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L-theanine attenuates abstinence signs in morphine-dependent rhesus monkeys and elicits anxiolytic-like activity in mice

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Abstract

L-theanine, 2-Amino-4-(ethylcarbamoyl) butyric acid, an amino acid found in green tea (*Camellia sinensis*), is sold in the United States as a dietary supplement to reduce stress and improve cognition and mood. The observations that L-theanine has been shown to inhibit caffeine's stimulatory effects and that caffeine produces precipitated withdrawal signs in opioid-addicted monkeys and some opioid withdrawal signs in some normal monkeys, suggest that L-theanine may suppress opioid withdrawal signs. Additionally, L-theanine produces anxiolytic effects in humans indicating that it has anti-anxiety properties. Thus, in these studies we determined whether L-theanine attenuates opioid-withdrawal signs in morphine-dependent rhesus monkeys, a model for spontaneous opioid withdrawal in human opioid addicts. We also evaluated whether L-theanine decreases anxiety-like behavior in mice, using the elevated plus maze and marble burying assays. L-theanine significantly attenuated designated opioid withdrawal signs, including fighting, rigid abdominal muscles, vocalizing on palpation of abdomen, pacing, retching, wet-dog shakes, and masturbation. It had a relatively quick onset of action that persisted for at least 2.5 h. L-theanine also produced anxiolytic-like effects in the elevated plus maze and the marble burying assay in naïve mice at doses that did not significantly affect motor behavior. The results of these studies suggest that L-theanine may be useful in the pharmacotherapy of treating opioid withdrawal as well as anxiety-associated behaviors.

Keywords

L-theanine; opioid dependence; withdrawal; anxiety; elevated plus maze; marble burying; midazolam; mice; rhesus monkeys

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1. Introduction

Morphine and other opiates are prescribed for the treatment of various types of severe pain and are also widely abused substances. The long-term use of these compounds results in tolerance and physical dependence. Abstinence from opioids in dependent individuals results in severe withdrawal symptoms that contribute to continued drug use. In humans, opioid withdrawal is characterized by behavioral (e.g., anxiety, anhedonia, and restlessness), gastrointestinal (e.g., diarrhea, and emesis), and other physiological effects (e.g., hypertension, tachycardia, tremors, dehydration, and body aches; American Psychiatric Association, 1994). Opioid withdrawal signs have also been described in rhesus monkeys that include, drowsiness, agitation, rigidity of abdominal muscles, restlessness, tremors, coughing, and wet-dog shakes (Aceto, 1990). Opioid withdrawal symptoms reflect a significant impediment to the treatment of opioid dependence and, consequently, new treatment strategies to reduce opioid withdrawal symptoms that lack abuse potential are needed.

L-theanine, 2-amino-4-(ethylcarbamoyl) butyric acid, an amino acid structurally related to glutamic acid, found in green tea (*Camellia sinensis*) and mushrooms (*Boletus badius*), is sold in the United States as a dietary supplement and is designated as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration. This substance is absorbed after oral administration, readily crosses the blood-brain barrier, and has been shown to inhibit caffeine's EEG stimulatory effect at almost equimolar concentrations in rats (Kakuda et al., 2000). Additionally, L-theanine blocked caffeine-induced convulsions in mice (Kimura and Murata, 1971) and inhibited caffeine-induced spontaneous motor activity (Kimura and Murata, 1980).

Theophylline has long been known to produce a "quasi-morphine abstinence" in naive rats that was intensified by naloxone and suppressed by heroin (Collier et al., 1974). Similarly, caffeine (4.0-32.0 mg/kg, s.c.) produced commonly observed withdrawal signs as those precipitated by naloxone in morphine-dependent rhesus monkeys (Aceto et al., 1978). Given the observations that L-theanine inhibits caffeine's stimulatory effects and that caffeine produces opioid withdrawal signs, the first objective of the present study was to test whether L-theanine would suppress opioid withdrawal signs in *rhesus monkeys*.

L-theanine has been reported to produce anxiolytic effects in humans (Haskell et al., 2008; Lu et al., 2004), including increasing alpha wave activity, which is associated with relaxed alertness (Gomez-Ramirez et al., 2007). Interestingly, caffeine induces panic attacks and anxiety at high doses (Lee et al., 1985; Rowlands, 1987) and also reduced sleep efficiency (Bonnet and Arand, 1992), an effect that was partially blocked by L-theanine (Jang et al., 2012). Additionally, co-administration of L-theanine (10 mg/kg) and midazolam (1.5 mg/kg) reduced anxiogenic-like effects in rats, as assessed in the elevated plus maze (Heese et al., 2009). These findings suggest that L-theanine possess anti-anxiety properties. Thus, in order to evaluate the effect of L-theanine on anxiety, we tested the anxiolytic-like effects of this compound in naive mice in the elevated plus maze and the marble burying assay, another behavioral test that can be used to evaluate anxiety-like behavior in mice (Broekkamp et al., 1986; Kinsey et al., 2011; Njung'e and Handley, 1991). In order to compare the effects of L-theanine to an established anxiolytic, the benzodiazepine midazolam was employed as a positive control in these anxiety models.

2. Materials and Methods

2.1 Subjects

2.1.1 Young adult male and female rhesus monkeys (*Macaca mulatta*) served as subjects—Twenty-four monkeys in the weight range 3.5-7.5 kg comprised the group tested. All monkeys had prior exposure to drugs and to the behavioral procedures in which they were tested. Food and water was freely available. Their diet consisted of Purina Lab Diet Fiber-Plus Monkey Biscuits #5049 (PMI Feeds, Inc., St. Louis, MO) supplemented with fresh fruit twice weekly. A 13 h light/11 h dark cycle was in effect (lights on from 0600-1900 h).

The health of the monkeys was monitored daily by technical and veterinary staff. Monkeys had visual, auditory and olfactory contact with each other throughout the study. Monkeys also had access to puzzle feeders, mirrors and chew toys to provide environmental enrichment. Nature videotapes or music were played at least twice a week in all housing rooms.

2.1.2 Adult male outbred ICR mice were purchased from Harlan Laboratories—(Indianapolis, IN, USA). Subjects were housed 4-6 mice per cage in a temperature-controlled (20-22° C) environment, with a 12-h light/dark cycle and ad libitum access to food and water.

All procedures were carried out in accordance with the “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academy Press, 2011) and were approved by the Institutional Animal Care and Use Committee of Virginia Commonwealth University. The American Association for the Accreditation of Laboratory Animal Care (AAALAC) certified these facilities.

2.2 Drugs

In the monkeys, L-theanine (purchased from Sigma Aldrich, St Louis, MO) and morphine sulfate (purchased from Mallinckrodt Inc., Hazelwood, IL) were dissolved in a vehicle solution of sterile water for injection (Hospira, Inc., Forest Hills, IL). Injections were given through the s.c. route of administration in a volume of 1 ml/kg. In the mouse studies L-theanine (purchased from Sigma Aldrich, St Louis, MO) and midazolam (salt form, midazolam maleate, purchased from Sigma Aldrich, St Louis, MO) were dissolved in saline. Injections were given through the intraperitoneal (i.p.) route of administration in a volume of 10µL/g of body mass.

2.3 Procedures

2.3.1 Single-Dose Substitution Test (SDS) in Morphine-Dependent Monkeys—Subjects were made morphine dependent using modified methods previously described (Deneau, 1956). Morphine was given subcutaneously (s.c., 4 mg/kg) daily at 06:00, 12:00, and 18:00 h for at least three months to produce maximal dependence. The criteria for maximal physical dependence development on morphine included: 1) the development of complete tolerance to morphine’s acute behavioral effects; 2) precipitated withdrawal signs when naltrexone, an opioid antagonist, was administered 30, 60 and 90 days after the initiation of the morphine regimen; and 3) abrupt or spontaneous withdrawal signs before testing began.

A minimum of four monkeys/treatment was used and a minimal two-week wash out period was imposed between testing in monkeys that were tested in more than one treatment group (i.e., injected with L-theanine, morphine, or vehicle during test sessions). Withdrawal was

assessed as previously described (Aceto et al., 1977; Aceto et al., 1986). The test drug or control substance (morphine or vehicle) was administered s.c. in monkeys that had not received morphine for 14–15 h and showed definite signs of withdrawal. Each animal was randomly chosen to receive one of the following treatments: a) L-theanine (vehicle, 1, 4, or 8 mg/kg); b) morphine sulfate control (4.0 mg/kg); and c) vehicle control (1.0 mL/kg). Withdrawal signs were scored, absent or present, once during each of five consecutive 30-min observation periods. Withdrawal signs included: drowsiness (sitting with eyes closed and lethargic or being indifferent to surroundings), fighting, vocalizing, rigidity of abdominal muscles, vocalization during palpation of abdominal muscles, restlessness (pacing), tremors, coughing, wet-dog shakes, and masturbation. The observer was “blind” regarding the assignment of treatments.

2.3.2 Elevated Plus Maze Test in Mice—Mice were acclimated to the test room for 30 min and then were injected (i.p.) with L-theanine (vehicle, 8, 16 or 24 mg/kg) 60 min before testing in the elevated plus maze. The benzodiazepine midazolam (vehicle, 1 or 2 mg/kg) was administered 30 min before testing in the elevated plus maze. The elevated plus maze (Hamilton–Kinder, Poway, CA, USA) consisted of a plus-shaped platform elevated 60 cm from the floor. Two opposite arms (35×5 cm) were enclosed by 15-cm high walls, whereas the other two arms had no walls. A central platform (5×5 cm) allowed access to each of the four arms. As previously described (Naidu et al., 2007), naive mice were placed in the central platform facing an open arm at the beginning of each test session. The percentage of time spent in the open arms and number of open arm entries were evaluated for 5 min using an array of photocells connected to a MotorMonitor® system (Hamilton–Kinder, CA, USA). To assess motor behavior, the number of entries into the closed arms, the total distance traveled in the closed arms, and the speed (total distance traveled/ total time) in the closed arms were determined. The plus maze was cleaned with dilute 1% ethyl alcohol after each test and with a dilute ammonia-based cleaning solution at the end of each testing session (i.e. at the end of the day).

2.3.3 Marble Burying Assay in Mice—Mice were acclimated to the test room for 30 min prior to drug treatment. L-theanine (vehicle, 8, 16 or 24 mg/kg; i.p.) was given 60 min before testing and midazolam (vehicle, 0.5, 1 or 2 mg/kg; i.p.) was administered 30 min before testing. The testing apparatus consisted of a polycarbonate mouse cage (internal dimensions: 33 cm long × 21 cm wide × 19 cm high) filled to a depth of 5 cm with pine wood bedding (Harlan Sani-Chip, Indianapolis, IN). Cages were placed in a sound-attenuating chamber lighted by a bank of white LEDs (75 lx) in which white noise and ventilation were supplied by a PC fan. Marble burying behavior was assessed as previously described (Deacon, 2006; Kinsey et al., 2011; Thomas et al., 2009). Prior to each test, 20 clear, glass marbles (10 mm diameter) were arranged in an evenly spaced, grid-like fashion across the surface of the bedding. A single mouse was placed into each cage, which was then covered with a transparent, Plexiglas lid with air holes. At the conclusion of the 20 min test, the subjects were removed from the test chambers and the number of buried marbles (defined as 50% or more of the marble covered by bedding) was summed. To evaluate motor behavior, immobility, defined as a lack of movement for at least 1250 ms, was simultaneously evaluated during testing using ANY-maze software (Stoelting, Kiel, WI).

2.3.4 Data analysis—All data are reported as mean ± SEM. The data from the Single-Dose Substitution Test (SDS) in Morphine-Dependent Monkeys were analyzed using the Kruskal-Wallis Analysis of Variance (ANOVA) test. The L-theanine data were then analyzed using nonparametric post hoc tests (Siegel and Castellan, 1988) that compared each L-theanine treatment condition to vehicle at the respective time point. The Mann Whitney test was used to analyze the positive control morphine versus saline treatment at

each time point. Data from the elevated plus maze and marble burying assays in mice were analyzed using one-way between-subjects ANOVA. Dunnett's test was used for post hoc comparison in which each dose was compared to vehicle. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1 SDS Monkey Assay: L-theanine reduces opioid-withdrawal signs in morphine-dependent rhesus monkeys

The results for the effects of L-theanine on opioid-withdrawal signs in morphine-dependent rhesus monkeys are found in Fig 1. Kruskal-Wallis one-way analyses of variance were significant at 30 min ($H = 8.4$, $df = 4$, $p < 0.05$), 60 min ($H = 10.4$, $df = 4$, $p < 0.05$), 90 min ($H = 9.6$, $df = 4$, $p < 0.05$), 120 min ($H = 9.8$, $df = 4$, $p < 0.05$), and 150 min ($H = 11.1$, $df = 4$, $p < 0.05$). Nonparametric post hoc tests (Siegel and Castellan, 1988) revealed that 8 mg/kg L-theanine ($p < 0.05$) significantly decreased withdrawal scores as compared to vehicle-treated monkeys at the 30 and 60 min time points. At the 90, 120, and 150 min time points both 4 ($p < 0.05$) and 8 ($p < 0.05$) mg/kg L-theanine decreased withdrawal scores as compared to vehicle-treated monkeys. Mann-Whitney post hoc tests revealed significant differences between vehicle and morphine treatment at each time point ($p < 0.01$; all time points).

3.2 L-theanine elicits anxiolytic-like effects in mice

L-theanine significantly increased the percentage of time spent in the open arms [Fig. 2a; $F(3,44) = 6.6$, $p < 0.001$] and the number of open arm entries [Fig. 2b; $F(3,44) = 5.6$, $p < 0.001$]. L-theanine significantly increased open arm time at 16 ($p < 0.01$) and 24 ($p < 0.05$) mg/kg as compared to vehicle-treated mice. The number of entries into the open arms was also increased at 16 ($p < 0.05$) and 24 ($p < 0.01$) mg/kg of L-theanine. Although L-theanine significantly increased the number of closed arm entries [Fig. 2c; $F(3,44) = 2.9$, $p < 0.05$], no dose of L-theanine significantly differed from vehicle. While L-theanine did not affect distance traveled in the closed arms [Fig 2d; $p = 0.15$], a small but significant effect was found in L-theanine-treated mice for speed (distance traveled/time spent in closed arm) in the closed arms [Fig. 2e; $F(3,44) = 2.9$, $p < 0.05$]. Mice treated with 24 mg/kg ($p < 0.05$) L-theanine had faster speeds in the closed arms as compared to vehicle-treated mice.

The positive control midazolam significantly increased percentage of time spent in the open arms [Fig. 3a; $F(2,24) = 6.1$, $p < 0.01$]. Mice treated with 2 mg/kg midazolam ($p < 0.01$) spent more time in the open arms than the vehicle-treated mice. Midazolam had no effect on open (Fig. 3b; $p = 0.47$) or closed (Fig. 3c; $p = 0.39$) arm entries. The distance mice traveled in the closed arms was significantly decreased by midazolam treatment [Fig. 3d; $F(2,24) = 5.8$, $p < 0.01$]. The distance traveled in the closed arms was less in mice treated with 2 mg/kg midazolam than vehicle-treated mice ($p < 0.01$). Midazolam had no effect on speed in the closed arms (Fig. 3e; $p = 0.23$).

In the marble burying assay, L-theanine significantly decreased the number of buried marbles [Fig. 4a; $F(3,36) = 8.3$, $p < 0.001$]. Mice treated with L-theanine buried fewer marbles than vehicle-treated mice at 16 ($p < 0.01$) and 24 ($p < 0.01$) mg/kg. L-theanine had no effect on the time spent immobile [Fig. 4b; $F(3,36) = 2.0$, $p = 0.13$]. Midazolam significantly also decreased the number of buried marbles [Fig. 5a; $F(3,47) = 20.2$, $p < 0.0001$]; however, it significantly increased immobility time [Fig. 5b; $F(3,47) = 15.6$, $p < 0.0001$]. Midazolam significantly decreased the number of marbles buried and time spent immobile at 1 ($p < 0.01$) and 2 ($p < 0.01$) mg/kg compared to vehicle-treated mice.

4. Discussion

A long-term goal has been to develop a safe and efficacious nonopioid compound to alleviate the opioid withdrawal syndrome in humans, a major impediment in the treatment of opioid dependence. The morphine-dependent rhesus monkey model was used because of its high face validity with respect to the behavioral expressions of opioid effects in humans and its predictive value regarding opioid pharmacotherapy (Aceto, 1990). We have found that L-theanine reduces opioid withdrawal signs in morphine-dependent rhesus monkeys. Moreover, the drug acted relatively quickly and had dose-related effects. These findings indicate that L-theanine may be a potential candidate to alleviate withdrawal in opioid-dependent patients. Historically L-theanine has been recognized as producing relaxing effects and in humans L-theanine produces anxiolytic effects (Haskell et al., 2008; Lu et al., 2004); thus, after finding that L-theanine reduces opioid withdrawal signs rhesus monkeys we evaluated whether L-theanine has anti-anxiety properties in mice.

In the present study, we found that L-theanine reduces anxiolytic-like behavior in mouse elevated plus maze and the marble burying assays. L-theanine produced a small, but significant increase in the time spent in the open arms and decreased marble burying in these respective assays. Doses of L-theanine that produced anxiolytic-like effects did not elicit motor disruptive effects in either assay. Although the highest dose of L-theanine tested (24 mg/kg) increased the speed in the closed arm, these results indicate that the anxiolytic-like properties of L-theanine occur at doses that do not cause motor suppression. In contrast, the anxiolytic-like effects of midazolam in both assays occurred at doses that also reduced motor behavior.

L-theanine has been used for centuries for its purported propensity to reduce stress and improve cognition and mood without causing drowsiness. In contrast to the results reported here, a previous reported found that L-theanine (4, 20 or 100 mg/kg) had no effect on elevated plus maze behavior in C57BL/J mice when administered acutely or three times daily every other day for three weeks (Wakabayashi et al., 2012). Similarly, 10 mg/kg L-theanine (Heese et al., 2009) had no effect on elevated plus maze behavior in rats, but augmented the anxiolytic-like effects of midazolam (1.5 mg/kg), as well as decreased fine and basic motor movements in this assay (Heese et al., 2009). Other studies suggest that L-theanine produces antidepressant-like behavior in mice (Wakabayashi et al., 2012; Yin et al., 2011) and that when administered to newborn rats L-theanine improves performance in the object recognition memory test (Takeda et al., 2011).

The results of midazolam in the present study are similar to our previous report in which 2 mg/kg midazolam increased time spent in the open arms and decreased motor behavior, though 1 mg/kg midazolam increased time spent in the open arms without affecting motor behavior (Naidu et al., 2007). Another study found that midazolam increased the percentage of time and entries into the open arms without affecting entries into the closed arms (Nunes-de-Souza et al., 2000). It should be noted that the anxiolytic-like effects of midazolam in rats have been mixed. Midazolam was found to have anxiolytic-like effects in naïve rats evaluated in the elevated plus maze (Albrechet-Souza et al., 2007; Cruz-Morales et al., 2002; Leiter et al., 2011); however, other studies found that it lacked significant anxiolytic-like properties in rats (Heese et al., 2009) or that it increased open arm time at doses that also decreased basic and fine motor movements (Leiter et al., 2011). These findings indicate that the anxiolytic-like properties of L-theanine as well as midazolam in the elevated plus maze are species (i.e., rats versus mice) and strain (i.e., C57BL/J versus ICR mice) dependent.

The present study is the first of which we are aware that has evaluated L-theanine or midazolam in the marble burying test. Thus, the finding that L-theanine reduced marble burying behavior without affecting overt motor suppression represents a novel finding consistent with the notion that it may possess anxiolytic-like activity. In contrast to the effects of midazolam in the marble burying assay, where we found it decreased the number of marbles buried but also increased time immobile, the benzodiazepine diazepam has been found to decrease marble burying at doses that did not affect locomotor activity (Broekkamp et al., 1986; Kinsey et al., 2011; Njung'e and Handley, 1991). An alternative interpretation of marble burying behavior is that this response reflects repetitive and perseverative digging behavior rather than novelty-induced anxiety (Thomas et al., 2009). This assay has also been described as a model of neophobia (Ho et al., 2002) and obsessive-compulsive behavior (Hedlund and Sutcliffe, 2007). Nevertheless, in the present study L-theanine was active in two commonly used preclinical animal tests of emotionality that are thought to model anxiety.

In clinical studies, L-theanine increased alpha wave activity, which is associated with decreases in stress and anxiety (Gomez-Ramirez et al., 2009; Juneja et al., 1999). L-theanine had anxiolytic effects in comparison with placebo during a relaxed state, but lacked these effects during an experimentally induced anxiety state (Lu et al., 2004) suggesting that it can decrease baseline anxiety, but does not affect anticipatory anxiety. However, in an acute stress task L-Theanine reduced heart rate and salivary immunoglobulin A responses as compared to a placebo (Kimura et al., 2007). In another clinical study L-theanine increased calmness but decreased alertness (Haskell et al., 2008). Co-administered with antipsychotic treatments, L-theanine reduced anxiety symptoms in schizophrenia and schizoaffective disorder patients (Ritsner et al., 2011), an effect that was significantly associated with increases in serum levels of BDNF (Miodownik et al., 2011).

Although the mechanism of action for the pharmacological effects of L-theanine remain to be established, this compound increases CNS levels of GABA, dopamine, and serotonin, as well as inhibits glutamate receptors (Kimura and Murata, 1971; Yokogoshi et al., 1998). Moreover, studies indicate that L-theanine increases BDNF levels (Miodownik et al., 2011; Takeda et al., 2011; Wakabayashi et al., 2012) and L-theanine has also been shown to have antagonistic effects on N-methyl D-aspartate (NMDA) receptors (Kakuda et al., 2002). Thus, the observations that BDNF levels can be mediated by glutamate actions at NMDA receptors (Castren et al., 1993; Mattson, 2008; Wakabayashi et al., 2012; Zafra et al., 1991) suggest that the antagonistic properties of L-theanine on NMDA receptors may contribute to L-theanine's behavioral effects. Glutamate is released during opioid withdrawal (i.e., Noda and Nabeshima, 2004) and, thus, L-theanine's antagonistic effects on NMDA receptors (Kakuda et al., 2002) may account for its ability to reduce opioid withdrawal signs in morphine-dependent rhesus monkeys. Additionally, given the anxiolytic properties of serotonin and GABA (Gonzalez et al., 1998; Graeff, 2002; Graeff and Zangrossi, 2010; Nikolaus et al., 2010; Rupprecht et al., 2006), L-theanine may produce its anxiolytic-like actions via its effects on these neurotransmitters.

5. Conclusions

We have found that L-theanine reduces opioid-withdrawal signs in morphine-dependent rhesus monkeys. These findings suggest that this relatively safe nonopioid compound may be useful in the alleviation of the opioid withdrawal syndrome in humans, an established impediment in the treatment of opioid abuse. We also found that L-theanine produces significant anxiolytic-like effects in mice, as assessed in the elevated plus maze and the marble burying assays at doses that do not produce changes in motor behavior indicating

that it may reduce anxiety-associated behaviors. These results suggest that L-theanine may represent a viable treatment for opioid abuse and anxiety-related disorders.

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Abbreviations

(L-theanine)	2-Amino-4-(ethylcarbamoyl) butyric acid
(SDS)	Single-Dose Substitution
(s.c.)	subcutaneously
(i.p.)	intraperitoneal
(BDNF)	brain-derived neurotrophic factor
(NMDA)	N-methyl d-aspartate
(GABA)	gamma amino butyric acid

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Highlights

- L-theanine attenuated opioid-withdrawal signs in morphine-dependent rhesus monkeys
- L-theanine had anxiolytic-like effects in the elevated plus maze in naïve mice
- L-theanine had anxiolytic-like effects in the marble burying assay in naïve mice

Figure. 1.

Cumulative number of spontaneous withdrawal signs observed in morphine-dependent monkeys at 30, 60, 90, 120, and 150 min after receiving a subcutaneous injection of vehicle, L-theanine (1.0, 4.0, or 8.0 mg/kg), or morphine sulfate (4.0 mg/kg). * $p < 0.05$, ** $p < 0.01$, indicate significant differences from vehicle-treatment at each specific time point; Data are shown as the mean $\pm$ SEM, $N = 4$ monkeys/group for each dose of L-theanine; $N = 7$ monkeys for morphine treatment; $N = 6$ monkeys for vehicle treatment.

Figure. 2.

L-theanine produces an increase in the percentage of time spent in the open arms (a) and number of open arm entries (b) in the elevated plus maze. L-theanine did not significantly affect the number of closed arm entries compared with vehicle (c) and had no effect on the distance traveled in the closed arm (d). The highest dose of L-theanine significantly increased running speed in the closed arm (e). Mice were treated with L-theanine 60 min before testing in the elevated plus maze. * $p < 0.05$, ** $p < 0.01$, indicate significant differences from vehicle-treated mice; Dunnett's post hoc test. All data are reported as mean $\pm$ SEM. N = 15 mice/treatment group for vehicle; N = 8 mice/treatment group for 8 mg/kg L-theanine; N = 17 mice/treatment group for 16 mg/kg L-theanine; N = 7 mice/treatment group for 24 mg/kg L-theanine.

Figure.3.

Comparison of the effects L-theanine to those of midazolam (positive control) in the elevated plus maze. Midazolam (2 mg/kg) significantly increased the time spent in the open arms (a) as well as significantly decreased the distance traveled in the closed arm (d) in the elevated plus maze. Midazolam (1 and 2 mg/kg) had no effect on open (b) or closed (d) arm entries as well as the speed (c; distance traveled/time spent) in the closed arm. Mice were treated with midazolam 30 min before testing. ** $p < 0.01$, indicates significant differences from vehicle-treated mice; Dunnett's post hoc test. All data are reported as mean $\pm$ SEM. $N = 13$ mice/treatment group for vehicle; $N = 6$ mice/treatment group for 1.0 mg/kg midazolam; $N = 9$ mice/treatment group for 2.0 mg/kg midazolam.

Figure. 4.

L-theanine decreased marble burying (a), but had no effect on immobility time (b). Mice were treated with L-theanine 60 min before testing. ** $p < 0.01$, indicates significant differences from vehicle-treated mice; Dunnett's post hoc test. All data are reported as mean $\pm$ SEM. $N = 12$ mice/treatment group for vehicle; $N = 8$ mice/treatment group for 8 mg/kg L-theanine; $N = 12$ mice/treatment group for 16 mg/kg L-theanine; $N = 8$ mice/treatment group for 24 mg/kg L-theanine.

Figure. 5.

Comparison of the effects L-theanine to those of midazolam (positive control) in the marble burying assay. Midazolam significantly decreased the number of buried marbles (a) at doses (1 and 2 mg/kg) that also significantly increased time immobile (b) in the marble-burying test. Mice were treated with midazolam 30 min before testing. ** $p < 0.01$, indicates significant differences from vehicle-treated mice; Dunnett's post hoc test. All data are reported as mean $\pm$ SEM. $N = 18$ mice/treatment group for vehicle; $N = 10$ mice/treatment group for 0.5 mg/kg midazolam; $N = 15$ mice/treatment group for 1.0 mg/kg midazolam; $N = 8$ mice/treatment group for 2.0 mg/kg midazolam.