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Does alcohol withdrawal influence expression of coping behavior and activation of brain stress systems in adolescent female rats?

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Background: Adolescence is a critical developmental period during which individuals experience dramatic alterations in brain structure, hormones, and behavior, including marked increases in risk-taking, novelty-seeking, and also alcohol use (Spear, 2000). Since early experience with alcohol is a risk factor for later alcohol abuse (Grant, 1997), it is vital that we better understand the factors that contribute to use and misuse of alcohol during adolescence. Animal models have demonstrated that adolescent rats exhibit many of the same changes seen in human adolescents, making them invaluable tools for examining the effects of alcohol during adolescence (Spear, 2016). It is thought that the interaction between stress, alcohol, and alcohol withdrawal during adolescence could serve as a vulnerability factor for initiation of alcohol intake and excessive consumption on future occasions. Thus, the current research sought to examine whether alcohol withdrawal would alter active coping behavior (gnawing) and activation of brain regions implicated in stress and alcohol effects among adolescent female rats when confronted with a stressor (mild restraint). It was hypothesized that, during alcohol withdrawal, stress would lead to an even greater activation of stress-related brain regions, and that this would be accompanied by increases in chewing. Only females were studied since preliminary evidence has suggested that they are more likely to exhibit active coping strategies when exposed to stress (Doremus-Fitzwater et al., 2015).

Methods: Pair-housed adolescent female Sprague-Dawley rats (N = 50, n = 8-9/group) were given once-daily intragastric (i.g.) intubations of either alcohol (4 g/kg) or tap water for 3 consecutive days beginning on postnatal day (P) 33 (± 2). This was immediately followed by a 2-day rest period, after which all rats received another 3-day cycle of exposure to the same solution (ethanol or water). 24 hours after the final intubation, rats in the stress groups were placed in restraint for 30 minutes. Half of these stressed rats were presented with a wooden stick (balsa wood) that allowed them the opportunity to chew during the restraint session. Chewing behavior was recorded for later analysis. During stress exposure, non-stressed controls remained non-manipulated in the home cage. Immediately after the 30 minutes of stress (or at an equivalent time for non-stressed controls) brains and blood samples were collected and preserved. Bilateral punches of the paraventricular nucleus of the hypothalamus (PVN) and amygdala were obtained, and immediate early gene (IEG) expression in response to stress and/or alcohol withdrawal was analyzed using RT-PCR. The PVN and amygdala are both brain regions intimately involved in the brain's stress response (Herman et al., 2005), and are altered by alcohol withdrawal (Koob, 2014). Corticosterone was also measured after stress exposure as an indicator of the hormonal response to stress and withdrawal.

Results: Examination of chewing behavior during stress application indicated no behavioral difference between control (water-exposed) rats versus those experiencing alcohol withdrawal. When blood corticosterone was examined, the results showed that exposure to restraint increased levels of this stress hormone compared to non-stressed rats, but neither alcohol withdrawal nor the opportunity to chew during restraint modified this hormonal response. IEGs expressed during brain activation revealed that stress exposure profoundly increased expression of IEGs in the PVN. Furthermore, rats allowed to chew during restraint showed even larger increases in IEG expression in the PVN, and this was especially apparent in the group that was experiencing alcohol withdrawal. Amygdala samples are currently being processed for IEG expression across the experimental groups, and given the involvement of the amygdala in mediating responses to

stressors, anxiety, and alcohol withdrawal, we expect that these effects of stress and active coping may be even more pronounced in the amygdala.

Discussion: Under these circumstances, ethanol withdrawal did not increase active coping among female adolescents. It may be that adolescent females are generally insensitive to these behavioral consequences of withdrawal, or it may be that the level of alcohol exposure was insufficient to alter this behavioral response during withdrawal. However, examination of stress-related brain regions revealed that the opportunity to engage in coping behavior during a stressful situation augmented the activation of these brain regions. These results imply that brain stress systems are sensitive to an organism's available coping strategies/opportunities and future research will need to identify the behavioral consequences of such neural changes.

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