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COMMON EFFECTS OF FAT, ETHANOL, AND NICOTINE ON ENKEPHALIN IN DISCRETE AREAS OF THE BRAIN

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Abstract—Fat, ethanol, and nicotine share a number of properties, including their ability to reinforce behavior and produce overconsumption. To test whether these substances act similarly on the same neuronal populations in specific brain areas mediating these behaviors, we administered the substances short-term, using the same methods and within the same experiment, and measured their effects, in areas of the hypothalamus (HYPO), amygdala (AMYG), and nucleus accumbens (NAc), on mRNA levels of the opioid peptide, enkephalin (ENK), using *in situ* hybridization and on c-Fos immunoreactivity (ir) to indicate neuronal activity, using immunofluorescence histochemistry. In addition, we examined for comparison another reinforcing substance, sucrose, and also took measurements of stress-related behaviors and circulating corticosterone (CORT) and triglycerides (TG), to determine if they contribute to these substances' behavioral and physiological effects. Adult Sprague–Dawley rats were gavaged three times daily over 5 days with 3.5 mL of water, Intralipid (20% v/v), ethanol (12% v/v), nicotine (0.01% w/v) or sucrose (22% w/v) (approximately 7 kcal/dose), and tail vein blood was collected for measurements of circulating CORT and TG. On day five, animals were sacrificed, brains removed, and the HYPO, AMYG, and NAc processed for single- or double-labeling of ENK mRNA and c-Fos-ir. Fat, ethanol, and nicotine, but not sucrose, increased the single- and double-labeling of ENK and c-Fos-ir in precisely the same brain areas, the middle parvocellular but not lateral area of the paraventricular nucleus, central but not basolateral nucleus of the AMYG, and core but not shell of the NAc. While having little effect on stress-related behaviors or CORT levels, fat, ethanol, and nicotine all increased circulating levels of TG. These findings suggest that the overconsumption of these three substances and their potential for abuse are mediated by the same populations of ENK-expressing neurons in specific nuclei of the hypothalamus and limbic system.
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Key words: Hypothalamus, Amygdala, Nucleus accumbens, Rat, Sucrose.

INTRODUCTION

Recent evidence suggests that food rich in fat has a variety of common features with drugs of abuse, with animal studies showing dietary fat to have similar behavioral effects to those observed after acute or low doses of ethanol and nicotine. While the effects may vary depending on the specific experimental paradigm, these three substances can each cause overconsumption and signs of reward (Balfour, 1994; Lewis, 1996; Levin, 2005; Barrett and Bevins, 2012; Hilario et al., 2012) and also produce changes in emotional behaviors, such as arousal, anxiety, and impulsivity (Aragon et al., 1992; Prasad and Prasad, 1996; Langen et al., 2002; Balerio et al., 2005; Soulis et al., 2007; Chepulis et al., 2009), which themselves can contribute to the increase in intake (Koob and Volkow, 2010; Tsujino and Sakurai, 2013). These similar effects, along with clinical (Kesse et al., 2001) and animal (Olausson et al., 2001; Carrillo et al., 2004; Fornari et al., 2007) studies showing intake of one substance to stimulate or substitute for intake of another, may help to explain why these three substances are often simultaneously overconsumed (Morganstern et al., 2011; Barson et al., 2011b).

These common features of fat, ethanol, and nicotine lead one to ask whether these substances act through the same areas and neurochemical systems of the brain. While these three substances have not yet been directly compared within the same study, data collected in separate reports using different experimental paradigms show that fat (Rada et al., 2012), ethanol (Yoshimoto et al., 1992; Gonzales and Weiss, 1998), and nicotine (Nisell et al., 1994) each stimulates the release of accumbal dopamine, an effect that may underlie their common reinforcing properties. These substances also modulate endogenous expression of the opioid peptide, enkephalin (ENK), in brain structures involved in consummatory behavior, reward, and emotional aspects of eating and drug use (Chang et al., 2007, 2010a; Petruzziello et al., 2013). The changes observed in these studies, however, are not always

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Abbreviations: AMYG, amygdala; BLA, basolateral nucleus of the amygdala; CeA, central nucleus of the amygdala; CORT, corticosterone; DIG, digoxigenin; ENK, enkephalin; EPM, elevated plus maze; FISH, fluorescence in situ hybridization; GABA, γ-aminobutyric acid; HYPO, hypothalamus; ir, immunoreactivity; NAc, nucleus accumbens; NAcC, NAc core; NAcSh, NAc shell; PB, phosphate buffer; PVN, paraventricular nucleus of the hypothalamus; SSC, sodium chloride and sodium citrate; S.E.M., standard error of the mean; TG, triglycerides.

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consistent. For example, the expression of ENK is stimulated in the paraventricular nucleus of the hypothalamus (PVN) and central nucleus of the amygdala (CeA) by both fat and ethanol (Chang et al., 2007, 2010a) but only in the CeA by nicotine (Loughlin et al., 2006), and investigations of ENK in the nucleus accumbens (NAc) have yielded mixed results with these different substances (Mathieu et al., 1996; Kelley et al., 2003; Chang et al., 2010a). Studies of the immediate early gene, c-Fos, a transcription factor, have also yielded mixed findings in these brain areas. Whereas acute administration of fat, ethanol, or nicotine has a similar stimulatory effect in the PVN (Chang et al., 2004; Herring et al., 2004; Loughlin et al., 2006), and ethanol and nicotine both stimulate c-Fos in the CeA (Matta et al., 1993; Leriche et al., 2008), inconsistent effects have again been observed in the NAc (Bachtell et al., 1999; Shram et al., 2007). Further, studies involving chronic administration show c-Fos immunoreactivity (ir) to be increased in the CeA and NAc core (NAcC) after ethanol (Bachtell et al., 1999) and in the NAcC after nicotine (Pich et al., 1997), but they have failed to reveal any effect of ethanol on c-*fos* in the PVN or NAc shell (NAcSh) (Hansson et al., 2008). Whereas the neurochemical phenotype of the activated neurons is generally unknown, there are two studies focusing on ENK which show the density of neurons that double-label with c-Fos to be increased in the PVN after acute ethanol (Criado and Morales, 2000) and in the PVN and CeA after acute nicotine (Loughlin et al., 2006).

It remains unclear which specific features shared by fat, ethanol, and nicotine may contribute to their similar effects on ENK in hypothalamic and limbic brain regions. While some reports show these substances to induce psychological stress (Scheufele et al., 2000; Walker et al., 2010; Can et al., 2012), which itself may stimulate ENK and c-Fos (Shoji and Mizoguchi, 2010; Christiansen et al., 2011; Noh et al., 2012), there are others using low doses or short-term administration showing no such effects (Pohorecky, 1990; Villegier et al., 2010; Morganstern et al., 2012). These three substances also have reinforcing properties in common (Corrigall et al., 1994; Czachowski and Samson, 1999; Ackroff and Sclafani, 2014). While another substance, sucrose, is also reinforcing (Czachowski et al., 2003) and its intake similarly stimulates accumbal dopamine release (Hajnal and Norgren, 2001; Rada et al., 2005), its consumption is actually found to suppress ENK expression in the NAc (Kelley et al., 2003) and to have little impact on c-Fos-ir in the PVN, CeA, or NAc (Bachtell et al., 1999; Pomonis et al., 2000; Ulrich-Lai et al., 2007; Mitra et al., 2010). Thus, neither psychological stress nor reinforcement appears to be a primary factor involved in any similar neurochemical effects induced by fat, ethanol, and nicotine.

To clarify these issues, the present report provides a systematic analysis of the specific neuronal populations stimulated by dietary fat and drugs of abuse, to determine whether they are similar in their sites of action and neurochemical changes in the brain and, if so, whether these changes are accompanied by similar behavioral or physiological responses that may contribute to or explain their actions. The first goal was to examine fat, ethanol, and nicotine, as well as sucrose for comparison, within the same experiment and using the same mode and short period of administration. The second goal was to provide a more precise anatomical analysis of changes in three main brain structures, hypothalamus (HYPO), amygdala (AMYG), and NAc, with measurements of both single- and double-labeling of ENK mRNA and c-Fos-ir. The final goal was to take steps toward determining whether fat, ethanol, and nicotine have specific behavioral and physiological effects in common, which may be related to their similar actions in the brain. These analyses involved measurements of stress-related behaviors and blood levels of the stress hormone corticosterone (CORT) and also the lipids, triglycerides (TG), which under some conditions are increased by these substances (Balfour et al., 1975; Widmaier et al., 1992; Scheufele et al., 2000; Chattopadhyay and Chattopadhyay, 2008; Barson et al., 2009; Cippitelli et al., 2014) and can themselves stimulate expression of ENK (Ahima et al., 1992; Chang et al., 2004) and c-Fos (Chang et al., 2004; Herring et al., 2004; Loughlin et al., 2006). A 5-day exposure was used in order to examine animals beyond their first encounter with the substances, which generally stimulates c-Fos expression in the brain (Ryabinin et al., 1997; Salminen et al., 2000; Chang et al., 2004), but before they become dependent or obese, which can decrease basal levels of ENK (McLaughlin et al., 1986) and greatly reduce a c-Fos response (Ryabinin et al., 1997; Salminen et al., 2000; Chang et al., 2004), therefore masking direct effects of the substances themselves. By directly comparing fat, ethanol, and nicotine, the results of this study should allow a more definitive answer as to whether and why these substances are similar in their effects on the activity of ENK-expressing neurons in specific brain sites.

EXPERIMENTAL PROCEDURES

Subjects

Adult, Sprague–Dawley rats ($N = 84$, Charles River Laboratories International, Inc., Wilmington, MA, USA), weighing approximately 350–400 g at the onset of experiments, were individually housed (22 °C, 12:12-h light–dark cycle with lights off at 11:00 a.m.) in a fully accredited American Association for the Accreditation of Laboratory Animal Care facility. All animals were given one week to acclimate to laboratory conditions, during which time they were maintained *ad libitum* on laboratory chow (LabDiet Rodent Chow 5001) and water. All procedures were approved by the Rockefeller University Animal Care and Use Committee and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Only male rats were used, as the greater preference of females for fat, ethanol, and nicotine (Leibowitz et al., 1991; Torres et al., 2009, 2014) can fluctuate significantly across the estrus cycle (Forger and Morin, 1982; Leibowitz et al., 1998), which could introduce variability in the neurochemical responses examined in this study.

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