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contract research in the neurosciences

Evaluation of Anxiolytic Effect of XXX in Guinea Pig Maternal Separation Test.

DATE

This study was conducted under terms of a Services Agreement between NeuroDetective Inc. and CLIENT, entitled TITLE OF SERVICE dated…

OBJECTIVE

This study tested the efficacy of XXX in reducing anxiety-related behavior in the guinea pig using the maternal separation test. The study also assessed if this compound produced any behavioral impairment or sedation effects.

BACKGROUND

Guinea pigs have been shown to act as reliable and valid models for the assessment of pharmacological compounds on behaviors related to anxiety (2,9-14,17). Three types of behavioral assessments have been used on guinea pigs to assess anxiety; the maternal separation test (2,9,10), the elevated plus maze test (11-14) and the open field test (6,17). The maternal separation task involves separating young pups from their mothers for short periods of time (5 minutes) and counting the number of high-pitched vocalizations emitted by the infants. Higher scores indicate higher levels of anxiety. Pettijohn (1979) has shown that the maximum number of pup vocalizations is observed at two weeks of birth and then steadily declines over the next 12 weeks. Furthermore, he has also shown that the number of vocalizations is influenced by separation from mother, home cage and siblings, with the maximum number of vocalizations occurring when the pup is separated from all three.

Compounds that anesthetize, put their subjects into a catatonic state, or lead to severe impairments in coordination are of no value as anxiolytics. Thus, tests of general activity and coordination are needed in order to determine the type of effect a test compound is having. The righting task and the incline board task are two behavioral tasks that have been used to assess general locomotor status (7,8). In the righting task animals are placed on their backs and their speed to right is timed with higher scores indicating impairment. The incline board task involves placing a guinea pig on a textured board and slowly raising one end (at the rear of the animal). The degree of incline at which the animal braces itself and moves off the board serves as the measure of impairment, with higher scores indicating impairment.

STUDY DESIGN

This study used the maternal separation test to assay levels of anxiety and the incline board and righting tasks to assess sedation or behavioral impairment. Juvenile guinea pigs of the Hartley (albino) strain at least two weeks of age were the test subjects. Three doses of the test compound (XXX) were used: DOSE 1, DOSE 2, and DOSE 3 mg/kg, delivered intraperitoneally (i.p.), with each dose provided to separate groups of animals. In addition there were three comparison groups of animals: Vehicle (0.9% saline), Buspirone at 2 mg/kg (a positive control), and Untreated. The groups of animals receiving the XXX vehicle and buspirone were also dosed i.p. The endpoint measures analyzed were: (1) the number of distress vocalizations emitted by the juvenile guinea-pigs over a 5 minute period; (2) the degree of incline of a board at which animals placed on the board braced themselves; and (3) the time taken by the animals to right themselves after being placed in a supine position.

METHOD AND MATERIALS

Facilities

This study was conducted in the laboratory of, and under the direction of, Dr. G. Campbell Teskey at the Department of Psychology, University of Calgary, Calgary, Alberta, Canada. All animal housing and experimentation facilities have been inspected by the Canadian Council on Animal Welfare and are in good standing. The guinea pigs were housed in a room dedicated for that purpose and tested in a separate room. All procedures used were approved by the Life and Environmental Science Animal Care Committee at the University of Calgary.

Animals

Pregnant adult albino guinea-pigs (Hartley, albino) were obtained from the University of Calgary/NeuroDetective breeding colony at the University of Calgary. The females were housed in groups of 2 in large home cages in a dedicated animal colony and ear marked for individual identification. The litter sizes were between 1 and 5 pups. Newborn guinea pigs were ear marked for individual identification. The adult females remained housed two to a cage following partruition.

Vocalization Test

At two weeks of age all animals were weighed and placed in an observation chamber (clear acrylic, 30 cm long x 20 cm wide x 20 cm high) to determine whether they were "squeakers" or "non-squeakers". Only confirmed "squeakers" (defined as making more than 200 vocalizations in a 5-minute period [5]) were used in subsequent behavioral testing.

XXX injections

XXX treated animals received either an i.p. administration of 0.0, DOSE 1, DOSE 2 or DOSE 3 mg/kg XXX, depending on group designation, at a volume of 1.0 ml/kg (0.9% saline). Buspirone treated animals received an i.p. administration of 2.0 mg/kg at a volume of 1.0 ml/kg in the same 0.9% saline that was used for XXX and Vehicle. Untreated animals were removed from their mothers and held, and received no liquid administration. All animals were returned to their mothers following dosing for 0.5 hour, before being removed from their mothers for behavioral testing. There were 10 animals per group and a total of 6 groups. Each animal was tested twice in all three tests, with a 3-day rest period between the first dosing and testing and the second dosing and testing. All assessments were made by one trained assistant, blinded to the treatment of each animal.

Maternal Separation Test

At two weeks of age and at the specified time interval following drug dosing (0.5 hrs), a single guinea-pig pup was separated from its mother and placed in a test chamber (identical to the observation chamber described above). The number of vocalizations over a five-minute period was counted, using a hand-held counter. After completion of the second maternal separation test (the two tests separated by 3 days, see previous paragraph), a mean number of vocalizations made by each animal in the two tests was calculated and used in data analyses. Vocalizations were also recorded for later re-play and confirmation of the counter.

Incline Board Test

Following each maternal separation test, the juvenile guinea pigs were placed onto an inclined board with the rostral end of the animal facing the hinge. The board was slowly lifted and the degree incline at which the animal displayed bracing behavior was read off a protractor affixed to the table surface adjacent to the inclined board. The test was repeated once for a second score, following the second dosing, and the mean of the two scores calculated for later analyses.

Righting Test

Following each incline board test, the animals were placed on their backs on a table, held for 1 second, then released. This task was videotaped and the behavior scored from a slow motion replay of the tape. The latency to right, with all four limbs on the surface, was read from the videotape timer. This test was repeated once for a second score, following the second dosing, and the mean of the two scores calculated for later analyses. Following each righting test the animals were returned to the colony.

STATISTICS

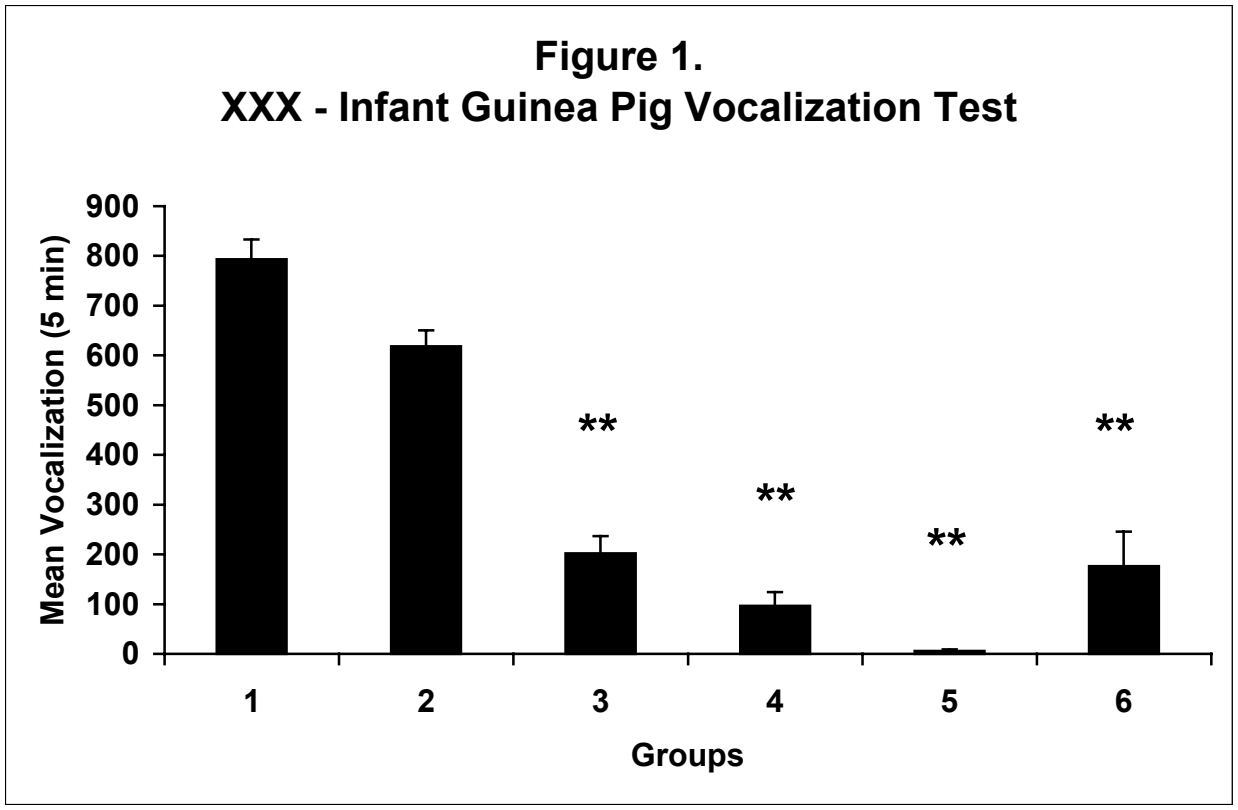
All dependent measures were analyzed using one-way Analysis of Variance, with the alpha level set at 0.05 (two-tailed). At the request of CLIENT, Dunnett's two-tailed follow-up tests were performed with the vehicle control as the comparison group. All statistical analyses were performed using GB-STAT.

RESULTS

XXX Maternal Separation Task

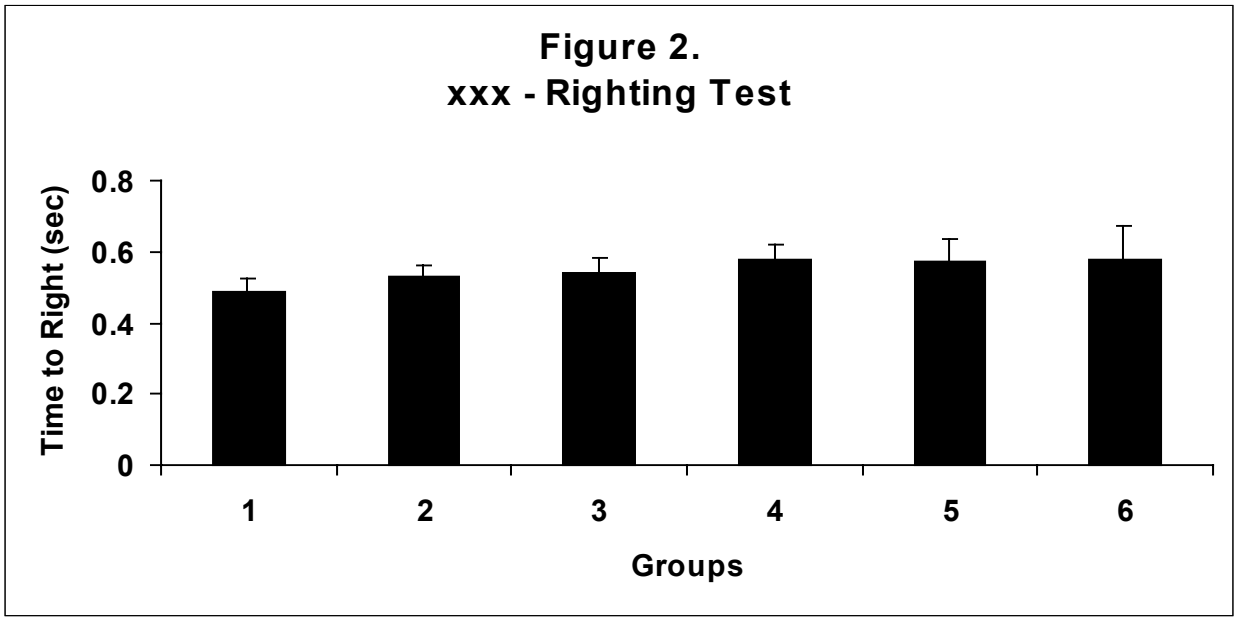
Figure 1 displays the mean (± S.E.M.) number of infant guinea pig distress vocalizations for (1) untreated, (2) 0.0 mg/kg vehicle control, (3) DOSE 1 mg/kg XXX, (4) DOSE 2 mg/kg XXX, (5) DOSE 3 mg/kg XXX, and (6) 2.0 mg/kg buspirone. A one-way ANOVA showed a significant main effect of treatment, $F(5,54) = 52.2$, $p<0.0001$.

Dunnett's 2-tailed follow-up procedure comparing vehicle control with all other groups indicated that all three drug groups (DOSE 1, DOSE 2, DOSE 3 mg/kg XXX), as well as the buspirone control, significantly reduced the number of distress vocalizations, $p < 0.01$. (** indicates significance at that level, see below.)



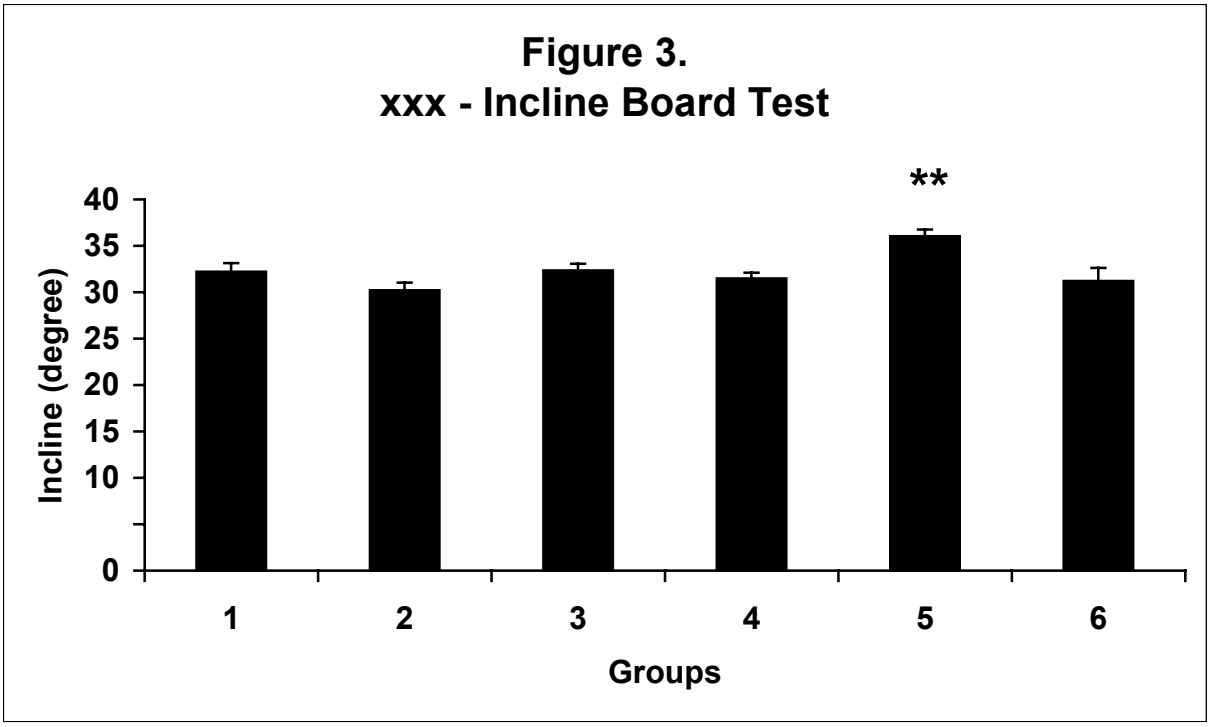
XXX Righting Task

Figure 2 shows the mean (± S.E.M.) latency to right (sec) for (1) untreated, (2) 0.0 mg/kg vehicle control, (3) DOSE 1 mg/kg XXX, (4) DOSE 2 mg/kg XXX, (5) DOSE 3 mg/kg XXX, and (6) 2.0 mg/kg buspirone. A one-way ANOVA showed a non-significant main effect of treatment, $F(5,54) = 0.45$, $p = 0.81$.



XXX Incline Board Task:

Figure 3 shows the mean (± S.E.M.) incline to brace (degrees) for (1) untreated, (2) 0.0 mg/kg vehicle control, (3) DOSE 1 mg/kg XXX, (4) DOSE 2 mg/kg XXX, (5) DOSE 3 mg/kg XXX, and (6) 2.0 mg/kg buspirone. A one-way ANOVA showed a significant main effect of treatment, $F(5,54) = 4.90$, $p < 0.0009$. Dunnett's 2-tailed follow-up procedure comparing vehicle control with all other groups indicated that the main effect of treatment was due solely to the highest dose of XXX (DOSE 3 mg/kg), which led to the animals' not bracing themselves until the board was inclined at a significantly greater degree, $p < 0.01$. (** indicates significance at that level, see below.)



CONCLUSIONS

1. XXX at DOSE 1, DOSE 2 and DOSE 3 mg/kg reduces anxiety, as measured by large and statistically significant decreases in distress vocalizations by infant guinea pigs when separated from their mothers.

2. Both control tasks (righting, incline board) showed that both the low and middle doses (DOSE 1 and DOSE 2 mg/kg XXX) did not produce behavioral impairments, indicating they did not have sedative or catatonic effects.

3. Taken together, the trend toward increased latency to right and the significant increase in degree of inclination before bracing, suggest that the high dose of XXX (DOSE 3 mg/kg) may produce a mild behavioral impairment.

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XXX Summary Statistics

1) ANOVA Summary Table for **XXX on Vocalization Test**

Source	Sum Sqres	df	Mean Sqres	F-Ratio	Prob
Between Grps	025638.25	5	805127.65	52.20458	<.0001
Within Grps	832817.65	54	15422.54907		
Total	4858455.9	59			

--Dunnett's Procedure (Treatments vs. Control)--

		Control VAR2
Untreated	VAR1	1.53137
Control	VAR2	0
DOSE 1	VAR3	7.31116**
DOSE 2	VAR4	9.22155**
DOSE 3	VAR5	10.84386**
Buspirone	VAR6	7.78021**

** p<.01 * p<.05

2) ANOVA Summary Table for **XXX on Righting Test**

Source	Sum Sqres	df	Mean Sqres	F-Ratio	Prob
Between Grps	0.06938	5	0.01388	0.45213	0.8099
Within Grps	1.65726	54	0.03069		
Total	1.72664	59			

3) ANOVA Summary Table for **XXX on Incline Board Test**

Source	Sum Sqres	df	Mean Sqres	F-Ratio	Prob
Between Grps	20DOSE 2	5	40.64	4.94215	.0009
Within Grps	444.05	54	8.22315		
Total	647.25	59			

--Dunnett's Procedure (Treatments vs. Control)--

		Control VAR2
Untreated	VAR1	1.55954
0	VAR2	0
DOSE 1	VAR3	1.6765
DOSE 2	VAR4	1.0137
DOSE 3	VAR5	4.56165**
Buspirone	VAR6	0.77977

** p<.01 * p<.05