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# Early maternal separation impacts cognitive flexibility at the age of first independence in mice

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ABSTRACT

Early life adversity is associated with increased risk for mental and physical health problems, including substance abuse. Changes in neural development caused by early life insults could cause or complicate these conditions. Maternal separation (MS) is a model of early adversity for rodents. Clear effects of MS have been shown on behavioral flexibility in rats, but studies of effects of MS on cognition in mice have been mixed. We hypothesized that previous studies focused on adult mice may have overlooked a developmental transition point when juvenile mice exhibit greater flexibility in reversal learning. Here, using a 4-choice reversal learning task we find that early MS leads to decreased flexibility in post-weaning juvenile mice, but no significant effects in adults. In a further study of voluntary ethanol consumption, we found that adult mice that had experienced MS showed greater cumulative 20% ethanol consumption in an intermittent access paradigm compared to controls. Our data confirm that the MS paradigm can reduce cognitive flexibility in mice and may enhance risk for substance abuse. We discuss possible interpretations of these data as stress-related impairment or adaptive earlier maturation in response to an adverse environment.



## 1. Introduction

Early life experiences are known to have a profound impact on brain development and behavior. Epidemiological data and clinical studies suggest a strong link between childhood maltreatment and the development of substance use disorders, mental health disorders, obesity, and other physical health problems (Heim and Nemeroff, 2001; Sánchez et al., 2001; Fishbein et al., 2009; Felitti et al., 1998; Dube et al., 2001; Jaffee et al., 2002; Gilbert et al., 2009). Changes in neural circuits supporting executive function caused by early neglect or maltreatment could both cause and/or exacerbate mental and physical health conditions. For example, executive function deficits may contribute to the development and management of substance use disorders (Goldstein and Volkow, 2011).

Our goal here was to develop a mouse model of effects of early life adversity on executive function with a focus on the subdomain of cognitive flexibility (also called updating in the RDoc system). Additionally, we sought to investigate how early life stress might contribute to the development of addiction-related behaviors by assessing ethanol consumption using an intermittent access "drinking in the dark" paradigm that leads to binge drinking episodes in mice (Rhodes et al., 2005; Thiele and Navarro, 2014). We choose to focus on mice to enable use of the wealth of tools for the study of neural circuits that are currently most developed in this species.

There is a large body of work that has used rats as a model system to study the effects of early adverse experience on anxiety and fear behavior and also, to a lesser extent, cognitive function. Many of these models involve disruptions of the infant-mother relationship, which is thought to be one of the most important relationships in early life (Levine, 1962; Bowlby, 1982). Some studies have focused on comparing the offspring of mothers that provide low versus high levels of maternal care (Liu et al., 1997), while others have manipulated the amount of bedding to induce maternal stress and erratic behavior (Gilles et al., 1996; Ivy et al., 2008) or employed more invasive separation paradigms which remove pups from their mother during the early postnatal period (Plotsky and Meaney, 1993; Francis et al., 2002). The most invasive separation studies have used artificial rearing with no dam care at all (Lovic and Fleming, 2004). Rat pups that have experienced low care levels or

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maternal separation (MS) have been shown by a wealth of studies to exhibit altered stress reactivity and anxiety behavior (Liu et al., 1997; Huot et al., 2002; Ladd et al., 2004; Lippmann et al., 2007; Aisa et al., 2007; Pryce and Feldon, 2003; Gilles et al., 1996; Dalle Molle et al., 2012).

A small but growing body of work has found evidence of cognitive changes in rats following early adverse experience. Adult rats that were artificially-reared (with no dam contact) have been shown to exhibit impairments in tests of cognitive flexibility in a 2-choice attentional set shifting paradigm (ASST) in which rodents learn to dig for cereal reward in scented or textured material and the rewarded contingency is reversed or the rewarded dimension is shifted (Lovic and Fleming, 2004). Impairments in 2-choice reversal in this same digging based task have also been found in adult rats that underwent 3 h of maternal separation during the first two weeks of life (Baudin et al., 2012). Working memory and flexibility in spatial tasks has also been found to be altered in adolescent and adult rats following early maternal separation (Brenhouse and Andersen, 2011; but see Wang et al., 2015).

Notably, there is less evidence of a maternal separation effect on cognitive flexibility in mice, a species in which we have greater access to the study of specific circuits. Furthermore, past studies in mice have found inconsistent results which call into question the reliability of the rodent model. One study found the effects of maternal separation on cognitive function was strain-dependent (Mehta and Schmauss, 2011). The Balb/c strain showed spatial working memory and set-shifting deficits in adulthood following maternal separation, while the adult C57Bl/6 strain showed no impairments across multiple cognitive domains. Importantly, this mouse study found no deficit in reversal learning in either strain. In contrast, other studies have found adult spatial learning impairments and working memory impairments, including deficits in spatial reversal in both C57Bl/6J (Fabricius et al., 2008) and Balb/cJ strain (Wang et al., 2011), and Y maze spontaneous alternation and temporal order memory in the C57Bl6 strain (Yang et al., 2015). Two of these positive findings however focus on spatial tasks dependent on the hippocampus rather than odor or texture based digging tasks that have been found to be dependent on the integrity of the frontal cortex of rodents (Birrell and Brown, 2000; Garner et al., 2006; Bissonette et al., 2008; Kim and Ragozzino, 2005; McAlonan and Brown, 2003).

We have previously reported developmental changes in reversal learning in mice (Johnson and Wilbrecht, 2011) using a 4-choice, odor-based digging task (similar to the ASST but with 4 choices). We also established this task is dependent on the integrity of the dorsomedial frontal cortex (dmPFC) (Johnson and Wilbrecht, 2011). Designing this current study, we hypothesized that maternal separation may alter the developmental trajectory of cognitive flexibility, and that the 4-choice task may be more sensitive to detecting this effect in mice. Four-choice tasks likely produce greater cognitive load and ambiguity or uncertainty than 2-choice tasks and can be argued to be more naturalistic in their resemblance to real world foraging environments (Ragozzino and Rozman, 2007). We chose to use the C57Bl/6 strain of mice for this study, as it is commonly used in labs that study neural circuits and behavior.

Exposure to early life stressful events has been postulated to be associated with increased vulnerability to develop substance use disorders. Human subject studies have found an association between early life adversity and increased incidence of alcohol use disorders (Macmillan et al., 2001; Green et al., 2010; Enoch, 2011). Maternal separation in rats (Huot et al., 2001) and mice (Cruz et al., 2008) has been shown to lead to increased ethanol consumption in adulthood, but the ethanol consumption paradigms used have differed and one study suggests handling protocols may impact later drinking (Ploj et al., 2003; Moffett et al., 2007). We sought to confirm if maternal separation experience in mice leads to greater consumption of ethanol using a more recent "drinking in the dark" model (Rhodes et al., 2005) that promotes binge-levels of drinking.

Here, we report that a fairly low dose of 60 min per day of maternal separation in mice for the first ten days of life can impact reversal performance measured using a 4-choice odor based task in juvenile mice tested at postnatal day 26. However, when testing in adulthood (~P60), early maternal separation experience did not lead to any difference in performance in the 4-choice reversal task, even after the daily separation time was raised to 180 min. We discuss how a change in the developmental trajectory of cognitive function may be interpreted as adaptive earlier maturation in adverse circumstances or stress induced impairment. Our data also strengthen links between early adversity and substance use disorders. When adult MS and littermate control mice were allowed intermittent access to 20% ethanol, we found a significant interaction between MS experience x time on cumulative ethanol consumption.

## 2. Materials and methods

### 2.1. Animals

Male and female C57Bl/6 *Mus musculus* (lines originally obtained from Charles River) were used for this study. Dams and sires were housed in pairs throughout the breeding and rearing period. Post-weaning, experimental mice were group housed in single sex cages, 2–5 per cage. All cages were on a 12/12 reverse light cycle (lights off at 10 AM). Testing took place during the dark period. All animals received nesting material and plastic hut in their home cage. All procedures were approved by the Ernest Gallo Clinic and Research Center and UC Berkeley Animal Care and Use Committees.

#### 2.1.1. Maternal separation (MS)

From postnatal (P) day 1 to P11, with a one day break on the weekend for some litters, half of each litter of pups was removed daily from their cage for either 60 or 180 min (MS group), while half of the litter remained with the dam (littermate, LM group). (Group sizes: Cohort 1 Juvenile 60 min: MS = 14, LM = 15; Cohort 2 Adult 60 min: MS = 12, LM = 8; Cohort 3 Adult 180 min: MS = 19, LM = 11). This particular paradigm was selected as a combination of elements from a broad variety of MS protocols used in other rodent studies (Bock et al., 2005; Monroy et al., 2010; Macri et al., 2008). During separation, MS pups were kept in a clean cage warmed by an electric pad. MS pups were able to hear and smell each other, but not touch during seperation due to a cardboard divider. The control group were littermates (LM group), forming the other half of the litter who were initially handled and marked the same as the MS half, but who were placed back in the home cage with the dam during the period of separation. All mice were identified by marker and then ear clipping. After P11 mice were not handled until weaning at P21, with the exception of one weekly cage cleaning. At weaning, the average weight of maternally separated mice was not distinguishable from littermates (MS = 8.99 ± 0.34, $n = 14$, LM = 8.83 ± 0.35 $n = 15$; $t(27) = 0.3258$, $P = 0.75$, cohort 1).

### 2.2. Four-choice odor discrimination and reversal task

Extensive methods for the four-choice reversal were published previously (Johnson and Wilbrecht, 2011). Briefly, testing took place over 5 days with 2 days of food deprivation, followed by habituation, shaping and one final day of discrimination and reversal testing. Juvenile mice started food deprivation at P22 and were tested at P26 (Fig. 1a.), while adult mice started food deprivation at P56 and were tested at P60 (Fig. 1b and c.). The same cohorts of mice that underwent the maternal separation or littermate procedure were food deprived (to 90% of *ad libitum* of adult weight or 90% of