Indian J Physiol Pharmacol 2006; 50 (3): 309–312

*SHORT COMMUNICATION*

# INTRASTRAIN VARIATIONS IN ANXIOLYTIC EFFECT OF NITRAZEPAM IN MICE

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**(Received on September 5, 2005)**

**Abstract :** This study investigated the individual differences in the baseline anxiety and anxiolytic effect of nitrazepam in Balb/c mice. Initially mice were sorted according into low, intermediate and high anxiety groups (LA, IA and HA) based on the number of entries to and time spent in open arms in elevated plus maze. Later, anxiolytic effect of nitrazepam (2 mg/kg, p.o) in LA, IA and HA mice was evaluated using hole board and light/dark tests. In Hole board test, LA mice made more number of head dippings and spent more time during head dippings, while HA mice made less number of head dippings and spent less time during head dipping when compared to that of IA mice. In light/dark test LA mice made more reentries to and spent more time in bright compartment, while HA mice made few reentries to and spent less time in bright compartment. Results suggest that mice of a single strain differ in their baseline anxiety and anxiolytic effect of nitrazepam.

**Key words :** anxiety nitrazepam anxiolytic effect mice

## INTRODUCTION

Anxiety is the response of the subject to real or potential threats that may impair homeostasis. Strain variations and between sex variations in the anxiety and anxiolytic effect of rodents have been reported in literature (1, 2, 3). But variations in baseline anxiety and anxiolytic effect of drugs within a single strain of mice are not reported in literature. Therefore in this study we aimed to investigate the individual differences in the baseline anxiety and anxiolytic effect of nitrazepam in Balb/c mice.

## MATERIAL AND METHODS

Inbred male Balb/c mice of 6–8 week old were used for the study. The animals were maintained on normal mice feed (Lipton feeds limited, Bangalore) and free access was given to food and water. Mice were maintained in clean cages at controlled temperature (21±1°C) and humidity (50±10%) under 12 h reversed light cycle (lights off at 0700 h). Clearance from the Institutional Ethical Committee was obtained prior to starting the study and CPCSEA (Committee for the purpose of

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control and supervision of experiments on animals) guidelines were followed throughout the study.

**Measurement of baseline anxiety and segregation of mice into different anxiety groups**

Mice (n=100) were taken to the psychopharmacology lab and kept for 1 hour for acclimatization. Baseline anxiety of mice was evaluated using elevated plus maze between 16:00 hours and 20:00 hours. The procedure followed was that of Montgomery et al (4). Each mouse was placed at the intersection of the four arms of the elevated plus maze so that its head was facing towards the open arm of the platform. Various indices of anxiety like number of entries to the open arms and closed arms, time spent in open arms and closed arms, total number of entries and total time spent in exploration were recorded for 5 min under dim light. Mice were sorted into low, intermediate and high anxiety groups (LA, IA and HA) depending on their performance with respect to indices of anxiety. For example, mice which made number of entries to open arms between mean ± 1SD value of the entire experimental subjects (100) were considered as intermediate anxiety (IA), and mice which made more entries to open arms than mean + 1SD value were considered as low anxiety (LA) and mice which made less entries to open arms than mean – 1SD value were considered as high anxiety (HA).

**Evaluation of anxiolytic effect of nitrazepam in LA, IA and HA mice**

LA, IA and HA mice were divided into control and test groups. Nitrazepam (2 mg/kg) (Anglofrench Drug company, Bangalore, India) was suspended in saline with a drop of Tween 80 and administered by oral route to test group mice 30 min before experiment. Control group mice received equal quantity of saline. Anxiolytic effect of nitrazepam in LA, IA and HA mice was evaluated using hole board and bright dark apparatus. LA, IA, HA mice were individually placed in the hole-board apparatus and observed for 5 min. The number of counts of nose poking/head dippings and duration of head dippings are observed for a period of 5 minutes as described by Bossier and Simon (5). Procedure described by Misslin et al (6) was followed for evaluating the anxiolytic effect in Light/dark test. LA, IA, HA mice were placed at the center of the bright compartment of light/dark apparatus and the number of reentries to bright compartment and time spent in bright compartment by each mouse were observed for 5 min. Statistical significance was determined by carrying out one-way ANOVA with posthoc Dunnet's 't' test.

## RESULTS

Mice made 3.1±2.2 entries to open arms and spent 144.5±29.4 seconds in the open arms of elevated plus maze. Segregation of mice into different anxiety groups (intermediate, low and high anxiety) based on the mean ± SD value of indices of anxiety revealed that 57 mice are of intermediate anxiety, 20 mice were of high anxiety and 23 were of low anxiety. LA mice were more explorative and made more entries (7.75±0.30) to open arms and spent more time (182.58±8.58 sec) in the open arms when compared to IA mice. HA mice were less explorative and made few entries

(4.00±0.32) and spent less time (29.00±8.74 sec) in open arms ($F_{(2,32)} = 170.06$; $P<0.001$) when compared to that of IA mice. The total arm entries and total time spent by LA mice was high and that of HA mice were low when compared to IA mice (Table I).

**Evaluation of anxiolytic effect of nitrazepam in LA, IA, HA mice**

Evaluation of anxiolytic effect of nitrazepam in LA, IA, HA subgroups in hole board revealed that nitrazepam significantly ($P<0.001$) increased the number of head dippings and duration of head dippings in LA, IA and HA groups of mice. LA mice made more head dippings ($P<0.05$) and spent more time in head dippings ($P<0.01$) while HA mice made few head dippings ($P<0.05$) and spent less time in head dippings ($P<0.01$) when compared to that of IA mice. One way ANOVA revealed significant differences in the number of head dippings ($F_{(2,15)} = 22.624$; $P<0.001$) and duration of head dippings ($F_{(2,15)} = 38.2$; $P<0.001$) (Table II). Evaluation of anxiolytic effect of nitrazepam in LA, IA and HA groups of mice in light/dark test also revealed similar differences in the anxiolytic effect of nitrazepam. LA mice made more reentries to bright compartment ($P<0.05$) and spent more time in the bright compartment ($P<0.01$), while HA mice made few reentries to bright compartment ($P<0.05$) and spent

TABLE I: Behavior of Balb/c mice in elevated plus maze.

| *Groups* | *Entries to OA* | *Entries to CA* | *Total entries* | *Time spent in OA (sec)* | *Time spent in CA (sec)* | *Total time (sec)* |
|---|---|---|---|---|---|---|
| LA | 7.75±0.30** | 2.25±0.30 | 10.00±0.34** | 182.58±8.58** | 82.16±17.00 | 251.71±8.72 |
| IA | 4.00±0.32 | 2.80±0.33 | 6.75±0.44 | 131.33±13.70 | 77.08±12.16 | 208.41±12.40 |
| HA | 0.80±0.11** | 5.08±0.64** | 6.08±0.64 | 29.00±8.74** | 157.10±15.49** | 184.83±18.80 |

N=12 in each group. Values are expressed as mean±SEM.
OA: Open arms, CA: Closed arms, LA: Low anxiety, IA: Intermediate anxiety, HA: High anxiety, LA vs IA and HA vs IA: One way Anova with posthoc Dunnet's 't' test: *=P<0.05, **P<0.01.

TABLE II: Anxiolytic activity of nitrazepam in emotionally different groups of Balb/c mice.

| *Animal model* | *Indices of anxiety* | *Treatment* | *Anxiolytic activity of nitrazepam (mean±SEM)* | | |
|---|---|---|---|---|---|
| | | | *LA* | *IA* | *HA* |
| Hole board | Number of Head dippings | Vehicle | 31.5±3.4* | 23.8±1.01 | 15.6±0.5* |
| | | Nitrazepam | 58±1.8** | 49±1.5 | 41.5±1.7* |
| | Duration of head dippings (sec) | Vehicle | 75.1±3.1** | 61.6±3 | 9.5±1.2** |
| | | Nitrazepam | 128.8±7.3** | 75.3±1.6 | 66.5±3.7$^{NS}$ |
| Light/dark test | Number of reentries to bright compartment | Vehicle | 3±0.3* | 1.5±0.2 | 0.5±0.2 |
| | | Nitrazepam | 5.8±0.6* | 3.8±0.5 | 2±0.2 |
| | Time spent in bright Compartment (sec) | Vehicle | 83±4.1** | 60±3.8 | 40.8±4** |
| | | Nitrazepam | 256.6±6** | 179.1±3 | 121±6** |

N=6 in each group. Values are expressed as mean±SEM.
LA: Low anxiety, IA: Intermediate anxiety, HA: High anxiety, Nitrazepam (2 mg/kg)
LA vs IA and HA vs IA: One way Anova with posthoc Dunnet's 't' test: *=P<0.05, **P<0.01.

less time in bright compartment (P<001) when compared to that of IA mice. One way ANOVA revealed significant differences in the number of reentries to bright compartment ($F_{(2,15)} = 20.37$; P<0.001) and the time spent in the bright compartment ($F_{(2,15)} = 146.33$; P<0.001) (Table II).

## DISCUSSION

Results revealed differences in the anxiolytic effect of nitrazepam in IA, LA and HA mice indicating the individual differences in the anxiolytic effect of mice which are identical in strain, sex and age. Previous studies reported such individual differences in the anxiety only in rats (7) and this study reports such individual differences in anxiolytic effect in a single strain of mice. Mice that were more explorative in elevated plus maze (LA) were found to be less anxious and exhibited better anxiolytic effect of nitrazepam in both hole board and bright dark test. On the contrary the mice that were less explorative (HA) exhibited more anxiety and less anxiolytic effect of nitrazepam in these models of anxiety. This suggests a link between exploratory behavior of Balb/c mice in elevated plus maze and behavior of mice in hole board and bright dark tests. Crawley and Davis reported association between exploratory behavior and anxiolytic effect of diazepam in different strains of mice (8).

## ACKNOWLEDGEMENTS

The authors thank Prof. B.G. Shivananda, Principal, Al-Ameen College of Pharmacy for providing necessary facilities to carryout the research work.

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