Chronic Reduction in Dietary Tryptophan Leads to Changes in the Emotional Response to Stress in Mice

Shusaku UCHIDA¹, Aya KITAMOTO¹, Hisahiro UMEEDA², Nobutoshi NAKAGAWA²,
Shoichi MASUSHIGE^{1,2} and Satoshi KIDA^{1,2,*}

¹Department of Agricultural Chemistry, Faculty of Agriculture, and ²Department of Bioscience,
Faculty of Applied Bioscience, Tokyo University of Agriculture,
1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

(Received December 18, 2004)

Summary Acute depletion of brain tryptophan (TRP) levels in humans has been used as a biochemical model of depression. In this study, we examined the effects of consumption of a diet low in TRP on emotional behavior in mice. Specifically, we assessed various parameters of emotional behavior in mice fed a TRP-limited diet for at least 1 mo. TRP-limited mice showed increased defensive, but not offensive, aggression in the resident-intruder test. In the social dominance tube test, these mice showed enhanced social dominance. Since defensive aggression is thought to be a reflection of not only aggression but also fear, these changes in the social behavior of TRP-limited mice are thought to reflect changes in their emotional status. TRP-limited mice also showed increased locomotor activity and mobility in the open field and forced swim tests, respectively, suggesting that their stress/emotional responsiveness was enhanced. Importantly, these mice displayed normal levels of anxiety and motor performance as determined by the elevated zero maze and open field tests, and the rotarod test, respectively, suggesting that their hyperactivity was not due to a reduction in anxiety levels or to enhancement of their motor performance. Thus, dietary TRP restriction appears to result in alterations in the emotional response to stress, in mice.

Key Words dietary tryptophan limitation, emotional response, mouse behavior, aggression, locomotor activity

Serotonin (5-HT) has been shown to play an important role in the regulation of emotions, including anxiety and aggression (1–5). Reduced brain levels of 5-HT induced by a lesion in the dorsal raphe containing cell bodies of serotonergic neurons reportedly led to increased aggression in rodents (6–8). Furthermore, abundant evidence suggests that brain serotonergic systems are involved in mediating anxiety and possibly depression (2–5, 9–14).

5-HT is synthesized from the essential amino acid L-tryptophan (TRP) by a two-step enzymatic reaction that involves the generation of 5-hydroxytryptophan as an intermediate (15, 16). In the first step, TRP is catalyzed by the rate-limiting enzyme tryptophan hydroxylase, the activity of which is normally limited by the availability of TRP (17). Thus, brain levels of TRP correlate closely with 5-HT levels. More importantly, brain and plasma 5-HT levels are directly influenced by dietary TRP intake (18–21); thus, dietary TRP likely influences behaviors that are regulated by 5-HT neurons.

Previous studies demonstrated that modulation of either TRP or 5-HT levels leads to similar changes in emotional behavior (6–8, 16, 22–34). In humans, acute TRP depletion induced by the ingestion of a balanced amino acid mixture that lacked TRP lead to an

increase in aggression and lowering of mood (22–24, 33, 34). This model of acute TRP depletion has been accepted as a biochemical model of depression (33, 34). Furthermore, rodents that had their dorsal raphe lesioned and those that were injected with serotonergic antagonists exhibited increased levels of aggression (6–8, 25, 35, 36). TRP depletion also increased mouse-killing behavior and shock-induced aggression in rats (26, 29–32). These studies strongly support the contention that dietary modulation of TRP might affect emotional behavior in mammals.

In light of the above data, it is important to investigate the effects of a chronic reduction in the dietary intake of TRP on anxiety and aggression. Accordingly, in this report, we examined the emotional behavior of mice fed a TRP-limited diet. Specifically we used the open field and elevated zero maze tests in order to assess the anxiety levels of mice, and also subjected them to resident-intruder and social dominance tube tests in order to evaluate their aggressiveness and social behavior, respectively.

MATERIALS AND METHODS

Animals. All experiments were conducted in accordance with the rules outlined in the Guide for the Care and Use of Laboratory Animals, Japan Neuroscience Society. Male C57BL/6N mice (Charles River, Kanagawa, Japan) were housed individually in cages in a

*Corresponding Author.
E-mail: kida@nodai.ac.jp

Table 1. Composition of diets.

Constituent (g/kg)	Control diets	TRP-limited diets
Casein (Vitamin-free)	90.00	90.00
Glycine	20.00	20.00
L-Threonine	0.78	0.78
L-Cystine	2.00	2.00
L-Tryptophan	0.68	0
Sucrose	776.54	777.22
Corn oil	50.00	50.00
Mineral mixture (AIN-93G-MX)	50.00	50.00
Vitamin mixture (AIN-93-VX)	10.00	10.00
Total content of tryptophan (%)	2.00	1.32

room that was kept on a 12 h light/dark cycle. This genetic background was used in studies investigating emotional behavior (12, 37–40). Mice were at least 12 wk of age until they were subjected to the behavioral tests described below, and all tests were carried out during the light phase. All experiments were conducted in a blinded fashion.

Limitation of TRP intake. Male mice (8 wk of age) were fed an L-TRP-limited diet (TRP-limited diet) for at least 4 wk, through their completion of the behavioral experiments. The composition of the experimental diet was based on the previous studies investigating the effects of the limitation of TRP intake (41–44). The mineral mixture and vitamin mixture was composed according to AIN-93-VX or AIN-93G-MX, respectively (45). Both the TRP-limited and control diets were freshly prepared weekly. The composition of the diets and their final TRP levels are tabulated in Table 1. Importantly, a previous study has shown that more decrease in TRP-intake leads to more abnormal change in not only 5-HT but also other neurotransmitters (46). Mice were allowed free access to either TRP-limited or control chow, as well as water.

Behavioral procedures

Resident-intruder test: To assess offensive aggression, resident mice were individually housed for 4 wk prior to the introduction of a wild-type male intruder mouse, the latter of which was previously housed in a group of five animals. To assess defensive aggression, mice that had previously played the role of resident animal became the intruder; a different group of wild type mice that had been individually housed for 4 wk, were used as the residents. Each resident mouse was paired with the same intruder mouse for 5 min for 3 consecutive days. The latency to first attack bite (attack latency) and the total number of attacks by the test mice were measured. Animal behavior was recorded using a video camera.

Social dominance tube test: To measure subordinate and territorial behavior such as competing responses to another mouse, the social dominance of TRP-limited and control mice was tested as previously described (47–49), using an acrylic tube (30 cm long and 3.5 cm

Fig. 1. TRP-limited mice (n=12) showed comparable body weight gain with control mice (n=12).

diameter). A TRP-limited and control mouse were released into opposite ends of the tube. The mouse that was left alone in the tube was assessed as the “winner” and its opponent the “loser.” Animal behavior was recorded using a video camera.

Open field test: Since mice fear novel, open spaces and generally avoid the center of open fields, this test is used to measure anxiety. Mice were placed into the center of a square open field chamber (40 cm long×40 cm wide×40 cm high) that was surrounded by white acrylic walls. The total length of the path the mouse traveled (locomotor activity), the number of times it stood on its hindlimbs (rearing events) and the time it spent in a center square (24 cm×24 cm; time in center) were measured over the course of 5 min using an automatic monitoring system (TARGET 2, Neuroscience Inc., Tokyo, Japan).

Elevated zero maze test: Since mice generally avoid open spaces where mice realize height, the duration of time that they spend in the open spaces and the number of times they enter such spaces are thought to reflect their state of anxiety. The zero-maze consisted of a circular path (5.5 cm width, inner diameter of 46 cm) that had two open and two closed sections (wall was 15 cm high) and was elevated 50 cm above the floor (50). Mice were initially placed in the closed section and their behavior observed for 5 min. The length of time that they spent in the open section (open section time) and the number of times they entered into the open section with two or four paws was measured. Animal behavior was recorded using a video camera.

Forced swim test: It is possible that an increased locomotor activity in TRP-limited mice as displayed in the open field test might be due to alteration of the stress response in the face of a dangerous, inescapable situation. To test this, mice were left in an acrylic cylinder (height 25 cm, diameter 15 cm) that contained 14 cm of water, for a total of 6 min. Their immobility time was measured during the final 5 min of the test using an automatic monitoring system (Image FS for Inter View, O'Hara & Co., Ltd., Tokyo, Japan). The increase in immobility time is thought to reflect behaviors associated with depression (51, 52).

Rotarod test: Mice were placed on a rotating drum (3 cm in diameter; O'Hara & Co., Ltd.). The drum was initially rotated at a speed of 4 rpm after which it was

Fig. 2. TRP-limited mice displayed increased defensive aggression as determined by the resident-intruder test. Offensive (A, B) and defensive (C, D) aggression were measured on 3 consecutive days. The latency to first attack bite (attach latency; A, C) and the number of attacks (B, D) are shown for TRP-limited ($n=15$) and control ($n=15$) mice. Asterisks indicate statistical significance at $p<0.05$.

accelerated gradually to 40 rpm over the course of 5 min. The amount of time that the mouse remained on the accelerating rod (running time) was recorded as an indicator of their motor performance, on 3 successive days. Animal behavior was recorded using a video camera.

Statistics. All values are expressed as the mean $\pm$ SE. Data were analyzed using an analysis of variance (ANOVA) and a single-factor ANOVA and *post hoc* comparisons were used to analyze differences between groups for the behavioral tests and TRP content measurement studies.

RESULTS

Effect of chronic restriction of dietary tryptophan on body weight gain

Figure 1 shows the body weight gain of TRP-limited and control mice. Male adult mice (8 wk of age) were fed a TRP-limited diet. A previous report using juvenile rodents showed that depletion of TRP from diet and similar restriction of TRP intake with this study led to a severe or mild impairment of body weight gain, respectively (30, 41). In contrast, the limitation of TRP intake for more than 2 wk did not affect the body weight gain of adult mice (Fig. 1). These results indicate that feeding adult mice a TRP-limited diet under our experimental conditions does not affect body weight gain.

Resident-intruder test

TRP-limited resident mice showed comparable offensive aggression, as assessed by their number of attacks and attack latency, compared to control resident mice (Figs. 2A and B). Regarding the results of their defensive aggression test, both TRP-limited intruder, and control

Fig. 3. TRP-limited mice showed increased social dominance in the social dominance tube test. The percent of times that TRP-limited mice were declared the winner is indicated by the black bar ($n=16$), while the percent of times that control mice were declared the winner is indicated by the white bar ($n=16$). The asterisk indicates statistical significance at $p<0.05$.

intruder mice showed similar results after their first trial (Figs. 2C and D). However, TRP-limited intruder mice showed significantly reduced attack latency from control intruder mice on the trials that were carried out on days 2 and 3 (Fig. 2C: day 2, $p<0.02$; day 3, $p<0.05$). Furthermore, test results on the third day revealed that TRP-limited mice also exhibited a significantly greater number of attacks than control mice (Fig. 2D: day 3, $p<0.01$). These results suggest that TRP-limited mice displayed increased defensive but not offensive aggression.

Social dominance tube test

Two hundred and fifty six trials were performed on a total of 16 control mice and 16 TRP-limited mice. In 150 of the 256 trials, TRP-limited mice were declared

Fig. 4. TRP-limited mice displayed increased locomotor activity in the open field test. The total path length (A), the number of rearings (B) and time spent in the center (C) are shown for TRP-limited (black bar, $n=17$) and control (white bar, $n=16$) mice. The asterisk indicates statistical significance at $p<0.05$.

Fig. 5. TRP-limited mice exhibited normal levels of anxiety as determined by the elevated zero maze test. The time spent in the open section (A) and the number of entries made into the open section (B) were similar for TRP-limited (black bar, $n=17$) and control (white bar, $n=17$) mice.

Fig. 7. TRP-limited mice showed normal motor performance in the rotarod test. The time it took TRP-limited mice to fall from the rotating bar (black bar, $n=17$) was similar to that seen in control mice (white bar, $n=17$).

Fig. 6. TRP-limited mice showed increased mobility in the forced swim test. The immobility times for the TRP-limited ($n=17$) and control ($n=17$) mice are indicated by the black and white bars, respectively. The asterisk indicates statistical significance at $p<0.05$.

the winner (Fig. 3; $p<0.05$), suggesting that they were more dominant than control mice, and further implying that TRP suppression affects social dominance as well as defensive aggressive behavior.

Open field test

TRP-limited mice exhibited significantly longer path lengths in the open field tests compared to control mice (Fig. 4A; $p<0.05$). They also displayed more episodes of rearing than control mice, though this difference was not statistically significant (Fig. 4B; $p=0.08$). These results suggest that TRP-limited mice exhibited increased locomotor activity. On the other hand, both TRP-limited and control mice spent a comparable amount of time in the center of the field (Fig. 4C;

$p=0.16$), leading to the conclusion that the increased locomotor activity in TRP-limited mice was not due to decreased anxiety.

Elevated zero maze test

TRP-limited mice spent a similar amount of time in the open section in this test, compared to control mice (Fig. 5A; $p=0.51$) and exhibited a comparable number of entries into the open section (Fig. 5B; $p=0.29$). These results suggest that chronic restriction of TRP intake did not affect anxiety levels in our animals.

Forced swim test

TRP-limited mice displayed significantly less immobility than control mice in this test (Fig. 6; $p<0.05$). These findings were consistent with our demonstration of higher locomotor activity in TRP-limited mice in the open field test. These results suggest that TRP intake limitation adversely affected the stress response in our animals.

Rotarod test

The results of the open field and forced swim tests suggested that limiting TRP intake leads to enhanced motor performance. To further investigate this possibility, we subjected mice to the accelerating rotarod test. Our results showed that TRP-limited and control mice displayed comparable degrees of motor performance (Fig. 7: day 1, $p=0.91$; day 2, $p=0.34$; day 3, $p=0.96$), suggesting that limitation of TRP intake did not lead to facilitation of motor coordination. Thus, the hyperactivity seen in TRP-limited mice in the open field and forced swim tests was not likely to have been due to increased motor performance.

DISCUSSION

Previous studies have shown that reduced TRP intake leads to lower brain TRP and 5-HT levels in mice (19–21), suggesting that the behavioral changes that we found in these animals might have been due to alterations in their brain 5-HT levels caused by the restriction of TRP-intake.

In humans, acute reductions in TRP induced by the ingestion of a TRP-free amino acid mixture significantly increased aggression (22, 24). In rodents, feeding TRP-free diets increased shock-induced aggression and mouse-killing behavior (26, 29–32). In our study, mice that were fed a diet low in TRP for 1 mo also displayed increased aggression (Fig. 2). Importantly, previous studies have shown that reductions in 5-HT levels, induced by a lesion of the dorsal raphe nucleus in animals, led to increased aggression (6–8). Therefore, it is suggested but not proved that the enhanced aggressiveness of our TRP-limited mice was due to reductions in their brain levels of 5-HT.

It is noteworthy that defensive, but not offensive, aggression was increased in the TRP-limited mice. Interestingly, heterozygous mutant mice that lack the gene that encodes α -Ca²⁺ calmodulin dependent kinase II (α CaMKII) were also reported to exhibit an increase in defensive, but not offensive, aggression (39). α CaMKII is known to phosphorylate and activate TRP-hydroxylase, the rate-limiting enzyme in 5-HT synthesis (53–55). Indeed, α CaMKII mutant mice exhibited reduced 5-HT release from serotonergic neurons in the dorsal raphe (39). Thus, it is possible that chronic reductions in TRP intake reduce 5-HT synthesis in the dorsal raphe nucleus, which in turn mediates the behavioral changes that are seen in these animals.

Limiting TRP intake in mice also increased their social dominance behavior (Fig. 3). Since defensive aggression has been thought to reflect not only aggression but also fear (56), the changes in social behavior that we observed in our animals suggest that the limitation of TRP intake adversely affected their emotional status.

Our TRP-limited mice showed increased locomotor activity in the open field test (Fig. 4), a finding that is consistent with previous reports (32). Furthermore, they also exhibited increased mobility in the forced swim test (Fig. 6). On the other hand, they displayed normal anxiety levels in the open field (Fig. 4) and elevated zero maze tests (Fig. 5), and normal motor performance in the rotarod test (Fig. 7). These observations suggest that the hyperactivity that we observed in TRP-limited mice in the open field and forced swim tests was a reflection of an altered state of emotion other than anxiety. As described above, TRP-limited mice also displayed an increase in defensive aggression in the resident-intruder test (Fig. 2). Since defensive aggression is triggered as a result of an attack by a resident mouse, this form of aggression is thought to represent the response of the animal to a dangerous, inescapable situation. Taken together, these data suggest that consump-

tion of a diet with markedly reduced TRP may lead to alterations in the ability to respond to the stress induced by exposure to an inescapable, dangerous situation.

Mutant mice that lack the 5-HT_{5A} receptor reportedly exhibited increased locomotor activity without any change in their anxiety-related behavior (40). Furthermore, mice that lack the 5-HT_{1A} receptor were shown to exhibit increased mobility in the forced swim test (57). These observations raise the possibility that the increase in locomotor activity seen in TRP-limited mice was due to reduced signal transduction through 5-HT_{5A} and 5-HT_{1A} receptors. However, 5-HT_{1A} receptor knockout mice displayed increased anxiety in the open field and elevated plus maze tests (11, 12). Furthermore, surprisingly, mutant mice that lack the 5-HT_{1B} receptor exhibited reduced anxiety in these tests (11), suggesting that the phenotypes that regulate emotional behavior in 5-HT_{1B} receptor mutant mice are opposite with those in 5-HT_{1A} receptor mutant mice. Since previous reports have clearly indicated that the different 5-HT receptors have unique distribution patterns in the brain (58, 59), more work will need to be done before we are able to sort out the serotonergic mechanisms that are involved in the regulation of emotional status.

In summary, chronic dietary TRP restriction lead to the expression of abnormal emotional behavior, i.e., increased defensive aggression and an alteration in the stress response to a dangerous, inescapable situation. These results suggest that moderate intake of TRP is necessary in order to maintain normal emotional status in mammals.

Acknowledgments

This work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology and a Grant-in Aid for High Technology Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

REFERENCES

- 1) Edwards DH, Kravitz EA. 1997. Serotonin, social status and aggression. *Curr Opin Neurobiol* **7**: 812–819.
- 2) Julius D. 1998. Serotonin receptor knockouts: a moody subject. *Proc Natl Acad Sci USA* **95**: 15153–15154.
- 3) Kahn RS, van Praag HM, Wetzler S, Asnis GM, Barr G. 1988. Serotonin and anxiety revisited. *Biol Psychiatry* **23**: 189–208.
- 4) Lesch KP, Zeng Y, Reif A, Gutknecht L. 2003. Anxiety-related traits in mice with modified genes of the serotonergic pathway. *Eur J Pharmacol* **480**: 185–204.
- 5) Murphy DL, Wichems C, Li Q, Heils A. 1999. Molecular manipulations as tools for enhancing our understanding of 5-HT neurotransmission. *Trends Pharmacol Sci* **20**: 246–252.
- 6) Hole K, Johnson GE, Berge OG. 1977. 5,7-Dihydroxytryptamine lesions of the ascending 5-hydroxytryptamine pathways: habituation, motor activity and agonistic behavior. *Pharmacol Biochem Behav* **7**: 205–210.
- 7) Kostowski W, Valzelli L. 1974. Biochemical and behavioral effects of lesions of raphe nuclei in aggressive mice.

- Pharmacol Biochem Behav* **2**: 277–280.
- 8) Valzelli L, Bernasconi S, Bohlken S. 1976. Effect of mid-brain raphe lesion on avoidance learning in aggressive mice. *Biol Psychiatry* **11**: 575–582.
 - 9) Briley M, Chopin P, Moret C. 1990. Effect of serotonergic lesion on “anxious” behaviour measured in the elevated plus-maze test in the rat. *Psychopharmacology (Berl)* **101**: 187–189.
 - 10) Treit D, Robinson A, Rotzinger S, Pesold C. 1993. Anxiolytic effects of serotonergic interventions in the shock-probe burying test and the elevated plus-maze test. *Behav Brain Res* **54**: 23–34.
 - 11) Zhuang X, Gross C, Santarelli L, Compan V, Trillat AC, Hen R. 1999. Altered emotional states in knockout mice lacking 5-HT1A or 5-HT1B receptors. *Neuropsychopharmacology* **21**: 52S–60S.
 - 12) Gross C, Zhuang X, Stark K, Ramboz S, Oosting R, Kirby L, Santarelli L, Beck S, Hen R. 2002. Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature* **416**: 396–400.
 - 13) Hendricks TJ, Fyodorov DV, Wegman LJ, Lelutiu NB, Pehek EA, Yamamoto B, Silver J, Weeber EJ, Sweatt JD, Deneris ES. 2003. Pet-1 ETS gene plays a critical role in 5-HT neuron development and is required for normal anxiety-like and aggressive behavior. *Neuron* **37**: 233–247.
 - 14) Hariri AR, Weinberger DR. 2003. Functional neuroimaging of genetic variation in serotonergic neurotransmission. *Genes Brain Behav* **2**: 341–349.
 - 15) Cooper JR, Melcer I. 1961. The enzymic oxidation of tryptophan to 5-hydroxytryptophan in the biosynthesis of serotonin. *J Pharmacol Exp Ther* **132**: 265–268.
 - 16) Sandyk R. 1992. L-Tryptophan in neuropsychiatric disorders: a review. *Int J Neurosci* **67**: 127–144.
 - 17) Fernstrom JD, Wurtman RJ. 1971. Brain serotonin content: physiological dependence on plasma tryptophan levels. *Science* **173**: 149–152.
 - 18) Fernstrom JD, Wurtman RJ. 1972. Brain serotonin content: physiological regulation by plasma neutral amino acids. *Science* **178**: 414–416.
 - 19) Biggio G, Fadda F, Fanni P, Tagliamonte A, Gessa GL. 1974. Rapid depletion of serum tryptophan, brain tryptophan, serotonin and 5-hydroxyindoleacetic acid by a tryptophan-free diet. *Life Sci* **14**: 1321–1329.
 - 20) Gessa GL, Biggio G, Fadda F, Corsini GU, Tagliamonte A. 1974. Effect of the oral administration of tryptophan-free amino acid mixtures on serum tryptophan, brain tryptophan and serotonin metabolism. *J Neurochem* **22**: 869–870.
 - 21) Stancampiano R, Melis F, Sarais L, Cocco S, Cugusi C, Fadda F. 1997. Acute administration of a tryptophan-free amino acid mixture decreases 5-HT release in rat hippocampus in vivo. *Am J Physiol* **272**: R991–994.
 - 22) Young SN. 1986. The effect on aggression and mood of altering tryptophan levels. *Nutr Rev* **44** (Suppl): 112–122.
 - 23) Young SN, Leyton M. 2002. The role of serotonin in human mood and social interaction. Insight from altered tryptophan levels. *Pharmacol Biochem Behav* **71**: 857–865.
 - 24) Cleare AJ, Bond AJ. 1995. The effect of tryptophan depletion and enhancement on subjective and behavioural aggression in normal male subjects. *Psychopharmacology (Berl)* **118**: 72–81.
 - 25) Olivier B, Mos J. 1992. Rodent models of aggressive behavior and serotonergic drugs. *Prog Neuropsychopharmacol Biol Psychiatry* **16**: 847–870.
 - 26) Gibbons JL, Barr GA, Bridger WH, Leibowitz SF. 1979. Manipulations of dietary tryptophan: effects on mouse killing and brain serotonin in the rat. *Brain Res* **169**: 139–153.
 - 27) Gibbons JL, Barr GA, Bridger WH, Leibowitz SF. 1981. L-Tryptophan's effects on mouse killing, feeding, drinking, locomotion, and brain serotonin. *Pharmacol Biochem Behav* **15**: 201–206.
 - 28) Giammanco S, Ernandes M, Lopez de Onate R, Paderni MA. 1990. Short term diet of precooked corn meal almost lacking in tryptophan and interspecific rat-mouse aggressive behaviour. *Arch Int Physiol Biochim* **98**: 23–26.
 - 29) Giammanco S, Paderni MA, Hernandez M, Geraci V, La Guardia M. 1982. The mouse-killing behaviour of the rat, kept on a long-lasting diet devoid of tryptophan. *Boll Soc Ital Biol Sper* **58**: 1271–1274.
 - 30) Kantak KM, Hegstrand LR, Whitman J, Eichelman B. 1980. Effects of dietary supplements and a tryptophan-free diet on aggressive behavior in rats. *Pharmacol Biochem Behav* **12**: 173–179.
 - 31) Kantak KM, Hegstrand LR, Eichelman B. 1981. Dietary tryptophan reversal of septal lesion and 5,7-DHT lesion elicited shock-induced fighting. *Pharmacol Biochem Behav* **15**: 343–350.
 - 32) Vergnes M, Kempf E. 1981. Tryptophan deprivation: effects on mouse-killing and reactivity in the rat. *Pharmacol Biochem Behav* **14** (Suppl 1): 19–23.
 - 33) Benkelfat C, Ellenbogen MA, Dean P, Palmour RM, Young SN. 1994. Mood-lowering effect of tryptophan depletion. Enhanced susceptibility in young men at genetic risk for major affective disorders. *Arch Gen Psychiatry* **51**: 687–697.
 - 34) Smith KA, Fairburn CG, Cowen PJ. 1997. Relapse of depression after rapid depletion of tryptophan. *Lancet* **349**: 915–919.
 - 35) Malick JB, Barnett A. 1976. The role of serotonergic pathways in isolation-induced aggression in mice. *Pharmacol Biochem Behav* **5**: 55–61.
 - 36) Bell R, Hobson H. 1993. Effects of pindobind 5-hydroxytryptamine1A (5-HT1A), a novel and potent 5-HT1A antagonist, on social and agonistic behaviour in male albino mice. *Pharmacol Biochem Behav* **46**: 67–72.
 - 37) Ohl F, Sillaber I, Binder E, Keck ME, Holsboer F. 2001. Differential analysis of behavior and diazepam-induced alterations in C57BL/6N and BALB/c mice using the modified hole board test. *J Psychiatr Res* **35**: 147–154.
 - 38) Mayorga AJ, Lucki I. 2001. Limitations on the use of the C57BL/6 mouse in the tail suspension test. *Psychopharmacology (Berl)* **155**: 110–112.
 - 39) Chen C, Rainnie DG, Greene RW, Tonegawa S. 1994. Abnormal fear response and aggressive behavior in mutant mice deficient for alpha-calcium-calmodulin kinase II. *Science* **266**: 291–294.
 - 40) Grailhe R, Waeber C, Dulawa SC, Hornung JP, Zhuang X, Brunner D, Geyer MA, Hen R. 1999. Increased exploratory activity and altered response to LSD in mice lacking the 5-HT(5A) receptor. *Neuron* **22**: 581–591.
 - 41) Shibata K. 1995. Conversion ratio of tryptophan to niacin in rats fed with a nicotinic acid-free, tryptophan-limiting diet. *Biosci Biotechnol Biochem* **59**: 715–716.
 - 42) Shibata K, Kondo T, Marugami M, Umezawa C. 1996.

- Increased conversion ratio of tryptophan to niacin by the administration of clofibrate, a hypolipidemic drug, to rats. *Biosci Biotechnol Biochem* **60**: 1455–1459.
- 43) Hayakawa T, Shibata K, Iwai K. 1991. Why does a difference in growth rate between rats of the Wistar and Sprague Dawley strains occur when a niacin-free and tryptophan-limiting diet is fed? *Adv Exp Med Biol* **294**: 649–651.
 - 44) Shibata K, Shimada H, Kondo T. 1996. Effects of feeding tryptophan-limiting diets on the conversion ratio of tryptophan to niacin in rats. *Biosci Biotechnol Biochem* **60**: 1660–1666.
 - 45) Reeves PG, Nielsen FH, Fahey GC Jr. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* **123**: 1939–1951.
 - 46) Venero JL, Herrera AJ, Machado A, Cano J. 1992. Changes in neurotransmitter levels associated with the deficiency of some essential amino acids in the diet. *Br J Nutr* **68**: 409–420.
 - 47) Lijam N, Paylor R, McDonald MP, Crawley JN, Deng CX, Herrup K, Stevens KE, Maccaferri G, McBain CJ, Sussman DJ, Wynshaw-Boris A. 1997. Social interaction and sensorimotor gating abnormalities in mice lacking Dvl1. *Cell* **90**: 895–905.
 - 48) Lindzey G, Winston H, Manosevitz M. 1961. Social dominance in inbred mouse strains. *Naturwissenschaften* **191**: 474–476.
 - 49) Messeri P, Eleftheriou BE, Oliverio A. 1975. Dominance behavior: a phylogenetic analysis in the mouse. *Physiol Behav* **14**: 53–58.
 - 50) Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R, Schutz G. 1999. Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat Genet* **23**: 99–103.
 - 51) Porsolt RD, Le Pichon M, Jalfre M. 1977. Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266**: 730–732.
 - 52) Cryan JF, Markou A, Lucki I. 2002. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* **23**: 238–245.
 - 53) Ehret M, Cash CD, Hamon M, Maitre M. 1989. Formal demonstration of the phosphorylation of rat brain tryptophan hydroxylase by Ca^{2+} /calmodulin-dependent protein kinase. *J Neurochem* **52**: 1886–1891.
 - 54) Isobe T, Ichimura T, Sunaya T, Okuyama T, Takahashi N, Kuwano R, Takahashi Y. 1991. Distinct forms of the protein kinase-dependent activator of tyrosine and tryptophan hydroxylases. *J Mol Biol* **217**: 125–132.
 - 55) Furukawa Y, Ikuta N, Omata S, Yamauchi T, Isobe T, Ichimura T. 1993. Demonstration of the phosphorylation-dependent interaction of tryptophan hydroxylase with the 14–3–3 protein. *Biochem Biophys Res Commun* **194**: 144–149.
 - 56) Brain PF, Smoothy R, Benton D. 1985. An ethological analysis of the effects of tipludom on social encounters in male albino mice. *Pharmacol Biochem Behav* **23**: 979–985.
 - 57) Ramboz S, Oosting R, Amara DA, Kung HF, Blier P, Mendelsohn M, Mann JJ, Brunner D, Hen R. 1998. Serotonin receptor 1A knockout: an animal model of anxiety-related disorder. *Proc Natl Acad Sci USA* **95**: 14476–14481.
 - 58) Saudou F, Hen R. 1994. 5-Hydroxytryptamine receptor subtypes in vertebrates and invertebrates. *Neurochem Int* **25**: 503–532.
 - 59) Andrade R. 1998. Regulation of membrane excitability in the central nervous system by serotonin receptor subtypes. *Ann NY Acad Sci* **861**: 190–203.