

# Investigation of the Anxiolytic Effects of Linalool, a Lavender Extract, in the Male Sprague-Dawley Rat

**MAJ Michael Cline, CRNA, MSN, ANC, USA**

**MAJ John E. Taylor, CRNA, MSN, ANC, USA**

**MAJ Jesus Flores, CRNA, MSN, ANC, USA**

**CPT Samuel Bracken, CRNA, MSN, ANC, USA**

**Suzanne McCall**

**LTC Thomas E. Ceremuga, CRNA, PhD, ANC, USA**

*The purpose of our study was to investigate the anxiolytic effects of linalool and its potential interaction with the GABA<sub>A</sub> receptor in Sprague-Dawley rats. Lavender has been used traditionally as an herbal remedy in the treatment of many medical conditions, including anxiety. Linalool is a major component of the essential oil of lavender. Forty-four rats were divided into 4 groups: control, linalool, midazolam (positive control), and flumazenil and linalool. The behavioral and the neurohormonal/physiological components of anxiety were evaluated. The behavioral component was examined by*

*using the elevated plus maze (open arm time/total time) and the neurohormonal/physiological component by measuring serum catecholamine and corticosterone levels. Data analysis was performed using a 2-tailed Multivariate Analysis of Variance and Sheffe post-hoc test. Our data suggest that linalool does not produce anxiolysis by modulation of the GABA<sub>A</sub> receptor; however, linalool may modulate motor movements and locomotion.*

**Key Words:** Anxiolysis, elevated plus maze, lavender, linalool, rat.

**A**nxiety is an uneasy feeling of dread or discomfort, the source of which may or may not be known to the individual experiencing it. It is often accompanied by an autonomic nervous system response.<sup>1</sup> Anxiety also includes the apprehension and anticipation that enable an individual to prepare for imminent danger.<sup>1</sup> An estimated 19 million adults in the United States have some form of anxiety disorder, at an estimated cost of more than \$42,000 million a year.<sup>2</sup> The 1-year prevalence rate for all anxiety disorders exceeds 16% of adults ages 18 to 54, and there is significant overlap with substance abuse and mood disorders.<sup>3</sup> These disorders are not limited to the United States, but are present across other cultures.<sup>3</sup>

Anxiety can activate the stress response<sup>4</sup> with endocrine and neurotransmitter release. Corticosteroids are elevated to modulate the biological response to stress. The sympathetic nervous system, is also activated by stress-producing anxiety releasing catecholamines (ie, epinephrine and norepinephrine) from the adrenal medulla.<sup>4</sup> This fight-or-flight response includes elevated heart and respiratory rates, glycolysis, and the release of various neurohormonal mediators. As the exposure to these mediators and neurotransmitters continues, fatigue and a weakened immune system may result.

The anticipation of surgery creates anxiety in most patients. Anxiety increases risk of adverse outcomes and

can result in a more complicated anesthetic plan. The activated stress response results in increased neuroendocrine mediators (elevated catecholamines and cortisol levels). These increased levels may cause harm to the patient whose status is already tenuous, perhaps resulting in an adverse outcome or poor wound healing.<sup>5,6</sup> When patients have feelings of well-being and less anxiety, their satisfaction increases and their recovery times may decrease.<sup>7</sup> Thus, the administration of anxiolytic drugs preoperatively to avert or blunt a patient's stress response is an important part of the anesthesia care plan.

Benzodiazepines are anxiolytic drugs frequently administered by the anesthetist and have been reported to reduce anxiety, pain, and cardiovascular activation in the treatment of chest pain.<sup>8</sup> An early study found that patients with decreased preoperative anxiety required less pain medication and were discharged an average of 2.7 days sooner.<sup>9</sup> Further research demonstrated that premedication with benzodiazepines improved patients' comfort during needle localization procedures and significantly reduced intraoperative anxiety levels before breast biopsy procedures without prolonging discharge times.<sup>10</sup>

Many Americans with anxiety disorders attempt to treat themselves with herbal medications. These remedies may have similar mechanisms of action as drugs manufactured for the treatment of anxiety and other disorders. A majority of drugs manufactured for anxiolysis have

adverse effects such as hypotension, sedation, and a high potential for addiction and abuse. Herbal medications are not associated with a large incidence of abuse or addiction.<sup>11</sup> Enthusiasm for herbal medications as alternative treatments has increased rapidly in America. In 1997, 12% of US consumers reported using herbal medications, representing a 380% increase since 1990.<sup>12</sup> According to the Dietary Supplement Health and Education Act of 1994, there is no requirement for evidence of efficacy, safety, or quality-control standards for supplements, which increases the risk of adverse effects related to herbal preparations.<sup>13</sup> In the United States alone, between 1993 and 1998, the US Food and Drug Administration documented approximately 2,600 adverse events, including 100 deaths, related to herbal medications.<sup>12</sup> Currently, there is no central recording of adverse effects suspected to be associated with herbal remedy interactions; therefore, the true number of adverse effects may be much higher than reported. This lack of data demonstrates the need for scientific research concerning herbal medications and their possible adverse effects and interactions with traditional medicines.

Many surgical patients believe that herbal remedies are benign and often fail to report use to anesthesia providers. Although herbal medications are used widely, limited information is available regarding their effects during the perioperative period.<sup>12</sup> The steep rise in herbal medication use may be associated with an increase in morbidity and mortality in the perioperative period as a consequence of interactions with prescribed medications (polypharmacy) or herbal-induced alterations in physiology.<sup>14</sup> Cardiovascular problems (myocardial infarction and stroke), altered hemostasis, prolonged or insufficient anesthesia, organ transplantation rejection, and drug interactions have been reported as complications of herbal supplements.<sup>14</sup> Many herbal medications interact with prescription medications, exhibiting adverse effects such as arrhythmias, bleeding, and prolonged sedation.<sup>12</sup> Up to 70% of herbal medication users do not reveal their usage to healthcare providers, exacerbating the problem.<sup>15</sup> While herbal preparations have numerous purported benefits, very little scientific research has been conducted concerning their pharmacologic properties.

Lavender has been one of the most widely used herbs in history. The Romans used its fragrance for bathing and cooking. The herb was strewn over the stone floors of castles as a disinfectant and deodorant. Today, lavender is commonly found in perfumes, deodorizers, soaps, bath and talc powders, and candles, and as a flavor enhancer in teas, jellies, salads, and vinegars. Other popular uses today include aromatherapy and body massage. Lavender has been used for the medicinal treatment of anxiety, insomnia, itching, inflammation, bacterial infections, headaches, coughs, burns, insect bites, sunburns, colic, and muscle spasms.<sup>16,17</sup>

*Lavandula angustifolia* is one of the most common species of lavender with potential medicinal use. It contains 38 different compounds including linalyl acetate and linalool, which are the major constituents, by volume, of the essential oil of *Lavandula angustifolia*.<sup>17</sup> Linalyl acetate and linalool are considered to have sedative and local anesthetic effects but their site of action is unknown.<sup>17</sup> It has been suggested that linalool modulates nicotinic receptors at the neuromuscular junction.<sup>18</sup> Linalool is also purported to modulate the *N*-methyl-D-aspartate (NMDA) receptor in the cerebral cortex, inhibiting excitatory seizure activity.<sup>19</sup> The protective actions of an aqueous lavender extract during glutamate-induced neurotoxicity in cultures of cerebellar granular cells has been reported.<sup>20</sup> Furthermore, there is evidence that linalool may block calcium channels in the rodent model,<sup>21</sup> but there are no data pertaining to the anxiolytic effects of linalool.

The purpose of this study was twofold. The first objective was to determine if the lavender extract, linalool, has anxiolytic effects in the rat model. The second objective was to investigate possible modulation of the  $\gamma$  aminobutyric acid (GABA<sub>A</sub>) receptor by linalool in the rat central nervous system.

## Materials and Methods

Forty-four male Sprague-Dawley rats (Harlan Sprague Dawley Laboratories, Indianapolis, Indiana), each weighing 200-250 g, were used. They were housed in groups of 3 in polycarbonate shoebox cages lined with bedding. The animals went through a 14-day adaptation period in a temperature-controlled environment (22 ± 1°C, 60% humidity) with a light-dark cycle of 12 hours of light (6 AM to 6 PM) and 12 hours of dark (6 PM to 6 AM). They were allowed free access to food and water. The animals were handled for weighing, drug administration, and cleaning of cages only, and they were naïve to the elevated plus maze. The use of laboratory rats in this protocol was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and received Institutional Animal Care and Use Committee (IACUC) approval.

The rats were divided into 4 treatment groups (11 rats per group). Each animal received an intraperitoneal injection of: (1) vehicle (Tween 1%, solvent for linalool); (2) linalool (Sigma Chemical Co, St. Louis, Missouri) 125 mg/kg, dissolved in Tween 1%;<sup>19</sup> (3) midazolam (Roche, Basel, Switzerland) 1.5 mg/kg; (4) flumazenil 3 mg/kg (Sigma) dissolved in dimethyl sulfoxide (DMSO) and linalool, 125 mg/kg (dissolved in Tween 1%). The group receiving flumazenil (a known benzodiazepine receptor antagonist)<sup>22,23</sup> and linalool was used to evaluate the potential modulation of the benzodiazepine receptor site on the GABA<sub>A</sub> receptor by linalool. All animals received 1 mL total volume of the intraperitoneal injection.

In addition, all experimentation occurred on a timed schedule between 1 PM and 4 PM on 4 consecutive days to ensure that each treatment group was exposed to similar variability of corticosterone release related to the animals' circadian rhythm.

After the 30-minute period following the drug administration, each animal was placed in the center of the elevated plus maze (EPM) located in a darkened room. The EPM is a widely used instrument to measure anxiety in the rodent model<sup>24</sup> and has been validated by Pellow et al<sup>25</sup> based on the previous work by Montgomery.<sup>26</sup> Research on this instrument has supported its use as a standard measurement of anxiety, and specifically, benzodiazepine-induced anxiolysis in rodents.<sup>27</sup>

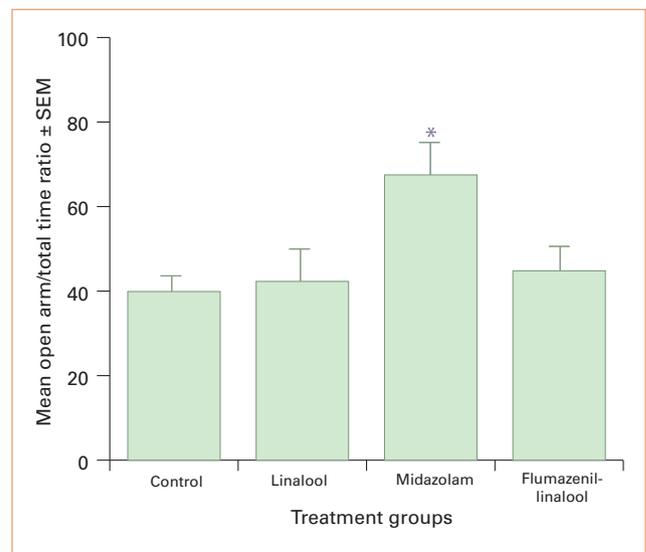
The rats were oriented facing an open arm for a 5-minute evaluation of behavioral response to the anxiety generated by the EPM. Each test was video recorded for verification of data validity. The EPM was networked with MotorMonitor software (Hamilton-Kinder, Poway, California) that tracked the number of entries into each type of arm (open vs closed), time spent in the open arms expressed as a percentage of the total time, and fine and basic motor movements. The EPM was cleaned with soap and water and dried between each animal run to limit variability.

Immediately following the 5-minute test on the EPM, the animals were removed from the testing room and humanely killed by guillotine. Trunk blood was obtained and collected in heparinized tubes. The blood samples were cooled for 5 minutes in ice and then centrifuged for 7 minutes. Following the separation of the plasma from the red blood cells, the plasma was withdrawn and transferred to microtubes and frozen for temporary storage. After all data collection was completed, the frozen plasma was sent on dry ice to Esoterix (Austin, Texas), a reference laboratory, for analysis of corticosterone and catecholamine (epinephrine and norepinephrine) levels.

## Results

Data were collected from 44 subjects with 2 subjects withdrawn from the study because the lights in the testing environment were inadvertently turned on during their time on the elevated plus maze. This unanticipated, extraneous light resulted in observable behavioral changes in the 2 subjects.

Analysis of the ratio of open arm time vs total time spent in the elevated plus maze revealed no statistically significant difference between the vehicle group, linalool, and flumazenil plus linalool group. However, expected significant differences ( $P = .031$ ) in the open arm, a measurement of behavioral changes associated with anxiolysis, were observed between the midazolam and control groups (Table 1 and Figure 1).



**Figure 1.** Ratio of Open Arm time/Total Time (in seconds) on the Elevated Plus Maze

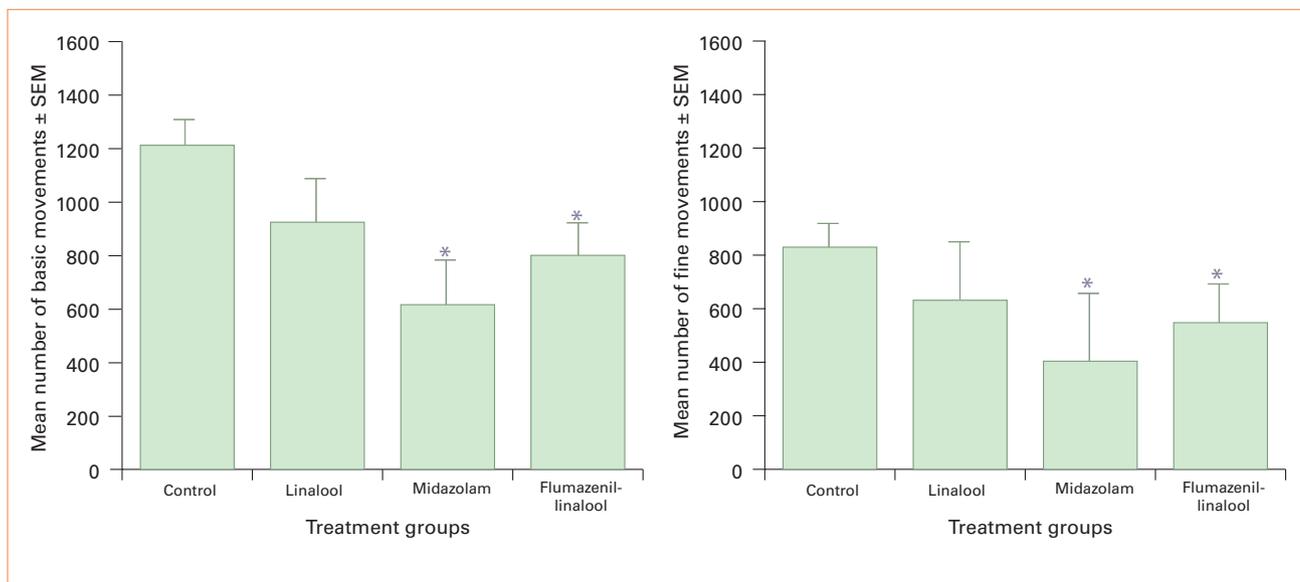
\*Indicates significant statistical difference of  $P < 0.05$ .

	<b>Control (n=11)</b>	<b>Linalool (n=11)</b>	<b>Midazolam (n=9)</b>	<b>Flumazenil + linalool (n=11)</b>
Ratio open/total, mean (SD), sec	39.9 (15.5)	42.3 (22.1)	*67.5 (23.4) $P = .031$	44.8 (19.1)
Basic movements, mean (SD)	1,210.8 (143.7)	923.4 (321.9)	*615.3 (319.0) $P < .000$	*799.6 (230.8) $P = .012$
Fine movements, mean (SD)	829.9 (91.8)	631.5 (217.1)	*403.9 (252.9) $P < .000$	*547.9 (144.1) $P = .008$

**Table.** Ratio of Open Arm Time/Total Maze Time (in seconds) and Number of Motor Movements on Elevated Plus Maze per Group

See Figures 1 and 2 for graphic representation of table data.

\*Indicates significant statistical difference of  $P < 0.05$ .



**Figure 2.** Basic and Fine Motor Movements on the Elevated Plus Maze

\*Indicates significant statistical difference of  $P < 0.05$ .

The total number of basic (gross) and fine motor movements tracked during time in the elevated plus maze were analyzed. Basic motor movements are simply the count of beam breaks in the elevated plus maze. Each time a photo beam is interrupted, the basic movement counter is increased. These movements reveal a gross measure of locomotion, but do not distinguish what type of activity is being performed. Fine motor movements are a compilation of small animal movements such as grooming, head weaves, or bobs. Analysis showed a significant decrease in movement of rats in both the midazolam and flumazenil and linalool groups compared with the control group (Table 1 and Figure 2). Although there is a similar trend of decremented movement, no statistically significant difference in the number of gross or fine motor movements was noted between the linalool and control groups (Table 1 and Figure 2). Analysis of the data showed no significant difference in serum catecholamine or corticosterone levels among all 4 groups.

## Discussion

Lavender and its extracts have a long history of central nervous system action such as sedating and calming effects. This study investigated the purported anxiolytic properties of linalool and its potential interaction with the GABA<sub>A</sub> receptor site.

Previous literature suggested that a linalool dose of 350 mg/kg<sup>19</sup> would be appropriate to examine anxiolysis in our study. In a pilot study, rats given this dose became unconscious and incapable of ambulating on the elevated plus maze. A dose-response pilot study examined 2 lower doses of linalool: 175 mg/kg and 125 mg/kg. Rats remained conscious with the ability to maintain locomotion, which is essential for participation in the elevated plus maze, at the

125 mg/kg dose, but not at the 175 mg/kg dose. Thus, we determined that 125 mg/kg was the optimal dose of linalool for rats to maintain the ability to ambulate in the elevated plus maze under the conditions of this study.

The behavioral measurements comparing the ratio of open arm time to total time spent in the elevated plus maze suggest that linalool may not produce anxiolysis. In addition, there was no difference between the flumazenil (benzodiazepine receptor antagonist) and linalool group and the linalool group, which suggests that linalool does not modulate the benzodiazepine receptor site, and the results do not support the hypothesis that linalool modulates the GABA<sub>A</sub> receptor.

Analysis of the neurohormonal data (catecholamines and corticosterone) did not demonstrate a significant difference among the groups. Possible explanations include an unintentional deviation of the assay protocol after shipment or an exposure time on the EPM that was not long enough to elicit a detectable hormonal response in the rat model.

Motor movements data demonstrated that midazolam and the combination of flumazenil and linalool significantly decreased both basic and fine motor movements. The linalool group motor activity data also trended downward. We are not sure how to interpret this data, but we speculate that it may be the result of the modulation of another neurotransmitter site in the central nervous system. Previous work by Rex<sup>28</sup> indicated that flumazenil may act as a weak partial agonist of the GABA<sub>A</sub> receptor in rats, therefore causing increased inhibition of the central nervous system. Re and colleagues<sup>18</sup> suggested that linalool modulates nicotinic receptors at the neuromuscular junction thus decreasing acetylcholine release and limiting motor activity. Elizabetsky<sup>19</sup> proposed that linalool

interacts with the NMDA receptor in the cerebral cortex, inhibiting excitatory seizure activity. Buyukokuroglu<sup>20</sup> examined the protective actions of a lavender extract during glutamate-induced neurotoxicity in cultures of cerebellar granular cells from 1-day-old Sprague Dawley rats and found that the extract blocked glutamate-induced neurotoxicity. Finally, Gilani<sup>21</sup> speculated that linalool may block calcium channels, which may result in anticonvulsant properties seen in the rodent model.

## Conclusion

Although our data did not support anxiolytic effects of linalool in the rat model, they suggested that linalool modulates the central nervous system by producing unconsciousness and degradation of motor movements. It is important to determine the molecular site of action of linalool to understand the biochemical effects of this herbal extract.

Future investigations might explore the motor effects of linalool using other balance and locomotion instruments such as the rotorod. Additional studies might include those designed to determine effects of linalool at glutamatergic receptors (eg, NMDA) and at the neuromuscular junction. Once the molecular mechanism of action is clearly defined, work may then focus on studying the significant clinical interactions of linalool and other pharmaceuticals. This future research could explore the efficacy of linalool as an anesthesia adjunct or its adverse interactions with anesthesia medications in the perioperative period.

## REFERENCES

- Venes D, Clayton T, Taber C, eds. *Taber's Cyclopedic Medical Dictionary*. 16th ed. Philadelphia, PA: FA. Davis Co; 1989;120.
- ADAA. Statistics and Facts About Anxiety Disorders. <http://www.adaa.org/aboutadaa/PressRoom/Stats&Facts.asp>. Accessed April 10, 2004.
- Surgeon General Report. Etiology of Anxiety Disorders. <http://www.surgeongeneral.gov/library/mentalhealth/toc.html#chapter4>. Accessed February 12, 2005.
- Selye H. Further thoughts on "stress without distress." *Med Times*. November 1976;104(11):124-144.
- Weissman C. The metabolic response to stress: an overview and update. *Anesthesiology*. 1990;73(2):308-327.
- Kain ZN, Sevarino F, Pincus S, et al. Attenuation of the preoperative stress response with midazolam: effects on postoperative outcomes. *Anesthesiology*. 2000;93(1):141-147.
- Kraft M. Holistic issues in the preoperative period. *Int Anesthesiol Clin*. 2002;40(2):201-212.
- Huffman JC, Stern TA. The use of benzodiazepines in the treatment of chest pain: a review of the literature. *J Emerg Med*. 2003;25(4):427-437.
- Egbert LD, Battit GE, Welch CE, Bartlett MK. Reduction of postoperative pain by encouragement and instruction of patients: a study of doctor-patient rapport. *N Engl J Med*. 1964;270:825-827.
- van Vlymen JM, Sá Rêgo MM, White PF. Benzodiazepine premedication: can it improve outcome in patients undergoing breast biopsy procedures? *Anesthesiology*. 1999;90(3):740-747.
- Dhawan K, Dhawan S, Chhabra S. Attenuation of benzodiazepine dependence in mice by a tri-substituted benzoflavone moiety of *Pasiflora incarnata* Linnaeus: a non-habit forming anxiolytic. *J Pharm Pharm Sci*. 2003;6(2):215-222.
- Ang-Lee MK, Moss J, Yuan CS. Herbal medicines and perioperative care. *JAMA*. 2001;286(2):208-216.
- Sabar R, Kaye AD, Frost EA. Perioperative considerations for the patient on herbal medicines. *Middle East J Anesthesiol*. 2001;16(3):287-314.
- Bovill JG. Adverse drug interactions in anesthesia. *J Clin Anesth*. 1997;9(6 suppl):3S-13S.
- Eisenberg DM, Kessler RC, Foster C, Norlock FE. Unconventional medicine in the United States: Prevalence, cost, and patterns of use. *N Engl J Med*. Cited by Lambert, CA. The New Ancient Trend in Medicine. <http://www.harvard-magazine.com/on-line/030221.html>. Accessed February 5, 2006.
- Gören A, Topcu G, Bilsel G, Bilsel M, Aydoğmuş Z, Pezzuto JM. The chemical constituents and biological activity of essential oil of *Lavandula stoechas* ssp. *stoechas*. *Z Naturforsch [C]*. 2002;57(9-10):797-800.
- Cavanagh HM, Wilkinson JM. Biological activities of lavender essential oil. *Phytother Res*. 2002;16(4):301-308.
- Re L, Barocci S, Sonnino S, et al. Linalool modifies the nicotinic receptor-ion channel kinetics at the mouse neuromuscular junction. *Pharmacol Res*. 2000;42(2):177-182.
- Elisabetsky E, Brum LF, Souza DO. Anticonvulsant properties of linalool in glutamate-related seizure models. *Phytomedicine*. 1999;6(2):107-113.
- Buyukokuroglu ME, Gepdiremen A, Hacimuftuoglu A, Oktay M. The effects of aqueous extract of *Lavandula angustifolia* flowers in glutamate-induced neurotoxicity of cerebellar granular cell culture of rat pups. *J Ethnopharmacol*. 2003;84(1):91-94.
- Gilani AH, Aziz N, Khan MA, et al. Ethnopharmacological evaluation of the anticonvulsant, sedative, and antispasmodic activities of *Lavandula stoechas* L. *J Ethnopharmacol*. 2000;71(1-2):161-167.
- Charney DS, Mihic SJ, Harris RA. Hypnotics and sedatives. In: Hardman J, Limbird L. *Goodman & Gilman's The Pharmacologic Basis of Therapeutics*. 10th ed. New York, NY: McGraw-Hill; 2001; 399-427.
- Nutt DJ, Malizia AL. New insights into the role of the GABA(A)-benzodiazepine receptor in psychiatric disorder. *Br J Psychiatry*. 2001;179:390-396.
- Treit D, Menard J, Royan C. Anxiogenic stimuli in the elevated plus maze. *Pharmacol Biochem Behav*. 1993;44(2):463-469.
- Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus maze as a measure of anxiety in the rat. *J Neurosci Methods*. 1985;14(3):149-167.
- Montgomery KC. The relation between fear induced by novel stimulation and exploratory behavior. *J Comp Physiol Psychol*. 1955;48(4):254-260.
- Kulkarni SK, Reddy DS. Animal behavioral models for testing antianxiety agents. *Methods Find Exp Clin Pharmacol*. 1996;18(3):219-230.
- Rex A, Voigt JP, Voits M, Fink H. Pharmacological evaluation of a modified open-field test sensitive to anxiolytic drugs. *Pharmacol Biochem Behav*. 1998;59(3):677-683.

## AUTHORS

MAJ Michael Cline, CRNA, MSN, ANC, USA, is a staff nurse anesthetist at the Womack Army Medical Center, Fort Bragg, North Carolina. He was a student in the US Army Graduate Program in Anesthesia Nursing, William Beaumont Army Medical Center, El Paso, Texas, when this article was written.

MAJ John E. Taylor, CRNA, MSN, ANC, USA, is the chief of Anesthesia Nursing at Reynolds Army Community Hospital in Fort Sill, Oklahoma. He was a student in the US Army Graduate Program in Anesthesia Nursing, Brooke Army Medical Center, San Antonio, Texas, when this article was written.

MAJ Jesus Flores, CRNA, MS, ANC, USA, is a staff nurse anesthetist at Eisenhower Army Medical Center, Fort Gordon, Georgia, and was a student in the US Army Graduate Program in Anesthesia Nursing, Brooke Army Medical Center, San Antonio, Texas, when this article was written.

CPT Samuel Bracken, CRNA, MS, ANC, USA, is a staff nurse anes-

thetist at William Beaumont Army Medical Center, El Paso. When this article was written, he was a student in the US Army Graduate Program in Anesthesia Nursing, also at William Beaumont Army Medical Center.

Suzanne McCall is a research assistant in the Department of Clinical Investigation, Brooke Army Medical Center, Fort Sam Houston, Texas.

LTC Thomas E. Ceremuga CRNA, PhD, ANC, USA, is the program

director in the US Army Graduate Program in Anesthesia Nursing, Fort Sam Houston, Texas.

#### **DISCLAIMER**

The views expressed in this article are those of the authors and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the US Government.