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Ethological Evaluation of the “Spontaneous” and stress-related Anxiety-like Behavior During Ethanol Withdrawal: Long-Term Impact of Alcohol Dependence

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Introduction

- Alcohol dependence is a chronic relapse disorder. Withdrawal symptoms, such as negative emotions, can persist for months or years following the removal of alcohol and are associated with relapse during long-term alcohol abstinence.
- Recent evidence suggested that neurochemical change during protracted withdrawal might be a time-dependent, progressive process. However, few behavioral studies had investigated time-dependent anxiety state during the protracted withdrawal and most available information on behavioral changes during protracted ethanol withdrawal is limited to “spontaneous” anxiety-like behavior.
- In the present study, we investigated “spontaneous” and stress-related anxiety in rats during the acute withdrawal, as well as short-term and long-term protracted withdrawal.



Material and Method (I)

Dependence induction

- Dependent rats ($n = 75$) were subjected to 28 d of dependence induction using ethanol vapor inhalation chambers in which rats were subject to 14-h intermittent (on, 20:00 pm; off, 10 am) ethanol exposure. The control rats ($n = 72$) were housed in vapor chambers but remained on non-ethanol-containing air.
- Blood samples were obtained at several time points during the vapor exposure period to determine blood alcohol levels (BALs). The blood samples were obtained right after turning off the pump.
- After the final cycle of vapor exposure, all rats were returned to the vivarium and stay there until the elevated plus maze test. Rats were being tested at different time points: 8 to 9 hours, 2 week, 6 week and 12 week after the last vapor exposure.



Material and Method (II)

Restrain stress

- Rat were restrained in a clear, vented Plexiglas tube fitted with a tail slot to prevent unnatural body postures, but designed to restrict nearly all movement. The restraint period will last 15 min.

Elevated Plus Maze

- Rats were placed individually onto the center of the maze facing a closed arm and removed after a 5-min period. The apparatus was wiped clean with water and dried after each subject. All rats were tested from 6 to 7 pm, which is corresponding to 8 to 9-h acute withdrawal time.



Statistic Analysis (I)

- The spatial and temporal distribution of behavior was calculated as percent total for both frequency (percent open entries, i.e. $100 * (\text{open arm entry} / [\text{open arm entry} + \text{closed arm entry}])$) and duration (percent time spend on open, i.e. $100 * (\text{open arm time} / [\text{open arm time} + \text{closed arm time}])$) data.
- The data were submitted to factor analysis using a principal component solution with an orthogonal rotation (varimax) of the factor matrix using SYSTAT 10.0 (SPSS Inc.). Factors were determined from an extracted correlation matrix. Varimax rotation, an orthogonal rotation method that minimizes the number of variables that have high loadings on each factor, was used. Factor solution were analyzed both by scree plot analysis and by retaining factors that had an eigenvalue > 1 (Kaiser HF).



Statistic Analysis (II)

- Factor scores were calculated and normalized by Z score transformation.
- Scatter Plots of Factor scores for factor 1, 2 and 3 were generated using SYSTAT 10.0 (SPSS Inc.).
- Effects of protracted withdrawal and restraint stress were analyzed separately by 4 x 2 mixed-factorial ANOVA with between subjects factor using SYSTAT 10.0 (SPSS Inc.). Significant interactions were followed by Fisher's PLSD post hoc tests to evaluate differences between each groups SYSTAT 10.0 (SPSS Inc.).



Table 1: Factor analysis of the elevated plus maze results combined dependent and non-dependent rats

	factor 1	factor 2	factor 3
	anxiety	locomotor	Conflict
open-entry	0.936	.	.
open-time	0.956	.	.
close-entry	.	0.986	.
close-time	.	.	-0.92
% open entry	0.945	.	.
% open time	0.956	.	.
center-entry	.	0.941	.
center-time	.	.	0.986
Total entry	.	0.942	.
% total variance explained	44.563	31.76	20.508

Fig 1. The effect of chronic ethanol exposure and restrain stress on anxiety measures(A) Factor 1Z score; (B) open arm time; (C) percent open arm time in elevated plus maze test at different withdrawal time points in dependent rats.

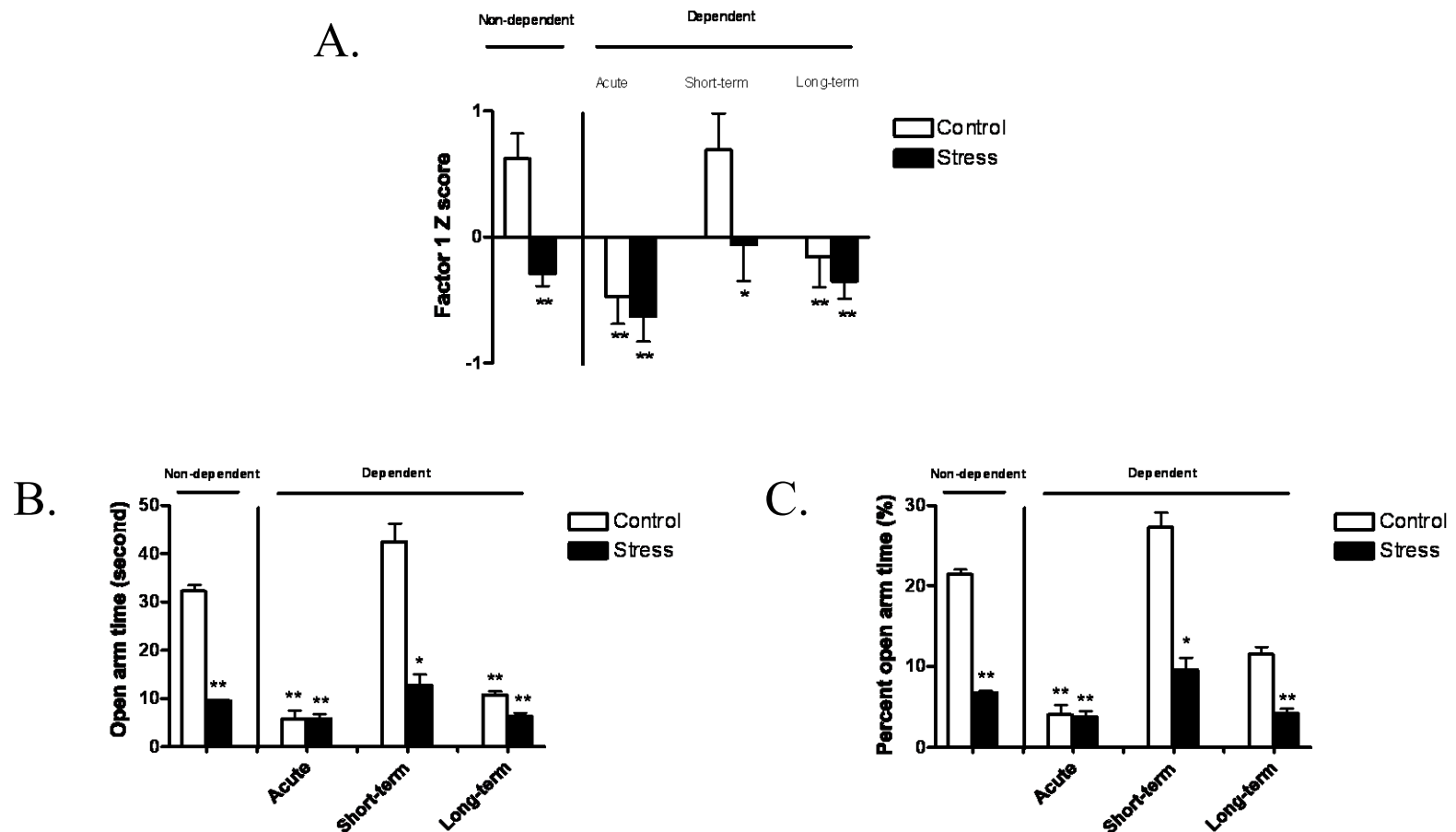


Fig 2. The effect of chronic ethanol exposure and restrain stress on locomotors activity measures (A) Factor 2 Z score; (B) close arm entry; in elevated plus maze test at different withdrawal time points in dependent rats.

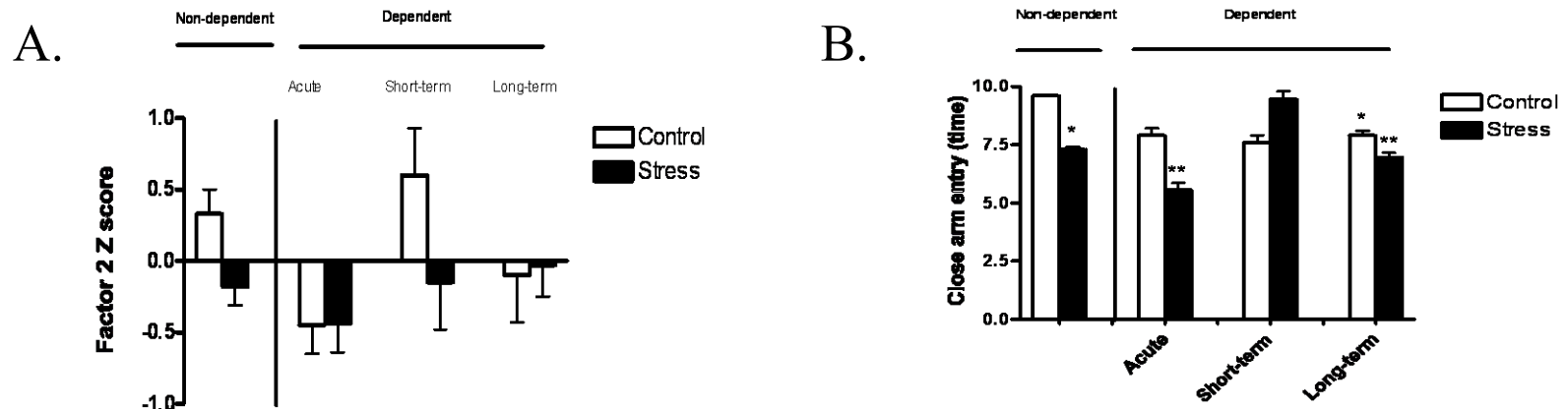


Fig 3. The effect of chronic ethanol exposure and restrain stress on conflict/decision making behavior measures (A) Factor 3 Z score; (B) center time in elevated plus maze test at different withdrawal time points in dependent rats.

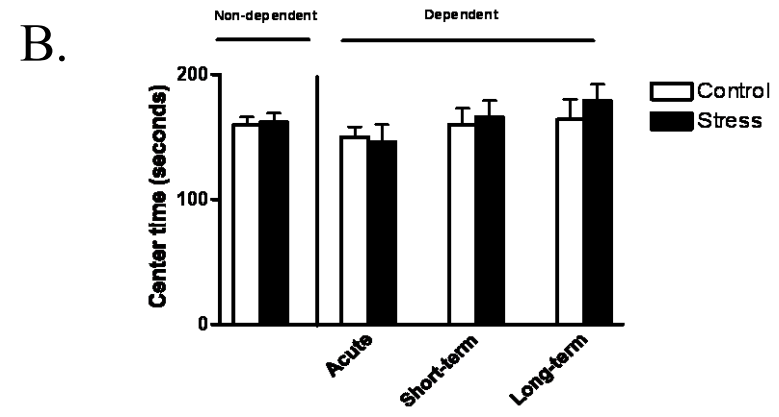
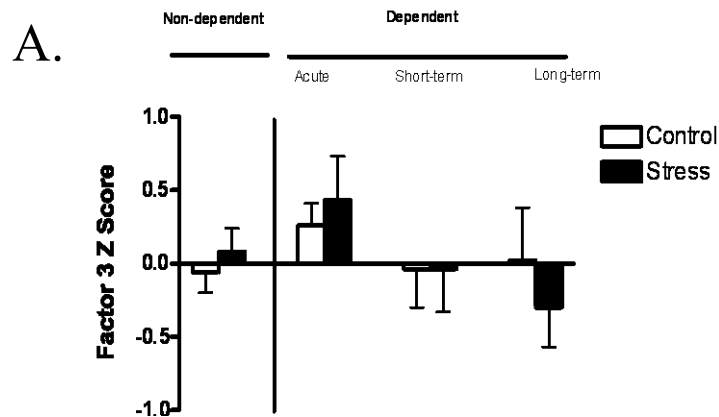


Fig 4. Scatter plots of Z scores of factor 1, 2 and 3 of individual rats of (A) non-dependent; (B) dependent acute withdrawal; (C) dependent short-term withdrawal; (D) dependent long-term withdrawal.

A. Control

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

B. Acute

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are needed to see this picture.

C. Short-term

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D. Long-term

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Summary

- The chronic ethanol exposure increased “spontaneous” anxiety-like behavior in elevated plus maze test in rats during acute withdrawal and the long-term protracted withdrawal, but not the short-term protracted withdrawal.
- Compare to the non-dependent control rats, the restraint stress significantly increased anxiety-like behavior in dependent rats, as well as in non-dependent rats.
- Chronic ethanol exposure had no effects on locomotor activity and conflict/decision making behavior of the elevated plus maze test.
- Chronic ethanol exposure changed the behavior profiles of elevated plus maze during acute withdrawal, short-term and long-term protracted withdrawal.



Conclusion

Chronic ethanol exposure caused a long lasting and time-dependent increase of “spontaneous” anxiety level and a changed behavioral profile of rats during ethanol withdrawal.

Acknowledgement

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