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GABA, Drug Effects, Anxiety, and 22-kHz Ultrasonic Vocalizations in Rats

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$GABA_A$ Drug Effects, Anxiety, and 22-kHz Ultrasonic Vocalizations in Rats

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Introduction

• Over the past twenty years, animal measures of anxiety have proved extremely useful in the development of new anxiolytic (anxiety-relieving) drugs to effectively treat a wide variety of anxiety disorders, including generalized anxiety and panic disorders. [5,9]
• Both agonists and antagonists for the neurotransmitters GABA (Gamma-aminobutyric acid) and Serotonin (5-Hydroxy-tryptamine(5-HT)) have been show to mediate anxiety. [4,5]
• Exploratory behavior in the Open Field Paradigm is used as a quantitative measure of their uneasiness, anxiety, or fear.[1,2]
• 22-kHz ultrasonic vocalizations (USVs) and other ethological measures have been used as indicators of anxiety in rats. [2,10,11]
• Unfortunately, many drugs used to treat anxiety may have sedative effects that decrease locomotion. [5,8,11] This could confound the results of studies that strictly use ethological measures of anxiety. Therefore, better measures must be developed to test the effects of neurotransmitter-modulating drugs.
• 22-kHz USVs are thought to be reflective of the animals' emotional state, since they are under their own cognitive control [1,2,3] and are mediated by several neurotransmitters. [4,5]
• Brudzynski et. al. (2003) have also suggested that the duration of the vocalization may indicate further emotive features of anxiety, as his study found two subpopulations of call duration, one ranging from 20-300 ms and the other from 310 to beyond 2000 ms.
• The aim of the present experiment was to investigate the relationship between 22-kHz vocalizations, established ethological measures of anxiety, and the GABA neurotransmitter system.

Methods

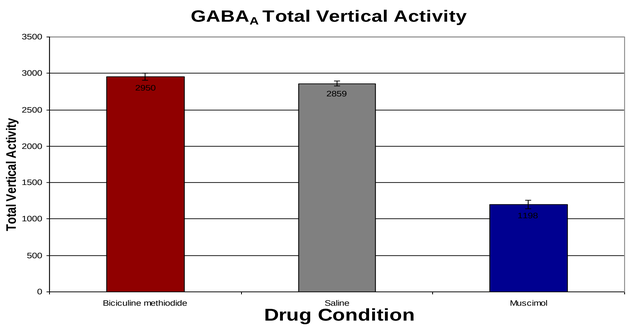
Subjects
60 adult Long-Evans rats (*Rattus norvergicus*) (30 males and 30 females)

$GABA_A$ Agonists and Antagonists

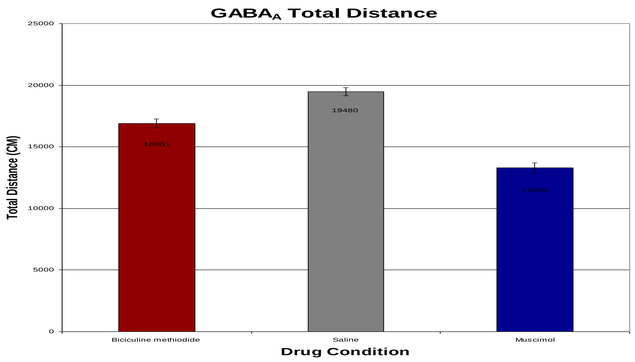
	Anxiogenic	**Anxioloytic**
$GABA_A$	Bicuculine methiodide	Muscimol

Experimental Procedure
• 20 Subjects (10 males and 10 females) were randomly assigned to each condition: anxiolytic, saline, or anxiogenic.
• 30 mins. after IP injection of drug, each rat was placed in a VERSAMAX experimental chamber, simulating the Open Field paradigm. Before each rat was placed in the chamber, 0.1 cc of fox urine (a stressor) was placed on filter paper 18 inches above the chamber.
• 22-kHz USVs were recorded using ultrasound detectors wired directly into video cameras. The detectors were positioned 1 meter above the field. (See Fig. 1.)
• Open field behavior and USVs were recorded over a period of three minutes.
• Each dependent variable was analyzed using a 2x3 (sex X drug condition) factorial ANOVA.
•None of the analyses revealed a significant main effect of sex of rat.

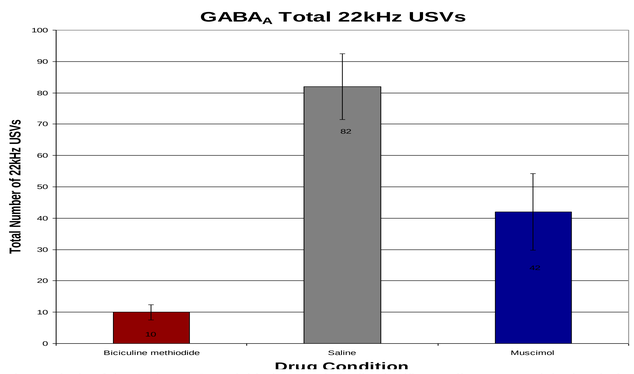
Results



Vertical activity is one indicator of exploratory behavior. It is most likely due to anxiety-related behaviors such as sniffing and scouting, which are used to determine the source of an anxiety causing stimulus (in this case, the predator odor). The analysis of vertical activity yielded a significant main effect of drug condition (F (2,60) = 21.2551, $p < 0.0001$). The bicuculline methiodide (stress-inducing) and the saline conditions resulted in significantly more vertical activity than the muscimol (stress-relieving) condition.



The total distance subjects traveled is another indicator of exploratory behavior. The analysis of the total distance yielded a significant main effect of drug condition (F (2,60) = 7.0577, p = 0.0019). Both the bicuculline methiodide (stress-inducing) condition and the saline condition induced more movement than the muscimol (stress-relieving) condition. Because the saline group yielded a significantly greater distance traveled than either drug, both drugs could be interpreted as having anxiolytic (stress-relieving) effects. See discussion for further comment.



The analysis of the 22-kHz USVs yielded no statistically significant effects at an alpha level of 0.05. This is likely due to several animals in the saline group showing an abnormally high number of USVs (based on USVs numbers from other previous studies).

Figure 2. Graphs of Behavioral and USV results.

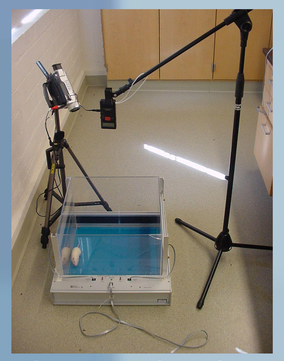


Figure 1. Experimental apparatus configuration.

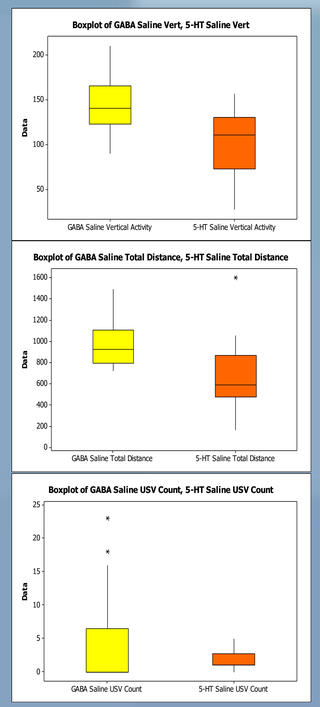


Figure 3. Comparison of the GABA vs. serotonin study saline groups.

Discussion & Conclusion

• In terms of vertical exploratory measures, the $GABA_A$ drugs showed the predicted effect, with the anxiogenic drug bicuculline methiodide inducing more rearing behavior than the anxiolytic drug muscimol. This result was similar to the results of one of our previous studies using serotonergic drugs (Niemiec & Au, 2005).

•In terms of horizontal exploratory measures, both saline and the $GABA_A$ anxiogenic bicuculline methiodide yielded greater total distance traveled than the anxiolytic muscimol. This was not as predicted, because greater exploratory behavior is typically noted in response to anxiolytic drugs.

• Locomotor confounds may be responsible for the decreased total/horizontal exploratory behavior in the muscimol condition, and the inverse relationship in the bicuculline methiodide condition.

•This is supported by experimenters' observations that the muscimol rats' muscles were more relaxed when picked up, and that attempted movements by the animals appeared to be inhibited by reduced muscular response.

• These behavioral results indicates that exploratory measures of anxiety may not be as valid as has traditionally been thought.

• Analysis of 22-kHz USVs did not reveal any significant effects. It is possible that the locomotor effects were so strong that they increased instead of decreased subjects' anxiety. Therefore, larger counts of distress USVs in muscimol rats could be explained by anxiety over the drug's physiological effects.

• These effects generate two possible conclusions: first, that USVs are less predictive of anxiolytic behavior in GABA drug effects than serotonin drug effects. Secondly, unusual distributions (Please see figure 3) in the saline (control) group may have skewed the results of this experiment and altered the observable pattern of drug effects.

• Fisher LSD tests indicate significant differences between some anxiogenic and anxiolytic conditions (Please see Results section). Fisher LSD tests indicate significant differences between some anxiogenic and anxiolytic conditions. Statistical significance (Vertical Activity) was found between the male Bicuculine methiodide group (M = 137.7, SD = 36.21) and the female Muscimol group (M = 27.3, SD = 21.1), $p < 0.01$; the female Bicuculine methiodide group (M = 157.3, SD = 61.5) and the male Muscimol group (M = 92.3, SD = 69.0), $p < 0.01$; the female Bicuculine methiodide group (M = 157.3, SD = 38.8) and the female Muscimol group (M = 27.3, SD = 21.1), $p < 0.01$.

•Boxplots comparing the distributions of the dependent variables obtained from this experiment with our previous serotonin research using the same methodology indicates greater variability in this experiment as well as other inconsistencies between the control groups in the two experiments, suggesting the groups were not equivalent. (See Fig. 3.)

•The reasons for these differences are not known, however, unanticipated changes in the the environmental conditions may have altered the animals' affective state/anxiety levels.

Citations

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Acknowledgements

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