as phenelzine sulfate (PHZ) and tranylcypromine (TR), are used as antidepressants [27, 28]. Some studies have reported that unborn children *in utero* or new-born children can be exposed to antidepressants taken during pregnancy and/or lactation *via* the placenta and/or breast milk, and this may induce behavioral as well as physical abnormalities in humans [29–32] and animals [33–36]. Furthermore, a single exposure to chemicals during pregnancy and/or the neonatal period can induce developmental and/or behavioral effects in rats and mice during adulthood [37–42].

The aim of the present study was to assess the delayed influence of temporal disturbances in the MAO-A system during the pre-weaning period on behavior in adulthood using a systematic behavioral test battery.

2. Materials and Methods

2-1. Animals: Twenty male C57BL/6J mice were used in this study (n = 10 in both the tranylcypromine and saline treated groups). Mice were obtained by mating commercially purchased male and female C57BL/6J mice (JCL, Tokyo, Japan). All mice were weaned at four weeks of age, and then housed 3–4 littermates per cage. At 10 weeks of age the mice were individually housed for one week prior to start of the behavioral experiments. The time schedule of the experiment is summarized in Fig. 1. The breeding and experimental rooms were air-conditioned (22 ± 1°C, 50–60% humidity), and a 12 h light-dark cycle was implemented (lights on at 0800). Food and water were freely available in the breeding cages. All behavioral experiments, except home cage activity measurements, were conducted during the light cycle between 1300 and 1700. All experimental procedures were performed in strict accordance with the guidelines of the Institute of Physical and Chemical

Research (RIKEN), and were approved by the institute's Animal Investigation Committee.

2-2. Drug treatment: Tranylcypromine (TRP: LKT Laboratories, MN, USA) was used at a dose of 10 mg/kg in this study. The drug was dissolved in physiological saline (0.9%, Otsuka, Tokyo, Japan) at room temperature (22 ± 1°C) before administration. The dose selected was determined by reference to the prescribing information of PARNATE™ (tranylcypromine sulfate: an antidepressant from GlaxoSmithKline Inc, Ontario, Canada) [43], and its safety was confirmed (no induction of sudden death, data not shown) from pilot experiments. The drug administration procedure was identical to that used in a previous study [42]. On postnatal day 14, each animal received either the drug or saline orally (per os [P.O.]; 10 μL/g body-weight) through a stainless-steel cannula (Muromachi-Kikai, Tokyo, Japan).

2-3. Behavioral procedures: Behavioral testing began when the mice were 11-weeks-old. The behavioral testing was performed as previously described [42, 44], and in the following order: assessment of sociality and aggressive behavior in the home-cage at 10 weeks of age (just prior to individual housing of the mice), home-cage activity measurement, open field (OF) test, light-dark (LD) box test, elevated plus maze (EPM) test, auditory startle response (ASR) and prepulse inhibition (PPI) test, Barnes maze (BM) test, hole-board (HB) test, and the classical fear conditioning (FZC) test. The inter-test intervals ranged from two to four days, depending on the task. The OF, LD box, EPM, ASR and PPI, and FZC tests were conducted in a sound proofed room as previously described [45]. During testing, the soundproofed room was dark and an electronic fan was running both for ventilation and background noise (35 dB). After each trial (except for home-cage activity measurements, and the ASR and PPI test, the various pieces of apparatus were wiped and cleaned with 80% alcohol and a damp towel. For the ASR and PPI test, the holding chambers were washed using tap water, wiped with a paper towel, and dried after each trial.

Assessment of sociality and aggressive behavior in the home-cage: Prior to the animals being individually housed, their social behaviors were observed to determine if the mice showed clear dominant-subordinate relationships in the home-cage. In addition, the fur condition and any damage to the body of each mouse were determined to rule out any fighting.

Home-cage activity measurement: The spontaneous home-cage activity of the mice was measured for 24 h per day for one week beginning in the afternoon on the day that they were transferred to the behavioral laboratory, and then daily for the next week using a 24-channel activity measurement system (O'Hara, Tokyo, Japan). The cages were placed individually into compartments made of stainless steel in a negative breeding rack (JCL, Tokyo, Japan). A piezoelectric sensor, which detected the movements of the mice (4–5 times per s), was fixed to the ceiling of each compartment. Following the completion of the home-cage activity assessments, the cages and bedding materials were changed, and the mice were then maintained throughout the remaining behavioral screening tests in micro-isolation racks (Allentown, NJ, US) that were identical to those used in the breeding rooms.

Open field test: The OF test arena was made of white plastic (50 (W) × 50 (D) × 40 (H) cm), and was illuminated by LED lights (70 Lux at the center of the field). Behavior was monitored by a charge-coupled device (CCD) camera fixed to the ceiling of the rack of the OF arena. Each mouse was placed in the center of the arena and allowed to move freely for 15 min. The total distance traveled (cm), and the time (s) spent in the center area of the arena (36% of the center of the overall arena) were recorded.

Light-dark box test: The light box was made of white plastic (20 (W) × 20 (D) × 20 (H) cm), and was illuminated by LED lights (250 Lux at the center of the box) with a CCD camera fixed to the ceiling. The dark box was made of black plastic (20 (W) × 20 (D) × 20 (H) cm) with an infrared camera fixed to the ceiling.

There was a tunnel with a sliding door in the center panel (3 × 5 cm) to allow the mice to transition between the light and dark boxes. Mice were introduced individually into the light box. The door to the tunnel automatically opened when the software detected that a mouse had been introduced into the testing arena. The mice were then allowed to move freely in the LD box for 10 min. The total distance traveled, percentage (%) distance traveled in the light box, % time spent in the light box, number of transitions made between the light and dark box, and the latency to enter the dark box for the first time were measured in this task.

Elevated plus maze test: A single-channel EPM (closed arms: 25 (W) × 5 (D) × 15 (H) cm; open arms 25 (W) × 5 (D) × 0.3 (H) cm) was set-up in the sound-proofed room. The floor of each arm was made of white plastic, while the walls of each closed arm and the ridges of each open arm were made of clear plastic. The closed and open arms were arranged orthogonally 60 cm above the floor. The lighting during testing was 70 Lux at the center of the maze platform (5 × 5 cm). The mice were placed individually on the center platform facing an open arm, and were then allowed to move freely in the maze for 5 min. The total distance traveled, % time spent in the open arms, and % open arm entries were measured in this task.

Auditory startle response and prepulse inhibition test: For the ASR test, each mouse was placed into a small plastic cage (30 or 35 mm in diameter and 12 cm long) that was set on a sensor block in a sound-proofed chamber (60 (W) × 50 (D) × 67 (H) cm). A dim light was fixed on the ceiling of the chamber (10 Lux at the center of the sensor block), and 65 dB white noise was provided as background noise. For the ASR test, the mice were acclimatized to the experimental apparatus for 5 min before the experimental session began. During the initial session, a 120 dB startle stimulus (40 ms) was presented ten times with a random inter-trial interval (10–20 s). During the second session, the startle responses to stimuli at various intensities were assessed. Each of the white noise stimuli (70, 75, 80, 85, 90, 95, 100, 110, and 120 dB; 40 ms) were presented five times in a quasi-random order, and with a random inter-trial interval (10–20 s). During the PPI test, the mice experienced five types of trials: no stimulus, startle stimulus only (120 dB, 40 ms), prepulse 70 dB and pulse 120 dB, prepulse 75 dB and pulse 120 dB, and prepulse 80 dB and pulse 120 dB. The prepulse duration was 20 ms, and the lead time (duration between the beginning of the prepulse and the beginning of the pulse) was 100 ms. Each type of trial was presented ten times in a quasi-random order with a random inter-trial interval (10–20 s). During the final session, only a 120 dB startle stimulus (40 ms) was presented ten times with a random inter-trial interval (10–20 s). The total duration of the ASR and PPI test was about 40 min.

Barnes maze test: A white plastic disk (100 cm in diameter) with 12 evenly spaced holes (3 cm in diameter) was placed 75 cm above the floor. The lighting was 150 Lux at the center of the maze. A habituation trial was conducted on the first day. For this, the mice were placed individually in the center of the maze and allowed to explore freely for 5 min. The escape training commenced following habituation. During the training session, the mice were placed individually in the center of the maze and led to an escape hole. The mice were then encouraged to escape into one of the 12 holes in the maze (by softly pinching the animal's tail if needed) into a black plastic box (17 (W) x 13 (D) x 7 (H) cm) that contained floor material from the home-cage of each mouse. This was repeated five times, changing the position of the escape hole in each trial. Maze training was conducted three times a day for four days. During the training sessions, the mice were placed into a small plastic container at the center of the maze for 10 s. The container was then opened to begin the trial. Each trial was terminated when the mouse escaped into the correct hole (escape cage without floor material) or 120 s had elapsed. The mice were left in the escape cage for 30 s and then returned to their home-cage. The inter-trial interval was approximately 15 min. A probe test was conducted after the final maze training session on the fifth day. The probe test was identical to the maze training, except that no escape cage was provided.

Hole board test: An OF arena made of gray plastic (50 (W) × 50 (D) × 40 (H) cm) with four equally-spaced holes (3 cm in diameter with an infrared sensor) in the floor was used (Model ST-1/WII, Muromachi-kikai,

Tokyo, Japan). The arena was illuminated by a fluorescent light (180 Lux at the center of the arena), and the background noise was approximately 50 dB. The behavior of each mouse was monitored by a CCD camera suspended about 1.5 m above the arena. During the HB test, the mice were introduced individually into the center of the arena and allowed to explore freely for 5 min. The total distance traveled (cm), latency to perform head-dips (s), number of head-dips, time spent engaged in head-dipping (s), number of rears, and the time spent engaged in rearing (s) were measured in this task.

Classical fear conditioning: The FZC test consisted of three parts: a conditioning trial (Day 1), a context trial (Day 2), and a cue trial (Day 3). Fear conditioning was carried out in a clear plastic chamber equipped with a stainless-steel grid floor (34 (W) × 26 (D) × 30 (H) cm). A CCD camera, which was connected to a video monitor and computer, was fixed to the ceiling of the chamber. The grid floor was wired to a shock generator. White noise (65 dB) was supplied from a loudspeaker as an auditory cue (conditioned stimulus; CS). The conditioning trial consisted of a 2 min exploration period followed by two CS-US (conditioned stimulus-unconditioned stimulus) pairings separated by a minute each. An unconditioned stimulus (US; foot shock: 0.5 mA, 2 s) was administered at the end of the 30 s CS period. A context test was performed in the same conditioning chamber for 3 min in the absence of the white noise CS 24 h after the conditioning trial. In addition, a cue test was performed using an alternative context with distinct cues, i.e., the testing chamber was different from the conditioning chamber described above in terms of brightness (almost 0–1 Lux), color (white), floor structure (no grid) with white bedding material on the floor (alpha-dri, Shepherd, TN, USA), and shape (triangular). The cue test was conducted 24 h after the contextual test was completed. The cue test consisted of a 2 min exploration period (no CS), to evaluate non-specific contextual fear conditioning, followed by a 2 min CS period (no foot shock) to evaluate cued fear. The frequency of the freezing response, characterized as immobility (other than respiration and heartbeat), was measured as an index of fear memory in these mice.

2-4. Data collection and statistical analysis: Image data were collected and analyzed using Image J OF4 (OF test), Image J LD4 (LD box test), Image J EPM (EPM test), Image J BM (BM test), and Image J FZ2 (FZC test) (O'Hara, Tokyo, Japan), and the CompACT VAS system (HB test) (Muromachi-Kikai, Tokyo, Japan). Mouse Startle (O'Hara, Tokyo, Japan) was used to analyze ASR and PPI test data.

Statistical analyses were conducted using SPSS™ version 19 statistical software (Japan IBM, Tokyo, Japan). A Student's *t*-test was used to compare the continuous data of the two groups, and a Mann-Whitney U-test was used to analyze the ratio data. Repeated testing paradigms were analyzed using a repeated measures analysis of variance (ANOVA; general linear model [GLM]). When Mauchly's hypothesis of sphericity was not supported, the degrees of freedom were modified using the Greenhouse-Geisser method [46]. Differences with a $p < 0.05$ were deemed statistically significant.

3. Results

The results of the behavioral assessments are summarized in Table 1. There were no abnormalities in gross development (e.g., appearance, body size or weight, or coat color) in the TRP-treated mice, and no evidence of social conflicts or aggression (body damage or loss of fur) in either the saline- or TRP-treated mice during the group housing condition.

In the home-cage activity test, the data from four cages were not collected due to a mechanical fault, so the data from sixteen mice (8 per group) were used in the analysis. TRP-treated mice showed a slight decrease in home-cage activity in the dark (active) phase, though this did not reach statistical significance (Fig. 2; repeated measures ANOVA, main effect of drug: $F(1, 14) = 2.05$, $p = 0.18$; main effect of day: $F(2.47, 34.60) = 28.93$, $p < 0.001$; main effect of phase (light period vs. dark period): $F(1, 14) = 47.80$, $p < 0.001$; *day* × *drug* interaction: $F(2.47, 34.60) = 1.01$, $p = 0.39$; *phase* × *drug* interaction: $F(1, 14) = 1.58$, $p = 0.23$; *day* × *phase* interaction:

F(2.97, 41.52) = 19.95, $p < 0.001$; *day* × *phase* × *drug* interaction: F(2.97, 41.52) = 0.37, $p = 0.79$). They also showed no anxiety-like behaviors in the OF test, the LD box, or the EPM test (see Table 1). In addition, the animals that were exposed to the drug showed intact spatial memory function in the BM test, and intact spatial and cued memory function in the FZC paradigm (Table 1).

Contrary to the above results, TRP-treated mice exhibited a greatly enhanced startle response to the auditory stimulus (Fig. 3A). A repeated measures ANOVA (GLM) revealed a statistically significant main effect of drug (F(1, 18) = 6.57, $p < 0.05$), tone intensity (F(2.25, 40.58) = 81.38, $p < 0.001$), and a *tone intensity* × *drug* interaction (F(2.25, 40.58) = 6.99, $p < 0.01$). As the interaction between the tone intensity and drug was statistically significant, the simple main effects of drug and tone intensity were analyzed. The simple main effects of both saline and TRP, and the tones used were statistically significant (saline: F(8, 144) = 21.63, $p < 0.001$; TRP: F(8, 144) = 66.75, $p < 0.001$; tone: 70 dB: F(1, 18) = 3.24, $p = 0.09$; 75 dB: F(1, 18) = 0.02, $p = 0.90$; 80 dB: F(1, 18) = 0.59, $p = 0.45$; 85 dB: F(1, 18) = 0.03, $p = 0.86$; 90 dB: F(1, 18) = 0.28, $p = 0.60$; 95 dB: F(1, 18) = 1.17, $p = 0.29$; 100 dB: F(1, 18) = 4.05, $p = 0.06$; 110 dB: F(1, 18) = 1.63, $p = 0.22$; 120 dB: F(1, 18) = 14.06, $p < 0.01$). The degree of startle in the TRP-treated mice was twice that of the saline-treated mice in the 120 dB condition. Furthermore, the saline-treated mice showed a decrease in the startle response in the final measurement compared with the initial measurement. On the other hand, the TRP-treated mice exhibited an increased startle response in the final measurement (Fig. 3B). A repeated measures ANOVA (GLM) revealed a statistically significant effect of drug (F(1, 18) = 4.93, $p < 0.05$), and *drug* × *tone repetition* (initial vs. final) interaction (F(1, 18) = 14.20, $p < 0.01$), though no effect of tone repetition was observed (F(1, 18) = 0.22, $p = 0.65$). As the interaction between drug and tone repetition was statistically significant, the simple main effects of drug and tone repetition were analyzed. The effects of both saline and TRP, and tone repetition were statistically significant (saline: F(1, 18) = 8.97, $p < 0.01$; TRP: F(1, 18) = 5.45, $p < 0.05$; tone repetition: initial: F(1, 18) = 0.23, $p = 0.33$; final: F(1, 18) = 2.91, $p < 0.01$). Despite the differences in the startle response, TRP-treated mice did not show any difference in the PPI trials with any prepulse condition (repeated measures ANOVA (GLM): drug: F(1, 18) = 0.31, $p = 0.59$; prepulse condition: F(1.54, 27.64) = 25.00, $p < 0.001$; *prepulse condition* × *drug* interaction: F(1.54, 27.64) = 0.18, $p = 0.78$).

In the HB test, TRP-treated mice showed a statistically increased number of head-dip responses (Fig. 4A: $t = -2.25$, $p < 0.05$), and an increase in the time spent engaged in head dipping (Figure 4B: $t = -2.29$, $p < 0.05$); however, there were no significant differences in the total distance traveled, the latency to head-dipping, the number of rears, or the length of time engaged in rearing ($t = 0.18$, $p = 0.86$; $t = 0.62$, $p = 0.54$; $t = 0.97$, $p = 0.35$; $t = 0.64$, $p = 0.53$, respectively) compared to the saline group.

4. Discussion

In the present study, mice that were exposed to an MAOI (tranylcypromine: TRP) in the pre-weaning period showed some emotional changes in adulthood. The results of this study are comparable to a previous study that showed that administration of an MAOI (clorgyline (CLO)) during the pre-weaning period induced emotional changes [19], and also parallels previous studies that reported that a single drug exposure during the early developmental period could cause both neural and behavioral abnormalities in adulthood [37–42].

Many studies have reported a relationship between impairments in the brain MAO system and aggression in both human and animals [3–5, 9–12, 19, 20]. Disturbances in the MAO system during the embryonic and/or early developmental stages might affect the impulsiveness of the individual, and enhance aggression. In the present study, TRP-treated mice did not show any evidence of social conflict or aggressive behavior in the group-housing conditions prior to the behavioral tasks. In previous studies, increased aggression was observed in MAO-A-deficient mice [18, 20], though inhibition of the MAO-A system by CLO during the developmental

period did not induce aggressive behaviors in adulthood [19]. This discrepancy between genetically-modified mice and drug-treated mice can, in part, be explained by the timing and duration of the disturbances to the MAO-A system. Furthermore, some reports have suggested that traumatic life events and/or maltreatment during the early developmental period may prompt the development of aggressive behavior in patients/animals with low MAO-A activity [9–12]. These results suggest that the lack of obvious social deficits and aggression in TRP-treated mice in the present study may be attributable to the timing of the inhibition of the MAO-A system, as well as the stable breeding conditions. In either case, more detailed studies are required to elucidate the relationship between temporal disturbances in the MAO-A system during the developmental period and the development of aggressive behaviors in adulthood.

In this study, TRP-treated mice showed a slight decrease in spontaneous home-cage activity, though this was not statistically significant (Fig. 2). However, the mice did not exhibit any differences in the OF, the LD box, or the EPM test. This is in contrast to the results of the study by Yu et al. who reported that the inhibition of the MAO-A-system by CLO during postnatal day (PD) 2 to 21 decreased locomotor activity and rearing in the OF [19].While the TRP-treated mice did not show any evidence of emotional changes in the OF, LD, or EPM, these results do not mean that the disturbances in the MAO-A system did not have any effect on emotionality as the results of previous studies using MAO-A-deficient mice are not consistent. Reduced activity in the OF and a lack of anxiety-like behaviors in the LD box and EPM have previously been reported [14], though equivalent locomotor activity and anxiety responses in wild-type and MAO-A-deficient mice have also been reported in the EPM [16]. Despite the inconsistencies in these results, changes in emotionality [13–18] and perseverative/stereotypical behaviors [14, 15] seem to be common features in MAO-A-deficient mice. Thus, the results from MAO-A-deficient mouse studies suggest that it is plausible that proper functioning of the MAO-A system during the pre-weaning period is necessary for the proper development of emotionality.

Contrary to the results from the tests mentioned above, TRP-treated mice exhibited a greatly enhanced startle response to an auditory stimulus (Fig. 3A). Furthermore, they showed enhancement of the startle response following repeated exposure to the stimulus (Fig. 3B). This suggests that TRP-treated mice may become sensitized to the auditory stimulus to which the control mice showed desensitization. This sensitization to the startle stimulus following repeated exposure to it suggests that TRP-treated mice alter their responsiveness to the auditory stimulus or information processing. As impairments in the MAO-A system lead to abnormal impulse behaviors in patients with Brunner syndrome [3, 4], the sensitization of TRP-treated mice to the startle stimulus may reflect enhanced impulsiveness.

In addition to the enhanced startle response, TRP-treated mice showed a significantly increased number and length of head-dipping responses in the HB test (Fig. 4A and B). Increased head-dipping responses are suggested to reflect a decrease in anxiety [47, 48]. These results suggest that TRP-treated mice show decreased anxiety-like behaviors in some unfamiliar environments, although the mice did not show altered anxiety-like behaviors in either the OF, LD, or EPM tests. TRP-treated mice had increased responsiveness to the holes in the HB apparatus, and enhanced startle responses in the ASR test (Fig. 3A). At this time, it cannot be determined whether the decreased anxiety-like behaviors or increased responsivity prompted the head-dipping responses in the HB test; thus, further detailed analyses are required to determine how emotionality is affected by disturbances in the MAO-A system. Changes in ASR and HB, which are closely related to some mental dysfunctions, have not been previously reported in MAO-A-deficient and/or drug-treated mice studies. Thus, these results provide new evidence about the relationship between MAO-A-system disturbances during early developmental stages and abnormal behaviors in adulthood. However, it must be noted that the behavioral changes observed in TRP-treated mice are limited, and the mechanisms of the changes discussed above are only speculation.

The present study demonstrated that a single incidence of MAO-A system inhibition during the early developmental period is enough to cause behavioral changes in mice during adulthood. These results suggest that exposure to MAOIs, as well as other antidepressants (e.g. tricyclic antidepressants, selective serotonin reuptake inhibitors [SSRIs]), during pregnancy and/or lactation may increase the risk of abnormal development in infants. Further systematic and detailed studies are required to elucidate the toxicity of MAOIs during development.

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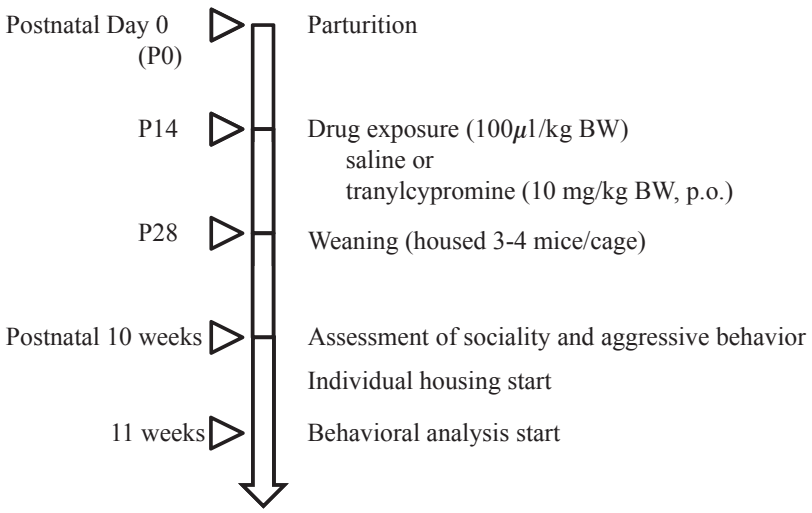

Fig. 1　Schematic drawing of the experimental procedure
BW: body-weight; p.o.: per os.

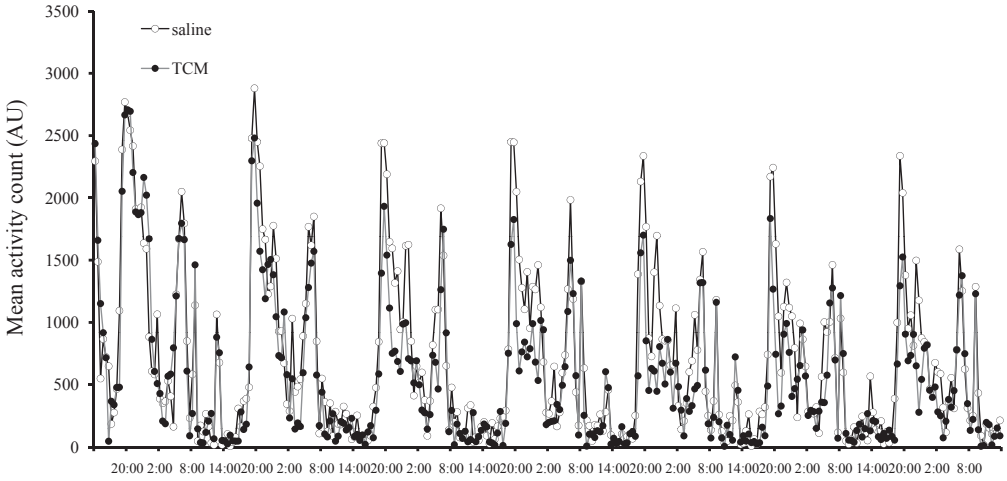

Fig. 2 Summary of the home cage activity measurement

Data represented as mean activity count. AU: arbitrary unit; SAL: saline-treated mice; TRP: tranylcypromine-treated mice.

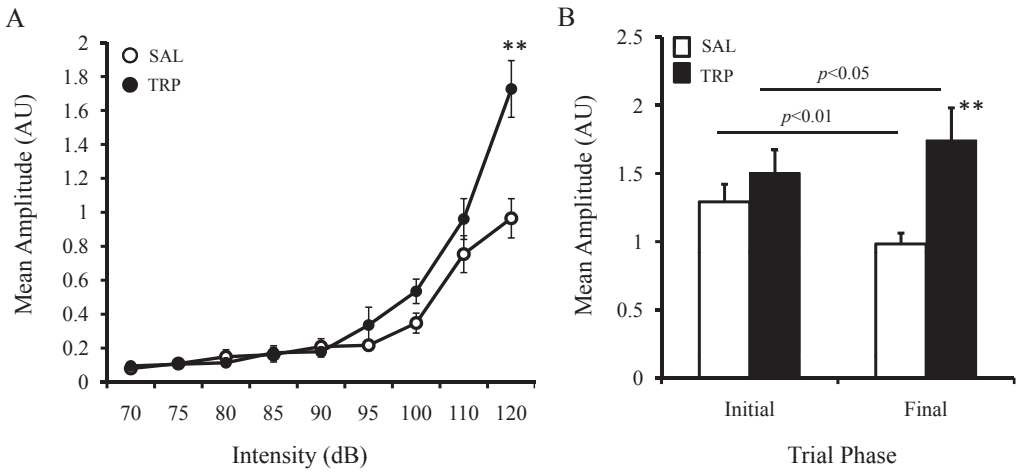

Fig. 3 Results of the auditory startle response (ASR) measurement

A) Mean response to each stimulus intensity. Data represented as mean ± S.E.M. ** $p < 0.01$ compared to saline-treated mice. B) Mean response to 120 dB stimulus in the initial and final 10 trials. Data represented as mean + S.E.M. The p-values represent statistically significant differences between the initial and the final trial phase. ** $p < 0.01$ compared to saline-treated mice. AU: arbitrary unit; SAL: saline-treated mice; TRP: tranylcypromine-treated mice.

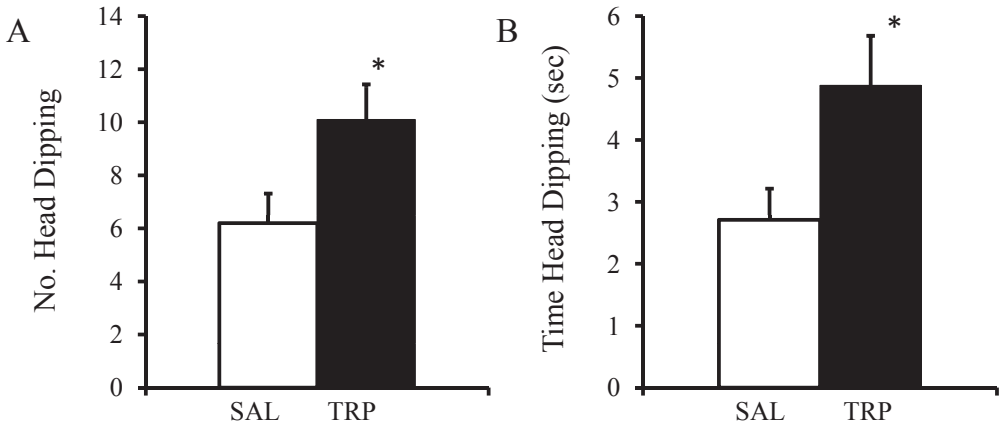

Fig. 4　Results of the hole-board (HB) test

A) Mean number of head-dips. B) Mean time spent head-dipping. Data represented as mean + S.E.M. * $p < 0.05$ compared to saline-treated mice. AU: arbitrary unit; SAL: saline-treated mice; TRP: tranylcypromine-treated mice.

Table 1 Summary of the behavioral assessment

Task/Index	Results (mean ± SEM)		Statistical significance
Task/Index	SAL	TRP	
Social behavior in the home-cage			
body damage	no evidence		
loss of fur	no evidence		
Home-cage activity measurement			
activity count (whole day) (AU)	715.5 ± 103.5	569.8 ± 48.9	t = 1.27, n.s.
Open field (OF) test			
total distance traveled (cm)	7033.8 ± 1041.1	7406.6 ± 1073.0	*t* = -0.788, n.s.
time spent in the center area (sec)	170.0 ± 43.7	186.0 ± 28.5	*t* = -0.966, n.s.
Light-dark box (LD) test			
total distance traveled (cm)	1543.5 ± 146.5	1576.8 ± 162.9	*t* = -0.910, n.s.
% distance traveled in the light box	42.7 ± 1.02	43.9 ± 1.55	U = 39, n.s.
% time spent in the light box	44.1 ± 1.44	45.9 ± 1.62	U = 39, n.s.
no. transitions between light and dark box	48.7 ± 10.8	49.2 ± 11.7	*t* = -0.10, n.s.
latency for the first enter into the dark box (sec)	18.0 ± 15.5	24.4 ± 14.1	*t* = -0.706, n.s.
Elevated plus maze (EPM) test			
total distance traveled (cm)	167.8 ± 225.8	1222.0 ± 256.0	*t* = -0.502, n.s.
% time spent in the open arms	30.5 ± 5.96	29.9 ± 5.77	U = 48, n.s.
% open arm entries	37.3 ± 4.92	39.0 4.62	U = 44, n.s.
Auditory startle response(ASR) and prepulse inhibition (PPI) test			
startle response at 120 dB	0.97 ± 0.12	1.73 ± 0.17	F(1, 18) = 14.06, $p < 0.01$
initial phase at 120 dB	1.29 ± 0.13	1.51± 0.17	F(1, 18) = 0.23, *n.s.*
final phase at 120 dB	0.98 ± 0.08	1.75 ± 0.24	F(1, 18) = 2.91, $p < 0.01$
Barnes maze (BM) test			
latency to escape (Day 4) (sec)	34.7 ± 7.43	34.7 ± 8.54	*t* = 0.001, n.s.
number of errors (Day 4)	4.60 ± 1.31	3.60 ± 0.74	*t* = -0.612, n.s.
Hole-board (HB) test			
total distance traveled (cm)	507.7 ± 171.9	115.7 ± 36.6	*t* = 0.182, n.s.
latency for head-dipping (sec)	10.4 ± 10.6	7.63 ± 9.53	*t* = 0.624, n.s.
number of head-dipping	6.20 ± 3.52	10.1 ± 4.20	*t* = -2.250, $p < 0.05$
time engaged in head-dipping (sec)	2.71 ± 1.59	4.88 ± 2.54	*t* = -2.289, $p < 0.05$
number of rearing response	0.50 ± 1.27	0.10 ± 0.32	t = 0.967, n.s.
time engaged in rearing response (sec)	0.26 ± 0.79	0.09 ± 0.28	*t* = 0.642, n.s.
Classical fear conditioning (FZC) test			
context test (% freezing)	48.7 ± 6.35	44.0 ± 6.96	U = 43, n.s.
cued test: without CS	18.1 ± 5.52	9.21 ± 3.58	U = 32.5, n.s.
with CS	49.8 ± 4.88	42.2 ± 4.96	U = 39.5, n.s.

Note) AU: arbitrary unit, SAL: saline treated mice, TRP: tranylcypromine treated mice.