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Research Report

Region-specific effects of isoflurane anesthesia on Fos immunoreactivity in response to intravenous cocaine challenge in rats with a history of repeated cocaine administration

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ABSTRACT

We have previously shown that acute intravenous (i.v.) administration of cocaine increases Fos immunoreactivity in rats under isoflurane anesthesia. Given that Fos expression is a marker of neural activation, the results suggested that isoflurane is appropriate for imaging cocaine effects under anesthesia. However, most imaging research in this area utilizes subjects with a history of repeated cocaine exposure and this drug history may interact with anesthetic use differently from acute cocaine exposure. Thus, this study further examined Fos expression under isoflurane in rats with a history of repeated i.v. cocaine administration. Rats received daily injections of either saline or cocaine (2 mg/kg, i.v.) across 7 consecutive days, followed by 5 days of no drug exposure. On the test day, rats were either nonanesthetized or anesthetized under isoflurane and were given an acute challenge of cocaine (2 mg/kg, i.v.). Additional saline-exposed controls received a saline challenge. Ninety min after the drug challenge, the rats were perfused under isoflurane anesthesia and their brains were processed for Fos protein immunohistochemistry. We found that challenge injections of cocaine following a regimen of repeated cocaine exposure resulted in Fos expression in the prefrontal cortex and striatum roughly equivalent to that found in rats who had received the cocaine challenge after a history of vehicle injections. Additionally, isoflurane anesthesia resulted in a heterogeneous attenuation of cocaine-induced Fos expression, with the most robust effect in the orbital cortex but no effect in the nucleus accumbens core (NAcC). These results indicate that cocaine-induced Fos is preserved in the NAcC under isoflurane, suggesting that isoflurane can be used in imaging studies involving cocaine effects in this region.

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1. Introduction

Functional magnetic resonance imaging (fMRI) is a popular technique for the study of drug effects throughout the brain in animal models (Ferrari et al., 2012; Salmeron and Stein, 2002). *In vivo* fMRI in animals requires immobilization and therefore almost always involves the use of general anesthesia (Steward et al., 2005). Isoflurane anesthesia is often used in brain imaging studies that require immobile animals (Liu et al., 2004; Masamoto et al., 2007) mainly because it is administered by the noninvasive route of inhalation and post-experiment recovery is rapid (Hanusch et al., 2007; Lukasik and Gillies, 2003). However, concerns remain over its impact on the coupling between neural activity and measurable physiological effects (Austin et al., 2005; Sicard et al., 2003). With the careful measurement of physiological variables, isoflurane was recently shown to allow robust fMRI examination of cocaine activation in drug-naïve rats (Schmidt et al., 2006). Additionally, we found that cocaine-induced Fos expression is partially maintained under isoflurane anesthesia in cocaine-naïve rats (Kufahl et al., 2009). Fos is the protein product of the immediate early gene, *c-fos*. Intracellular signaling in response to a variety of stimuli transiently increases Fos expression, and its expression is therefore considered a biomarker of neuronal activity in response to a stimulus (Morgan and Curran, 1995). Detection of Fos expression under isoflurane distinguishes this anesthetic from various injectable anesthetics, which have been shown to largely abolish cocaine-induced Fos expression (Kreuter et al., 2004; Kufahl et al., 2009; Ryabinin, 2000; Torres and Rivier, 1993).

An important issue that remains to be addressed is whether neural signaling that increases Fos expression is also preserved under isoflurane anesthesia in animals that have a history of repeated exposure to cocaine, given that animal models of cocaine dependence employ chronic administration regimens that often sensitize the behavioral and neurochemical responses to cocaine (Post and Rose, 1976; Shuster et al., 1977; White and Kalivas, 1998). For instance, daily cocaine administration reliably increases locomotor activity responses to a subsequent challenge dose, relative to an identical cocaine challenge in drug-naïve rats (Kalivas and Duffy, 1990). This sensitized cocaine-induced locomotor behavior persists up to two months after discontinuing a regimen of repeated cocaine injections (Henry and White, 1995) and is thought to be an effective model for examining neurobiological changes that may lead to addictive behaviors (see for review Everitt and Wolf, 2002; Koob and Le Moal, 1997; Robinson and Berridge, 1993). Furthermore, repeated cocaine administration is associated with long-lasting adaptations within multiple neurotransmitter systems (White and Kalivas, 1998; Wolf, 1998), including glutamate and GABA systems that are also affected by isoflurane anesthesia (Westphalen et al., 2011, 2013).

In the present study, we assessed the effect of isoflurane anesthesia on cocaine-induced Fos expression in rats that had undergone repeated injections of cocaine or saline. In addition, Fos expression of the anesthetized rats was compared to that of nonanesthetized rats with identical drug histories. Additionally, locomotor activity was measured in the nonanesthetized control rats to confirm that the repeated cocaine administration produced behavioral sensitization.

2. Results

2.1. Locomotor sensitization to cocaine

For seven consecutive days, rats were given daily intravenous (i.v.) injections of 2 mg/kg cocaine ($n=13$) or saline vehicle ($n=18$) and released into rectangular locomotor cages for 90 min. Horizontal crossovers between cage halves and vertical rearing were recorded from video and accumulated over the first 20 min of each session (Fig. 1). Repeated exposure to i.v. cocaine resulted in increased locomotor behavior from Day 1 to Day 7 of treatment of the cocaine-treated rats, as revealed by the presence of *repeated treatment* × *day* interactions in both crossovers ($F_{1,\ 23}=7.1$, $p<0.05$) and rearing ($F_{1,\ 23}=37.8$, $p<0.0001$). *Post hoc* comparisons confirmed that Day 7 crossovers (paired *t*-test, $t_5=2.7$, $p<0.05$) and rearing ($t_5=5.7$, $p<0.005$) were significantly greater in the cocaine-treated rats. In contrast, Day 7 rearing was significantly reduced in saline-treated rats ($t_8=7.3$, $p<0.005$), but the reduction in crossovers was not significant.

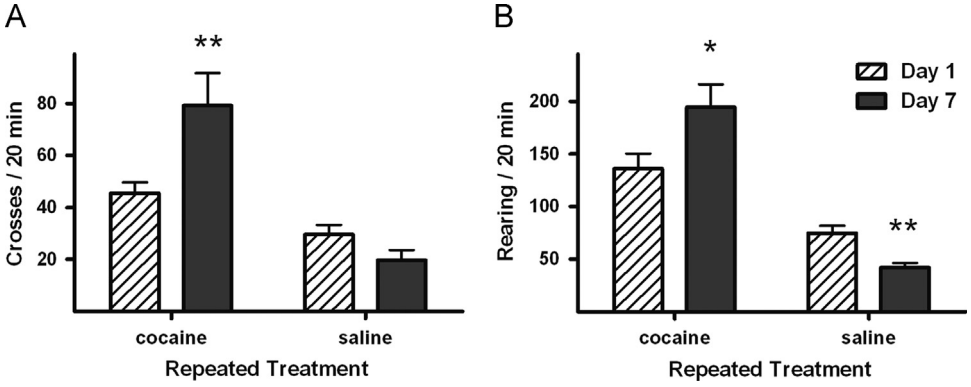

Fig. 1 – Effects of repeated treatment (cocaine, $n=14$ or saline, $n=17$) on horizontal crossovers (A) and rearing behavior (B), as assessed by comparisons between measurements made on Day 1 and Day 7. Values are presented as mean ± standard error of measurement (SEM). $^{*}p<0.05$ and $^{**}p<0.005$ difference between Day 1 and Day 7 (paired *t*-tests).