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Trans fat intake across gestation and lactation increases morphine preference in females but not in male rats: Behavioral and biochemical parameters

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ABSTRACT

The abuse of morphine has risen considerably in recent years, mainly due to the increase of its prescription in clinical medicine. Also, increased consumption of processed foods, rich in *trans* fatty acids (TFA), has caused concerns about human health. Thus, the aim of our study was to determine whether *trans* fat consumption in the perinatal period may affect preference for morphine in adolescent female and male rats. Dams were orally supplemented with water (C-control) or hydrogenated vegetable fat (HVF-rich in TFA) during gestation and lactation periods. On post-natal day 43, pups were exposed to morphine (4 mg/kg i.p., for 4 days) and assessed in the conditioned place preference paradigm. Anxiety-like symptoms were assessed, and oxidative status of the brain was estimated by reactive species (RS) generation. Female rats with HVF supplementation showed increased morphine preference and less anxiety-like symptoms. Additionally, both male and female rats from HVF-supplementation showed increased RS generation in the ventral tegmental area, whose level was similar in morphine-conditioned female rats. RS generation was increased in the hippocampus of morphine-conditioned female rats, regardless of the supplementation of their dams. We may infer that gender is a predictive factor to opioid preference, since adolescent female rats showed more susceptibility to addiction than males. Furthermore, *trans* fat consumption across the perinatal period is able to modify parameters of opioid preference in female rats, possibly due to TFA incorporation in phospholipid membranes, modifying the endogenous opioid system and the oxidative status in brain areas related to drug addiction.

1. Introduction

Addiction to opioids has become a problem of public health. In recent years, the US and some European countries have prescribed an excessive number of drugs involving opioids (Haffajee et al., 2015), contributing to the epidemic of abuse, which is predictive of addiction (SAMHSA, 2014). Opioids act at the cellular level by binding to μ, k and δ receptors, being μ-opioid receptors the most involved in pain relief, tolerance (Bailey and Connor, 2005), development of dependence (Al-Hasani and Bruchas, 2011), and abuse-related effects (Schwartz, 1998).

Conceptually, morphine is an alkaloid extracted from the opium poppy of the plant *Papaver somniferum* (Dewey, 2007), being one of most potent opioid analgesic drugs used. Together with pain relief, a smaller level of anxiety can be considered an additional beneficial effect of this drug (CSAT, 2005). However, morphine is often inappropriately used since it is a highly addictive drug (Zachariou et al., 2006). According to the Substance Abuse and Mental Health Services Administration (SAMHSA), opioids are the illicit drugs most frequently consumed among adolescents and young adults (SAMHSA, 2003). Between 2012 and 2013, in the United States, almost 55% of people who abused painkillers had access through a friend or relative, and only about 20% had a medical prescription. In 2013, 8.8% of youngsters from 12 to 17 years old used some kind of illicit drug, being 0.1% users of heroin (SAMHSA, 2014). In Brazil, women tend to consume more opioids compared to men (Carlini et al., 2001). In this sense, some

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reports have shown that gender also influences on the preference for drugs (Nazarian et al., 2004). Experimentally, female rats are more susceptible to rewarding effects (Karami and Zarrindast, 2008) and show lower tolerance to morphine antinociception than male rats (Craft, 2008). Although the mechanisms involved in gender difference are not clear, some hypotheses show that susceptibility may be due to neuroadaptation (Hedges et al., 2010), number or density of opioid receptors (Cicero et al., 2003), and different responses of males and females (Nestler, 2002).

Furthermore, life habits may also influence on drug addiction. Recent studies by our group have shown influence of dietary fats, especially processed foods that are rich in *trans* fatty acids (TFA), on the composition of neural membrane phospholipids, what modifies drug preference parameters (Kuhn et al., 2013, 2015). In fact, processed foods have been widely consumed in Western countries in recent decades (Santos et al., 2015), and this may cause an imbalance in essential fatty acids (Simopoulos, 2006), which are structural and functional components of neuronal membrane phospholipids (Rapoport, 2001). Besides, incorporation of TFA in the brain could also alter fluidity and biochemical properties of neuronal membranes associated with changes in the mesocorticolimbic system (Larqué et al., 2003; Acar et al., 2003), which is responsible for the rewarding effects of addictive drugs.

Considering that *trans* fat consumption during developmental periods may affect preference parameters for drugs (Kuhn et al., 2015, 2013), we have assessed its influence on addiction parameters related to morphine, also considering the gender of young rats.

2. Materials and methods

2.1. Animals and experimental procedure

All the experimental protocols were approved by the Research Ethics Committee of Universidade Federal de Santa Maria, affiliated to the Council for the Control of Animal Experiments (CONCEA), following international norms of animal care and maintenance. Animals were kept in Plexiglas cages with free access to food (Purina®) and water *ad libitum*, in a room with controlled temperature (23 °C ± 1) on a 12-h light/dark cycle with lights on at 7:00 a.m. throughout the experimental period. One week before mating, adult female Wistar rats (n=20) were supplemented by gavage (3 g/kg; p.o.) (Kuhn et al., 2013; Pase et al., 2013; Trevizol et al., 2014) with water (C-control) or hydrogenated vegetable fat (HVF-rich in *trans* fatty acids), and maintained under the same supplementation from conception until weaning of pups. Body weight gain of dams during gestation as well as body weight of pups at birth and their weight gain until weaning were assessed.

The FA profile of supplemented fat was determined as described previously (Pase et al., 2013) and is shown in Table 1. On post-natal day (PND) 41, adolescent male and female rats of each supplemented group (n=10) were exposed to the behavioral assessments described below. Mothers and offsprings were monitored during gestation and lactation periods (Table 2).

2.2. Drugs and solutions

Morphine sulphate (São Paulo, Brazil) was diluted in 0.9% saline (NaCl solution) and injected intraperitoneally (i.p.) in a dose of 4 mg/kg (Ma et al., 2008; Shi et al., 2004). Vehicle injections were 0.9% physiological saline.

Table 1
Fatty acid composition of different dietary supplementation (% of total fatty acids identified).

Fatty acids	Standard chow	Hydrogenated vegetable fat
Σ SFA	19.83	33.58
Σ MUFA	27.42	53.77
Σ PUFA	52.74	12.65
Σ n-6 PUFA	47.73	12.29
Σ n-3 PUFA	4.62	0.36
Σ TFA	0.20	13.43
Σ n6/n3 ratio	10.32	34.02

Abbreviations: SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, *trans* fatty acids.

2.3. Behavioral assessments

2.3.1. Conditioned place preference (CPP) procedure

This experimental paradigm was performed as described by Thanos et al. (2010). The CPP apparatus consisted of three compartments separated by manual guillotine doors, two boxes of equal size (45 × 45 × 50 cm) and equivalent intensity of light with different visual cues. One compartment had a smooth white floor and striped walls, whereas the other had a striped floor and smooth white walls. These two preference compartments were accessible by a central compartment (18 × 36 × 50 cm), gray with a smooth floor. Boxes were indirectly illuminated by incandescent light (40 W) of equal intensity at all times. The apparatus was cleaned with alcohol 20%, a wet sponge and paper towel before the introduction of each animal. CPP was performed through the following steps: habituation, pre-test, conditioning and test. On PND 41, all animals were placed in both compartments for 15 min for habituation. The purpose of this procedure was to exclude exploratory behavior that is common in new environments during both pre-test and conditioning steps, in order to avoid misinterpretations. On PND 42, rats were placed in the middle of the neutral area and time spent in each compartment was recorded for 15 min. Rats were discarded which showed strong unconditioned aversion (less than 25% of session time) or preference (more than 75% of session time) for any compartment (Vazquez et al., 2006). On PND 43, rats were confined in one chamber for 45 min immediately after morphine injection (4 mg/kg body weight, i.p.). After a 6 h interval, animals were confined for 45 min in the opposite chamber immediately after saline injection. Control animals were submitted to the same procedure, but they were injected with saline in both CPP sides. This procedure was continued for four consecutive days (Ma et al., 2008; Shi et al., 2004) (PND 43-46), when morphine was exclusively paired in the same CPP compartment, whereas vehicle was exclusively paired in the second environment (Carlezon et al., 2002). On CPP test day (PND 47), animals were not submitted to any invasive procedure, being placed in the middle chamber with doors open for 15 min and given access to both chambers. Total preference (%) was assessed for each of the two compartments by measuring time spent in each compartment (in seconds), which was divided by total time spent in all compartments (Brenhouse and Andersen, 2008; Thanos et al., 2010).

The rationale for choice of morphine dose (4 mg/kg) was based on a pilot study, when 4 mg/kg morphine sulphate showed preference in the CPP paradigm (data not shown). While literature has shown preference development in doses above 4 mg/kg of morphine sulphate (Dias et al., 2012; Tao et al., 2006; Gramage et al., 2015), here animals were conditioned with a dose immediately smaller than the one that showed preference for the drug, which was enough to show subtle differences between the groups born to mothers supplemented with *trans* fat and controls.