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STRESS-INDUCED CHANGES IN BRAIN SIGNALING PATHWAYS: IMPLICATIONS FOR PLASTIC BRAIN CHANGES CAUSED BY DRUGS OF ABUSE

D.B. Miller & J. P. O'Callaghan, Chronic Stress & Neurotoxicology Laboratory and Molecular Neurotoxicology Laboratory, Health Effects Laboratory Division, Centers for Disease Control and Prevention-NIOSH, Morgantown, WV 26505

Brain plasticity is believed to be responsible for the adaptations that accompany stress and drug addiction and likely involves the transduction of signals in a complex signaling network. Determining the key cellular events and multiple signaling pathways involved are an important step in understanding plasticity. Here we determined how acute and chronic stress affected multiple signaling pathways by evaluating a variety of stressors in the C57Bl6/J male mouse and examining the changes in the phosphorylation state of multiple proteins. Phosphorylation, the dominant mode of post-translation modification involved in cellular signaling is controlled through the enzymatic action of both kinases and phosphatases. As these enzymes remain active under a variety of post-mortem conditions it is essential to use a euthanasia method that preserves the state of phosphorylation. Consequently, all analyses were conducted on brain tissue obtained after microwave euthanasia, a procedure that ensures death within 50 msec and results in a brain temperature of ~ 90°C resulting in an immediate cessation of enzyme activity. We used a single 2-hr restraint, a single 5-min swim in 4° C water, 3 consecutive 10 min swims at room temperature separated by 5-min periods of non-swim, 7 days of continuous exposure to corticosterone through the implant of a 100 mg pellet or 21 consecutive days of each stressor with the exception of the corticosterone pellet. Hippocampus, cortex and striatum were collected immediately following the cessation of a stress session or at 24 hrs and 1 week later. A large-scale analysis of signaling networks was performed using kinome analysis (Kinetworks Phospho Site Screens KPSS 1.3 & 12.0), a novel technique involving the use of an array of phospho-specific antibodies covering proteins from many of the major signaling pathways currently known. Tissue homogenates were subjected to a simultaneous screen for the phosphorylation status of ~ 80 phosphoproteins and changes in phosphorylation were based on band intensity for individual phosphoproteins. Significant changes were found for a number of phosphoproteins including but not limited to CREB, ERK1/2, p38a MAPK, Msk1, Akt1, Src; the direction and intensity of the change depended on the type of stress model and the brain area examined. Most pertinent to the issue of the plasticity induced by stressors and drugs of abuse are the changes we observed in the phosphoproteins linked to cell structure including the cytoskeletal proteins □- and □-adducin, GSK3□and tau. Drug addiction is considered to be the result of plastic events and can result in changes to the same structural elements as stress including alterations in dendritic arborization and spine density. Our data suggest an examination of the signaling pathways involved in the control of the cytoskeleton may be warranted to increase our understanding of addiction.

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