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Differential long term effects of early diisopropylfluorophosphate exposure in Balb/C and C57Bl/J6 mice

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ABSTRACT

The long-term effect of postnatal administration of a sub-toxic dose of the irreversible acetylcholinesterase inhibitor diisopropylfluorophosphate (DFP) on depression and anxiety behavior was compared in two strains of inbred mice. C57BL/6J and Balb/C mice were injected for 7 consecutive days with either 1 mg/kg DFP or saline on postnatal days 14–20. Mice were tested at age 3–4 months for initial and learned anxiety using double-exposure elevated plus maze and to a novel enclosed environment. Depression was assayed using the sweet preference model of anhedonia and the forced swim test for despair. Postnatal DFP pretreatment led to less activity and more immobility in the elevated plus maze in both mouse strains in the first session. The effect was attenuated in the second session in the C57BL/6J strain but not the Balb/C strain. DFP did not affect the sweet preference or forced swim tests, suggesting a dissociation between the long-term effects of DFP on immobility in the context of approach-avoidance conflict (elevated plus maze) versus despair (forced swim).

1. Introduction

Acetylcholinesterase (AChE) is critical for different stages of cortical development (Hohmann, 2003; Lauder and Schambra, 1999; Olivera et al., 2003; Sherren and Pappas, 2005; Small et al., 1995). Consequently exposure to organophosphate (OP) pesticides, which inhibit AChE, can be expected to have adverse effects on the developing brain. Prenatal exposure to (OP) pesticides, including both occupational (Engel et al., 2007; Eskenazi et al., 2004, 2007) and residential exposure (Engel et al., 2007; Wolff et al., 2007), was associated with developmental deficits in infancy and childhood in two major studies in children of mothers exposed to pesticides during pregnancy. Lately, longitudinal studies from these groups have reported small, but significant differences in the intelligence quotient (IQ) of children who had gestational exposure to OPs (Rauh et al., 2006). In addition, gestational and childhood exposure were associated with cognitive deficits involving sensorimotor skills, attention, learning and inhibitory control in school-aged children (Grandjean et al., 2006; Lizardi et al., 2008; Ruckart et al., 2004).

In contrast to the substantial literature on the effects of OP pesticides in gestation and childhood on cognitive development, the potential risk for emotional disorders has received little attention, despite the fact that emotional and cognitive self-regulation share similar developmental trajectories and neural substrates (Berger et al., 2007). A significant increase in anxiety and depression was reported in adults who had been exposed to AChE inhibitors in the form of pesticides (Beseler and Stallones, 2008; Levin et al., 1976; Salmon et al., 2005) or nerve gas (Yanagisawa et al., 2006). Studies in laboratory animals (Byers et al., 2005) found anomalies in cortical development following exposure to OP substances, and early studies in infants indicate a developmental neurological delay (Engel et al., 2007; Wolff et al., 2007), leading to the hypothesis that delayed cortical development following pesticide exposure could affect emotional regulation. Considering the high rate of comorbidity of depression and anxiety, it was hypothesized that development exposure to OPs would be a significant risk factor for developing anxiety and depression disorders in adulthood.

Studies in rodents do indeed suggest that perinatal exposure to pesticides can alter anxiety, although interpretation of these studies is equivocal. Increased open arm exploration of the elevated plus maze (EPM), an assay for anxiety that has been validated

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pharmacologically by the anxiolytic effect of benzodiazepine anxiolytic drugs (File, 2001; Rodgers et al., 2002), was reported in male rats treated with CPF on postnatal days 1–4 (Aldridge et al., 2005), in male and female rats treated with parathion on postnatal days 1–4 (Timofeeva et al., 2008), and in male and female C57 mice treated with diisopropylfluorophosphate (DFP) on days 4–10 or 14–20 (Kofman and Ben-Bashat, 2006). However, re-exposure to the EPM after 48 h revealed that DFP-treated mice showed exacerbated learned anxiety which manifested itself in a drastic reduction in open arm exploration on re-exposure to the maze. In addition, adult female C57 mice, exposed postnatally to DFP, showed poorer passive avoidance at 24 h, but not at 72 h after training, whereas the males showed enhanced passive avoidance compared to controls (Kofman and Ben-Bashat, 2006). These studies suggested that the effects of early exposure to pesticides on anxiety behavior in rodents might be dependent on the type of measure used. Whereas, initial exposure to the EPM suggests that CPF treated rodents showed less anxiety behavior (Aldridge et al., 2005; Timofeeva et al., 2008), studies with DFP using the double-exposure EPM test, revealed increased behavioral inhibition rather than reduced anxiety (Kofman and Ben-Bashat, 2006).

The prominent reduction in exploration upon re-exposure to the EPM can also be related to increased habituation to the maze and not to increased anxiety. In order to examine this possibility, mice were also tested in a small, non-elevated arena that was approximately the same area as the EPM, for the same duration as the EPM exposure. In summary, previous studies suggested that the long-term effects of pesticides on anxiety might depend both on the baseline level of anxiety as well as the inherent proclivity to inhibit locomotion in the face of danger. Since the latter trait varies among inbred mouse strains, the present study compared C57Bl/J6 mice Balb/C mice, as in previous studies of the neurobiological underpinnings of anxiety and depression (Brinks et al., 2009, 2007; Caldji et al., 2004).

Two of the major symptoms of major depressive disorder are anhedonia and reduced motivation to escape an aversive situation, or helplessness. Chronic or acute administration of the widely used organophosphate, malathion, to adult rats, at doses that inhibit AChE activity, increased the duration of immobility in the forced swim test (FST) (Assini et al., 2005). In contrast, rats exposed to diazinon at doses below the threshold for AChE inhibition during development did not show a significant difference in FST immobility (Roegge et al., 2008). To date, this is the only study of the developmental exposure to OPs on the FST in adults. Thus, the current study examined this behavior in mice in order to compare the effects of assays of depression with the effects on assays of anxiety.

Anhedonia is often assessed in rodents by recording their preference for a sweetened drink. Mice that were developmentally exposed to chlorpyrifos (CPF) showed a decreased preference for chocolate milk versus water (Aldridge et al., 2005); however, preference for chocolate milk was not found to be affected by neonatal exposure to the organophosphate parathion (Timofeeva et al., 2008). Since the human and rodent adult studies suggest that both anxiety and depression are affected by exposure to AChE inhibitors, in the present study, the developmental effects of DFP on anxiety and depression were tested in the same cohort of mice.

DFP was used in this study as it is an irreversible organophosphate substance that was found to affect development of anxiety behavior in our lab (Kofman and Ben-Bashat, 2006) and to increase the expression of the rare readthrough splice variant of AChE, known as AChE-R (Kaufer et al., 1998; Meshorer et al., 2002). Although banned for use as a pesticide in the U.S.A., its mechanism of action and structure are similar to nerve gases like soman and is therefore DFP is of interest to behavioral neurotoxicology (Terry et al., 2011).

In order to extend previous findings and to relate them to documented strain differences in anxiety and depression (Livneh et al., 2010), this study compared Balb/C and C57BL/6J (C57) mice. Previous studies found that Balb/C mice show more signs of fear than C57 in the light/dark box (Anisman et al., 2001; Griebel et al., 2000; Lepicard et al., 2000) and novel environments (Ducottet and Belzung, 2005) compared to the C57 mice, leading to the prediction that they might also be more fearful in the EPM. However, a review of studies comparing these two strains in the EPM shows that if open arm exploration is used as an index of anxiety, BalB/C mice do not always appear to be more anxious than C57 mice. Various studies reported that compared to the Balb/C strain, C57 mice spend *more* (Brooks et al., 2005; Lepicard et al., 2000; Millstein and Holmes, 2007 females only) or *less* time (Chapillon et al., 1999; Priebe et al., 2005; Rogers et al., 1999) in the open arms of the EPM. Other comparison studies reported no difference between these two strains in EPM anxiety (Ducottet and Belzung, 2005; Griebel et al., 2000; Yilmazer-Hanke et al., 2003). Moreover, inconsistent results were reported among different laboratories using the same protocol in a canonical study (Wahlsten et al., 2003).

In order to overcome some of the methodological problems that might account for the inconsistent results, the current study took into account the possibility that the time spent in the open arms of the EPM would be greater in Balb/C mice because of their tendency to show more immobility, thus preventing them from discovering the 'safety' of the closed arms (Livneh et al., 2010). Anisman et al. (2001) showed that under conditions of dull illumination, Balb/C mice make more entries into the open arms compared to C57 mice although the reverse is true under direct open arm illumination. Hence, since the strain difference in the EPM reported above might have been confounded by the tendency of Balb/C mice to spend be more immobile in the open arms, we predicted that the Balb/C mice would show more immobility in a stressful situation and that this effect would be enhanced by post-natal exposure to DFP, as was shown to be the case for conditioned fear (Oriel et al., 2011).

The FST depression assay also reveals strain differences between Balb/C and C57 strains. Similar to findings in our lab (Livneh et al., 2010), Balb/C showed more immobility (Millstein and Holmes, 2007) and greater corticosterone elevations (Anisman et al., 2001) in response to FST compared to C57 mice. Although one lab reported no strain difference in immobility in the FST (Cervo et al., 2005; Guzzetti et al., 2008) and others reported that Balb/C mice showed less immobility (Sugimoto et al., 2008; Yoshikawa et al., 2002), in the present study, we expected to replicate the pattern found previously in our lab. Hence, it was predicted that Balb/C mice would show more immobility in the FST and that DFP would increase immobility in the FST and decrease sweet preference in the anhedonia test.

2. Materials and methods

2.1. Animals

All experiments were conducted on females of two inbred strains of mice: C57BL/6J and Balb/C, with two treatment groups in a 2×2 design. Females were chosen because they showed more behavioral reactions in learning and emotional tests after postnatal DFP in previous studies (Kofman and Ben-Bashat, 2006). Sires and dams were purchased from Harlan (Israel) and were mated in the lab. DFP was purchased from Sigma Ltd. and diluted to 0.1 μg/ml with 0.9% saline. Solutions were prepared daily. Mice were injected subcutaneously (s.c.) in a volume of 10 ml/kg with DFP (1 mg/kg) during PND 14–20 as in our previous studies (Kofman and Ben-Bashat, 2006; Levi et al., 2008). Control animals were injected with an equivalent volume of 0.9% saline. The pups of each litter were injected with the same substance and only one mouse per litter was tested in each test. The pups were weaned at PND 28 and housed with their female littermates with ad libitum access to food and water unless otherwise stated until testing at age 3–4 months. The colony was maintained in a temperature-controlled environment (21 ± 1 °C) under a 12-h light–dark cycle, with lights turned on at 19:00 h.