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THE ROLE OF THE GABAergic AND DOPAMINERGIC SYSTEMS IN THE BRAIN RESPONSE TO AN INTRAGASTRIC LOAD OF ALCOHOL IN CONSCIOUS RATS

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Abstract—The brain's response to ethanol intake has been extensively investigated using electrophysiological recordings, brain lesion techniques, and c-Fos immunoreactivity. However, few studies have investigated this phenomenon using functional magnetic resonance imaging (fMRI). In the present study, we used fMRI to investigate the blood oxygenation level-dependent (BOLD) signal response to an intragastric (IG) load of ethanol in conscious, ethanol-naive rats. An intragastrically infused 10% ethanol solution induced a significant decrease in the intensity of the BOLD signal in several regions of the brain, including the bilateral amygdala (AMG), nucleus accumbens (NAc), hippocampus, ventral pallidum, insular cortex, and cingulate cortex, and an increase in the BOLD signal in the ventral tegmental area (VTA) and hypothalamic regions. Treatment with bicuculline, which is an antagonist of the gamma-aminobutyric acid A ($GABA_A$) receptor, increased the BOLD signal intensity in the regions that had shown decreases in the BOLD signal after the IG infusion of 10% ethanol solution, but it did not affect the BOLD signal increase in the hypothalamus. Treatment with SCH39166, which is an antagonist of D1-like receptors, eliminated the increase in the BOLD signal intensity in the hypothalamic areas but did not affect the BOLD signal decrease following the 10% ethanol infusion. These results indicate that an IG load of ethanol caused both a $GABA_A$ receptor–mediated BOLD decrease in the limbic system and the cortex and a D1-like receptor-mediated BOLD increase in the hypothalamic regions in ethanol-naive rats.

Ethanol possesses complex pharmacological properties. Ethanol interacts directly with the gamma-aminobutyric acid A ($GABA_A$) receptors to allosterically alter their function in specific brain areas, which reinforces the effects of ethanol intake. In the central nucleus of the amygdala (AMG), ethanol enhances the GABAergic inhibitory postsynaptic potentials in rodents (Roberto et al., 2003). An agonist of the $GABA_A$ receptor facilitates ethanol intake in rats, whereas an agonist of the $GABA_B$ receptor does not (Smith et al., 1992; Boyle et al., 1993). Intragastric (IG) infusion of ethanol increases the c-Fos-positive GABAergic neurons in the medial prefrontal cortex, nucleus accumbens (NAc) and central nucleus of the AMG (Leriche et al., 2008). Ingested ethanol also increases GABA neurotransmission or changes in the GABA-binding in the AMG and in the striatum (Cowen et al., 1998; Roberto et al., 2003).

Several studies have investigated the brain circuitry underlying the rewarding effects of ingested ethanol (Vengeliene et al., 2008). The dopaminergic system originates from the ventral tegmental area (VTA) and projects to the NAc, AMG, hypothalamus, and medial prefrontal cortex in rats (Gessa et al., 1985; Zhou et al., 1999; Miller and Lonstein, 2009). The ethanol-induced conditioned place preference is mediated by dopamine (DA) receptors on neurons of the central nucleus of the AMG and NAc (Colombo et al., 1990; Smith et al., 1992; Stewart et al., 1996; Ciccocioppo et al., 1999; Gremel and Cunningham, 2008). Previous studies have revealed that D1-like receptors play important roles in increasing ethanol intake in rodents. IG infusion of ethanol causes post-ingestive flavor preference, and D1-like receptors in the lateral hypothalamus (LH) are one of the key molecules that are responsible for conditioned flavor preference in rats (Ackroff and Sclafani, 2001; Touzani et al., 2009). Chronic exposure to ethanol increases the sensitivity of D1-like receptors (Bailey et al., 2001). Ethanol-induced persistent activity in the VTA is enhanced by D1-like receptors (Tu et al., 2007).

One of the great advantages of awake functional magnetic resonance imaging (fMRI) is that we can investigate whole brain fMRI in conscious animals. The purpose of this study was to use awake fMRI to clarify the blood oxygenation level-dependent (BOLD) responses following IG administration of ethanol, thereby bypassing oral sensation. We further investigated the contribution of $GABA_A$ receptors and D1-like receptors to the IG ethanol-induced BOLD signal changes using bicuculline, which is a $GABA_A$ receptor antagonist, and SCH39166, which is a D1-like receptor antagonist.

EXPERIMENTAL PROCEDURES

Animals

BOLD fMRI measurements were performed on 32 male Wistar rats (10-week old at the start of the surgery, Charles River Laboratories Japan, Japan). They were assigned to one of the following five groups: distilled water group (n=6), ethanol group (n=6), ethanol+physiological saline (s.c.) group (n=6), ethanol+bicuculline (s.c.) group (n=6), and ethanol+SCH39166(s.c.) group (n=8). The rats were housed individually in wire-mesh cages under controlled temperature (23±0.5 °C) and light (1:00–13:00 h) conditions and were given free access to water and food (CRF-1, Oriental Yeast,

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Abbreviations: AMG, amygdala; BOLD, blood oxygenation level-dependent; DA, dopamine; fMRI, functional magnetic resonance imaging; $GABA_A$, gamma-aminobutyric acid A; IG, intragastric; LH, lateral hypothalamus; NAc, nucleus accumbens; VTA, ventral tegmental area.

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Tokyo, Japan). All animal procedures in the study were approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animal surgery

To facilitate awake MRI measurements, the rats received cranioplastic surgery under pentobarbital anesthesia (50 mg/kg body weight, i.p.), as has been described previously (Tsurugizawa et al., 2010). Briefly, IG cannulation was performed by passing one end of a silicone tube through the abdomen under the skin of the back and holding the tube to the head. Cranioplastic acrylic cement was applied to the skull with two holes molded on each side to serve as receptacles for the four glass-fiber bars that were used for head fixation during the MRI session. The other end of the silicone tube was inserted into the gastric fundus and ligated with silk thread. After surgery, the rats were allowed to recover for more than 1 week.

Acclimation training

The acclimation method has been described previously (Tsurugizawa et al., 2010). The rats were trained for 5 days to allow them to adapt to the fMRI conditions. Each rat was trained at the same time each day to minimize the effects of circadian rhythm variations. A pseudo-MRI system consisting of a non-magnetic bore and a head positioner was used during the first 3 days. At first, the rats were lightly anesthetized for less than 5 min using 2% isoflurane. They were then immediately set in the home-made head positioner by fixing four bars to the cranioplastic acrylic mount, and their bodies were gently restrained with elastic bands. To reduce any possible stress induced by the scanning noise, the rats were made to wear earplugs throughout the experiment. They were then kept in the pseudo-MRI apparatus for 30 min on the first day and 90 min on the second and third day after recovery from anesthesia. During the fourth and the fifth day, the rats were placed in a real MRI system under the conditions that would be used for the actual MRI measurements. The heart rate and the respiration rate were measured throughout the training period using an MR-compatible monitoring system throughout the training period (Model 1025, SA Instruments, Stony Brook, NY, USA). We confirmed that the respiration and heart rates on the fifth day were at normal levels.

MRI procedure

All MRI measurements were performed during the dark period after the rats had undergone fasting for 12–15 h. Rats equipped with a gastric cannula and cranioplastic acrylic cement were anes-

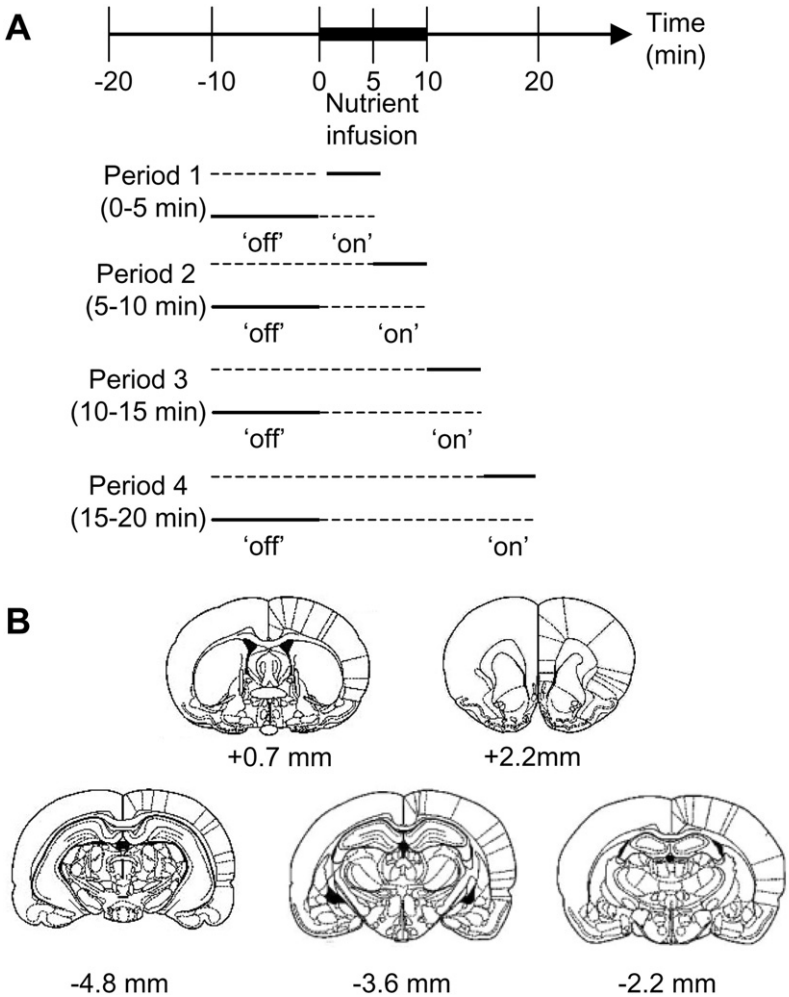

Fig. 1. (A) Diagram of the fMRI analysis. Functional data were obtained at 15 s intervals for 40 min, and the boxcar function was composed of an "off" period and an "on" period. The "off" period was the 10-min period prior to nutrient administration, and the "on" period covered four 5-min intervals after the onset of nutrient administration, for a total of 20 min (B) The coronal figures of the Paxinos atlas at +2.2 mm, +0.7, −2.2, −3.6 mm, and −4.8 mm from the bregma.