Abstract: Narcotic analgesics cause addiction by poorly understood mechanisms, involving mu opioid receptor (MOR). Previous cell culture studies have demonstrated significant basal, spontaneous MOR signaling activity, but its relevance to narcotic addiction remained unclear. In this study, we tested basal MOR-signaling activity in brain tissue from untreated and morphine-pretreated mice, in comparison to antagonist-induced withdrawal in morphine-dependent mice. Using guanosine 5'-O-(3'-[35]S)thio)triphosphate ([35]S)GTP gamma S) binding and adenylyl cyclase activity assay in brain homogenates, we demonstrated that morphine pretreatment of mice enhanced basal MOR signaling in mouse brain homogenates and, moreover, caused persistent changes in the effects of naloxone and naltrexone, antagonists that elicit severe withdrawal in dependent subjects. Naloxone and naltrexone suppressed basal [(35)S]GTP gamma S binding (acting as "inverse agonists") only after morphine pretreatment, but not in drug-naive animals. Moreover, naloxone and naltrexone stimulated adenylyl cyclase activity in striatum homogenates only after morphine pretreatment, by reversing the inhibitory effects of basal MOR activity. After cessation of morphine treatment, the time course of inverse naloxone effects on basal MOR signaling was similar to the time course of naltrexone-stimulated narcotic withdrawal over several days. The neutral antagonist 6 beta-naltrexol blocked MOR activation without affecting basal signaling (G protein coupling and adenylyl cyclase regulation) and also elicited substantially less severe withdrawal. These results demonstrate long-lasting regulation of basal MOR signaling as a potential factor in narcotic dependence.