

The influence of parity, pups, and gestational stress on cognitive flexibility during the postpartum
period.

Research Thesis

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by

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Introduction

Females of all mammalian species undergo fundamental behavioral changes during pregnancy and motherhood that are focused primarily on the care of offspring. Pregnancy and motherhood can also affect cognitive behaviors, particularly on hippocampal-dependent tasks assessing spatial learning and memory (Galea et al., 2014). Specifically, early pregnancy is associated with improved cognition, whereas late pregnancy and early postpartum are associated with impaired hippocampus-dependent spatial cognition compared to nulliparous controls (Darnaudéry et al., 2007; Workman et al., 2012). Motherhood also influences hippocampal-dependent learning beyond the early postpartum period but in a positive way such that spatial cognition is improved during the late postpartum period just prior to weaning as well as postweaning (Kinsley et al., 1999; Workman et al., 2012). These beneficial effects of maternal experience are attributable to a combination of pregnancy and mothering and persist throughout the lifespan (Kinsley et al., 1999; Lemaire et al., 2006; Pawluski et al., 2006; Workman et al., 2012). Although improved spatial cognition is most pronounced in first time (primiparous) mothers, it also occurs in multiparous mothers, although to a lesser extent (Pawluski et al., 2006).

Less is known regarding the effects of reproductive experience on performance in cognitive tasks that do not depend on the integrity of the hippocampus. The medial prefrontal cortex (mPFC) is a brain region that modulates cognitive functions including attentional processes and behavioral flexibility that can be assessed in rodents using the attentional set shifting task (Birrell & Brown, 2000; Fox et al., 2003). In this task, animals are required to learn a series of associations between stimuli of certain sensory modalities and food rewards and then switch these associations to other sensory modalities to effectively solve the task. Shifting attention from one sensory stimulus to another, referred to as an extradimensional shift (EDS),

requires the mPFC (Birrell & Brown, 2000; Fox et al., 2003). We have previously shown that postpartum females during the late postpartum period (20-24 days after parturition) perform better on the EDS of an attentional set shifting task (Leuner & Gould 2010) suggesting enhanced cognitive flexibility during motherhood. However, it is unknown whether these changes occur earlier in the postpartum period or, like hippocampal-dependent learning, would be impaired. Furthermore, whether the effects of motherhood on PFC-dependent cognitive function are attributable to pregnancy alone or require mother-pup interactions has yet to be fully investigated, as does the issue of parity. To examine these questions, we assessed attentional set shifting performance in mothers during the early (PD10-11) vs. late (PD20-21) postpartum periods as well as mothers whose pups were removed shortly after birth. We also compared attentional set shifting performance in primiparous (first time) vs. biparous (second time) mothers.

The postpartum period is often a time when women are more vulnerable to mental illness including depression. One of the strongest predictors for the emergence of postpartum depression is exposure to chronic stress during pregnancy. Similarly in rodents, chronic gestational stress induces depressive-like behavior during the postpartum period (Smith et al., 2004; Leuner et al., 2014; Haim et al., 2014). While the effects of gestational stress on cognitive function of the offspring have been well examined, relatively little is known about the effects of gestational stress on the cognitive function of the mother. Thus, we also examined attentional set shifting performance in mothers (PD21-22) that were exposed to chronic gestational stress.

Materials and Methods

Experiment 1: Cognitive flexibility in early vs late postpartum females and following pup removal

Virgin female and timed pregnant female Sprague-Dawley rats were purchased from Taconic (Germantown, NY). Pregnant animals arrived on gestation day 4 (GD4), rats were

housed individually on a 12 h light/dark cycle and provided food and water ad libitum. The day of birth was designated as postpartum day 0 (PD0) and litters were culled to either 0 or 10 pups (4-6 males, 4-6 females) on PD1. Mothers with litters were tested on the AST during either the early (PD11, n=9) or late (PD20-21, n= 6) postpartum. Mothers with pups removed (n=9) were tested early postpartum (PD10-11). Virgin animals (n=12) were housed for an equivalent amount of time as mothers tested in the early/mid postpartum period. Lavage samples were taken daily, and virgin animals were tested on the same day as mothers in the early/mid postpartum.

Experiment 2: Cognitive flexibility in primiparous vs biparous mothers

Virgin female Sprague-Dawley rats were purchased from Taconic (Germantown, NY) and bred in-house. For each pregnancy, males were paired with females for five days and allowed to mate. Each day, vaginal swabs were taken to confirm copulation. Biparous animals had different mates for each pregnancy. Rats were housed individually (when not paired) on a 12 h light/dark cycle and provided food and water ad libitum. Biparous (n=4), primiparous (n=6), and virgins (n=8) were age-matched and were subject to the AST on PD10-11 of the second pregnancy of biparous animals. For all litters, the day of birth was designated as postpartum day 0 (PD0) and litters were culled to 10 pups (4-6 males, 4-6 females) on PD1. The first litter for biparous animals were weaned on PD21.

Experiment 3: The effect of gestational stress on cognitive flexibility

Timed pregnant female Sprague-Dawley rats were purchased from Taconic (Germantown, NY). Upon arrival on gestation day 4 (GD4), rats were housed individually on a 12 h light/dark cycle and provided food and water ad libitum. The day of birth was designated as postpartum day 0 (PD0) and litters were culled to 10 pups (4–6 males, 4–6 females) on PD1.

Animals were randomly assigned to the stress (n=10) or no stress (n=9) groups. From GD7-13, pregnant rats in the stress group were subjected to 20 min of inescapable swim stress twice daily by being placed into a cylindrical container (37×30 cm) of room temperature water (25±0.5°C). From GD14-20, pregnant rats were placed into Plexiglas restrainers twice daily for 30 min of restraint stress. On each day, the stressors were administered at least 4 h apart during the light phase. Unstressed females were left undisturbed. For all litters, the day of birth was designated as postpartum day 0 (PD0) and litters were culled to 10 pups (4-6 males, 4-6 females) on PD1. Rats were tested on the AST on PD21

Attentional set shifting

In the attentional set shifting task (AST), rats were trained to recover a food reward (Froot Loop) buried in terra cotta digging pots filled with digging medium and covered with various textures. The set shifting apparatus was an opaque Plexiglas box (50 x 40 x 30 cm) divided into 3 areas: a starting area (16 x 40 x 30 cm) and two chambers (34 x 20 x 30 cm) which each held a digging pot. The chambers were separated from each other with a fixed opaque divider and separated from the starting area by a sliding opaque divider (Figure 1).

Rats were food restricted and maintained at 85% of their baseline weight for 9 (Experiments 1 and 2) or 10 (Experiment 3) days prior to testing to ensure sufficient motivation to perform the task. On each of these days, rats were placed in the apparatus and allowed to freely explore for 30 min without the presence of pots or the dividers. During the last day of habituation, rats were placed in the apparatus with the fixed divider in place. A pot filled with cob bedding with a reward on top was located in each section of the testing area. Following the habituation period, rats began the first of two phases of training occurring on consecutive days. On each of these days, rats were brought to the testing room and allowed to habituate for 30 min. Day 1 training began with rats being placed in the starting area with the dividing wall in place. Two plain pots filled with cob bedding were baited with the food reward and placed

behind the divider, one in each of the two chambers. A trial began when the divider was removed, giving the rat access to both pots. Rats were initially given four exploratory trials (120 s each) to freely explore the chambers and dig in both pots. Following the exploratory trials, rats underwent training in 4 stages in which the reward was: 1) placed on top of the cob bedding; 2) placed under a thin layer of cob; 3) buried beneath ~2 cm of cob; 4) buried under ~4 cm of cob. For all stages, rats were given 120 s to retrieve one reward. If rats failed to do so, the trial was repeated. Progression to the next stage required that rats retrieve one reward within 120 s for 6 consecutive trials. During Day 2 training, rats were taught to differentiate between two perceptual dimensions by presenting them with pairs of pots that differed on only one dimension. These included a digging medium discrimination and a texture discrimination. Rats were not exposed to the stimuli used in training again during testing. Throughout training, the correct pot was baited with the food reward while the incorrect pot contained crushed Froot Loop in the digging medium to prohibit the animal from using the scent of the reward to guide its behavior. The left-right positioning of the baited container across trials was randomized. Prior to the start of each trial, the rat was confined within the starting of the apparatus with the divider wall in place. The trial began when the divider wall was lifted giving the animal access to the two test sections, each containing a pot. Rats were initially given four exploratory trials (120 s each) to explore both chambers and dig in both pots. Following the exploratory trials, the rat was given 120 s to dig in either pot. In the event of an incorrect choice, the divider wall was immediately replaced so that the animal was not allowed access to the alternate pot. If an animal did not dig within 120 s, the partition was lowered, forcing the rat back into the waiting area. In either case, the trial was aborted and recorded as an error. Trials for each discrimination were continued until the animal reached a response criterion of 6 correct consecutive digs in the baited pot. On Day 3, rats underwent testing on a series of 5 discriminations presented in a fixed order for all rats (Table 1). For all testing trials, rats had access to both containers, only one of which was baited with a reward. As in training, the left-right positioning of the baited container across trials

was randomized and the first four trials were treated as exploration trials. For the remainder of the testing stage, the rat was given 120 s to dig in either pot. An error was recorded if the rat made an incorrect choice and dug in the unbaited pot or if the rat failed to dig. In either case, the trial was terminated and the divider wall was replaced forcing the rat back into the waiting area. Progression to the next stage of the task occurred once criterion performance (6 consecutive correct responses) was achieved. If 5 consecutive no dig trials occurred, the test was terminated and continued on the following day. Testing began with the presentation of a simple discrimination (SD) in which rats discriminated between 2 digging media in untextured containers, one of which predicted the food reward (positive stimulus). Next, in a compound discrimination (CD), a new texture dimension was introduced, but the positive stimulus was the same as in the SD. This was followed by an intradimensional attentional shift (IDS) involving two new stimuli from each stimulus dimension with digging medium remaining as the relevant dimension. The IDS was then reversed (REV), such that the formerly negative stimulus became the positive stimulus. Finally, in the extradimensional attentional shift (EDS), two new stimuli from each dimension were introduced, and the formerly task-irrelevant dimension (texture) became relevant.

Statistics

Trials to criteria on the AST were analyzed using two-way repeated measures ANOVA with group (experiment 1: virgin, early postpartum, late postpartum, postpartum pups removed; experiment 2: virgin, primiparous, biparous) and task phase (SD, CD, IDS, REV, EDS) or stress (experiment 3: chronic gestational stress or no stress) and task phase (SD, CD, IDS, REV, EDS) as independent variables. Significant interactions were followed by Bonferonni multiple comparison test. Statistical significance was set a p values < 0.05.

Results

Enhanced cognitive flexibility emerges early postpartum and requires mother-pup interactions

Figure 2 shows the trials to reach criterion for each phase of the attentional set shifting task in virgin, early postpartum females, late postpartum females and early postpartum females whose pups were removed shortly after birth. Two-way repeated measures ANOVA revealed main effects of group ($F_{3,33} = 2.84, p < 0.05$) and task phase ($F_{4,132} = 6.20, p < 0.001$) and a task phase by group interaction ($F_{4,132} = 2.60, p < 0.01$). Post hoc analysis showed that mothers during the early and late postpartum periods required fewer trials to reach criteria on the EDS phase of the AST as compared to virgin females and early postpartum females whose offspring were removed shortly after birth (p 's < 0.05). There were no significant differences among the groups on any other task phase (p 's > 0.05).

Parity does not influence performance on the attentional set shifting task

Figure 3 shows the trials to reach criterion for each phase of the attentional set shifting task in virgin females, primiparous mothers and biparous mothers. Two-way repeated measures ANOVA revealed main effects of group ($F_{2,15} = 8.63, p < 0.005$) and task phase ($F_{4,60} = 7.33, p < 0.001$) and a task phase by group interaction ($F_{4,60} = 2.83, p < 0.01$). Post hoc analysis showed that primiparous and biparous mothers required fewer trials to reach criteria on the EDS phase of the AST as compared to virgin females (p 's < 0.05) but did not differ from one another ($p > 0.05$). There were no significant differences among the groups on any other task phase (p 's > 0.05).

Gestational stress impairs reversal learning and extradimensional set shifting

Figure 4 shows the effects of chronic gestational stress on the trials to reach criterion for each phase of the attentional set shifting task. Two-way repeated measures ANOVA revealed main effects of group ($F_{1,10} = 8.63, p < 0.05$) and task phase ($F_{4,40} = 17.27, p < 0.0001$) and a

task phase by group interaction ($F_{4,40} = 4.04$, $p < 0.01$). Post hoc analysis showed that postpartum females stressed in pregnancy required more trials to reach criterion on the REV ($p < 0.05$) and EDS phases ($p < 0.01$) relative to unstressed postpartum females. There were no significant differences between stressed and unstressed postpartum females on any other task phase (p 's > 0.05).

Discussion

In this study, we extend previous work which demonstrated that motherhood has a beneficial effect on prefrontal cortex function (Leuner & Gould, 2010). Specifically, we show that cognitive flexibility is improved in mothers during both the early and late postpartum periods and is not affected by parity since both first time and second time mothers showed the enhancement in attentional set shifting performance. Moreover, we found that cognitive flexibility in mothers is dependent on the presence of pups, as removal of pups removed shortly after birth prevented the enhancement in EDS performance on the AST task. Lastly, we demonstrate that chronic stress during pregnancy impairs cognitive flexibility during the postpartum period (Leuner et al., 2014). Together, these data provide insights into the temporal dynamics and experiences which regulate prefrontal cortex dependent cognitive function during motherhood.

Enhanced behavioral flexibility emerges early postpartum regardless of parity status and requires mother-pup interactions.

The early postpartum postpartum period (days 1-4) is associated with impaired hippocampal-dependent spatial cognition compared to nulliparous controls (Darnaudéry et al., 2007; Workman et al., 2012) while the late postpartum period is accompanied by improved spatial cognition. In contrast, here we found that *both* early and late postpartum females show enhanced cognitive flexibility. It is important to point out however, that early postpartum females

in our study were tested on PD10-11 due to technical limitations requiring adequate food restriction to ensure digging behavior and this could explain the discrepancy.

The enhancement in AST performance was not evident in mothers whose pups were shortly removed after birth. Likewise, removal of pups prevents improved spatial learning and memory during motherhood (Pawluski et al., 2006). A possible reason why postpartum females do not show improved cognitive ability after pup removal may be because pregnancy and parturition without mothering experience is a stressor which increases anxiety levels that could interfere with their ability to perform the task. Despite this caveat, the data nonetheless suggest that pregnancy alone does not contribute to improved cognitive function but rather that mother-pups interactions are important regardless of whether the task is hippocampal or PFC dependent. It is possible that sensory stimulation provided by a litter of offspring is akin to an enriched environment, which is known to be beneficial to cognitive function (Kinsley et al., 1999). Along similar lines, the expression of maternal behavior itself may be an important factor driving the improvement in cognitive function. Future studies could address the role of sensory stimulation and the expression of maternal care by examining cognitive flexibility in sensitized female rats that are exposed to pups until they behave maternally. Such studies have been done using spatial tasks and these have shown enhancements in spatial memory after sensitization, pup-exposure, or pup-directed maternal behaviors alone, but these are transitory (Kinsley et al., 1999; Lambert et al., 2005; Pawluski et al., 2006) unlike the improvements in hippocampal-dependent spatial learning that persist long after weaning in postpartum rats that experience pregnancy, parturition and mothering (Workman et al., 2013). In this regard, it is worth noting that maternal experience does not persistently alter prefrontal cortical dependent learning after weaning as assessed using an operant set shifting task (Workman et al., 2013).

Although improved spatial cognition is most pronounced in first time (primiparous) mothers, it also occurs in multiparous mothers to a lesser extent (Pawluski et al., 2006). In contrast, our results indicate that improved performance in cognitive flexibility is retained in

biparous mothers. Spatial memory performance was assessed well after pups had been weaned, which further suggests that cognitive enhancements in motherhood could be dependent on the presence of pups. Whether cognitive benefits persist with additional maternal experience beyond two has yet to be determined.

Gestational stress impairs performance on AST

Here we show that two weeks of stress during pregnancy impaired performance on the EDS phase of the AST task as well as reversal learning (Leuner et al., 2014). These data are in line with previous work showing that other postpartum-related adaptations such as attenuated anxiety (Hillner et al., 2011) and improved spatial ability (Lemaire et al., 2006) are adversely impacted by gestational stress. Moreover, gestational stress is a risk factor for postpartum depression, which is also accompanied by cognitive deficits (Pio de Almeida et al., 2012). It is important to note that while both EDS and reversal learning are measures of cognitive flexibility, EDS relies on the mPFC while reversal learning is supported by the orbitofrontal cortex (OFC, Birrell & Brown, 2000; McAlonan & Brown, 2003). Thus, while the effects of motherhood appear to be restricted to the mPFC, gestational stress has more widespread effects and also impacts the OFC.

Possible hormonal correlates

When taken together, our data and others (Workman et al., 2013) shows that improved cognitive flexibility is dependent on pups and does not persist beyond the postpartum period suggesting that factors associated with the postpartum period have an 'activational,' rather than 'organizational' effect on cognitive functioning. As noted above, one possible factor is social enrichment provided by pups, although hormonal factors may also be involved. Although there are many hormonal changes that accompany motherhood, oxytocin is likely candidate for mediating the effects on cognitive flexibility. Oxytocin is a neuropeptide involved in maternal

care and has been linked to improved spatial learning in mother rats (Tomizawa et al., 2003). Oxytocin receptors (OTr) are located in the mPFC (Smeltzer et al., 2005) and future studies will investigate whether pharmacological blockade of these receptors prevents the postpartum enhancement in cognitive flexibility.

Neural mechanisms

In the mPFC, neurons undergo experience-dependent structural reorganization including changes in the size, shape, and number of dendritic spines, sites of excitatory synapses in the brain (Sorra & Harris, 2000). Spine alterations have been proposed to be the basis of changes in various types of behavior (McEwen & Morrison, 2013). Consistent with this, a previous study found an increase in dendritic spine density in the mPFC of primiparous rats compared to nulliparous rats, which corresponded with improved cognitive flexibility during the postpartum period (Leuner & Gould, 2010). Moreover, impaired performance on the AST task was accompanied by reduced mPFC dendritic spine density in postpartum females subjected to chronic stress during pregnancy (Leuner et al., 2014). Thus, dendritic spines may serve as a potential neural mechanism underlying changes in cognitive flexibility during the postpartum period (Leuner et al., 2010).

Significance

The postpartum period is a time when the survival and well-being of the offspring critically depends on the mother's ability to attend to her infant's needs which in turn requires that she be able to easily shift her attention depending on situational demands and adapt her behaviors accordingly (Barret & Fleming, 2011; Olazábal et al., 2013). Thus, enhanced cognitive flexibility during motherhood may be an adaptive response that enables successful mothering. In support of this, it has been shown that mother rats who perform better on attentional tasks are better mothers overall- they are less easily distracted, more attentive to their

litter and lick their pups more (Lovic & Fleming, 2004). This may also be true in humans where it has been shown that reduced maternal sensitivity and difficulties during mother-infant interactions are positively correlated with attentional deficits (Gonzales et al., 2012). By interfering with these functions, gestational stress may compromise the mother's capacity to adequately care for her young and may thus contribute to caregiving deficits seen in depressed mothers (Fleming et al., 1988).

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Discrimination	Change	Relevant Dimension	Irrelevant Dimension	Exemplar Combinations		
				Pair	+	-
Simple Discrimination (SD)	-	Medium	-	A	Beads	Gravel
Compound Discrimination (CD)	Add irrelevant dimension	Medium	Texture	A	Beads/Leather	Gravel/Denim
				B	Beads/Denim	Gravel/Leather
Intradimensional Shift (IDS)	New exemplars	Medium	Texture	A	Drierite/Fur	Wood Shavings/Reverse Fur
				B	Drierite/Reverse Fur	Wood Shavings/Fur
Reversal (REV)	Switch positive stimulus within dimension	Medium	Texture	A	Wood Shavings/Fur	Drierite/Reverse Fur
				B	Wood Shavings/Reverse Fur	Drierite/Fur
Extradimensional Shift (EDS)	Switch relevant dimension New exemplars	Texture	Medium	A	Velvet/Hair Ties	Reverse Velvet/Hair Ties
				B	Velvet/Hamster Bedding	Reverse Velvet/Hamster Bedding

Table 1. An example of a typical series of discriminations in the AST. Correct exemplars are in **bold** and can be paired with either exemplar from the irrelevant dimension.

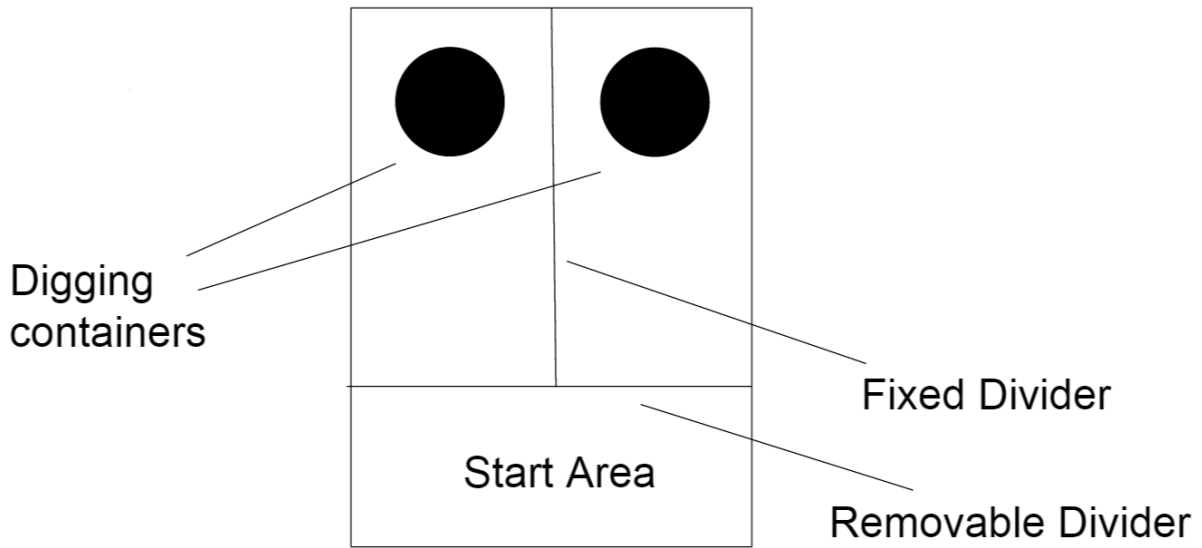


Figure 1. Diagram of the attentional set shifting apparatus. Trials begin with the rat in the start area and the displacement of the removable divider.

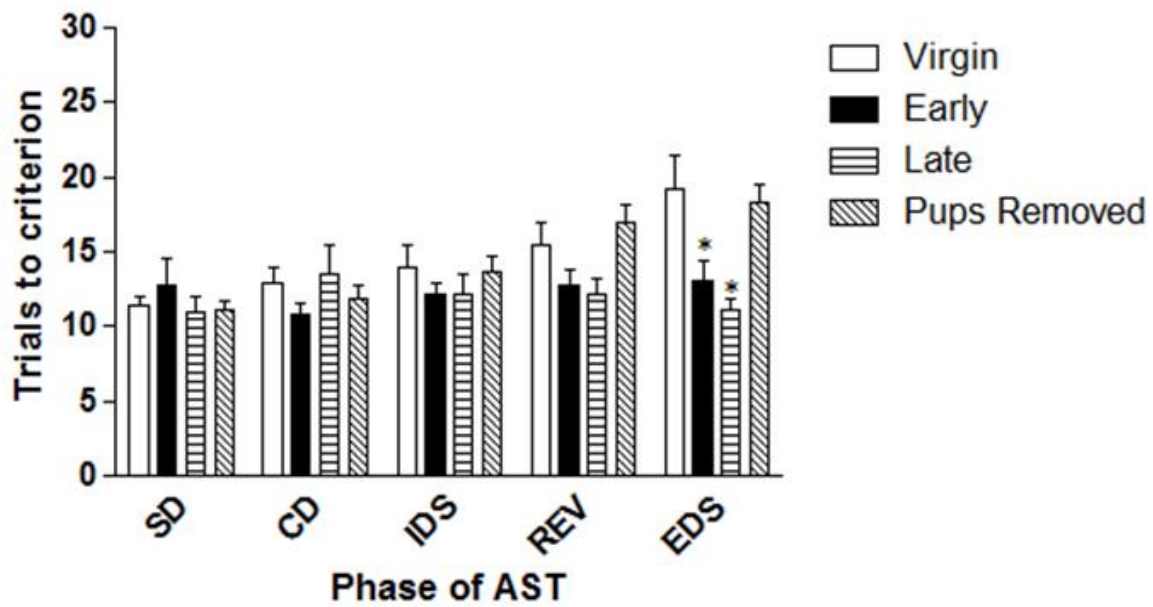


Figure 2. Mothers during the early and late postpartum periods required fewer trials to reach criteria on the EDS phase of the AST as compared to virgin females and early postpartum females whose offspring were removed shortly after birth (*p's < 0.05). There were no significant differences among the groups on any other task phase. Bars represent mean + SEM.

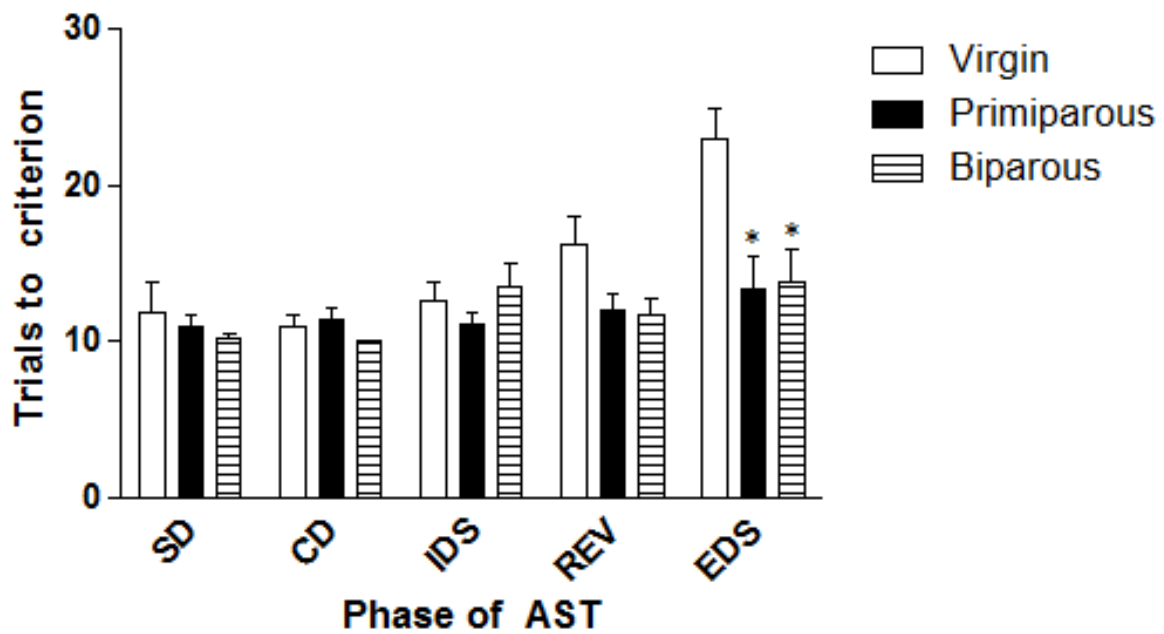


Figure 3. Both primiparous and biparous mothers required fewer trials to reach criteria on the EDS phase than virgin females (*p's < 0.01). There were no significant differences among the groups on any other task phase. Bars represent mean + SEM.

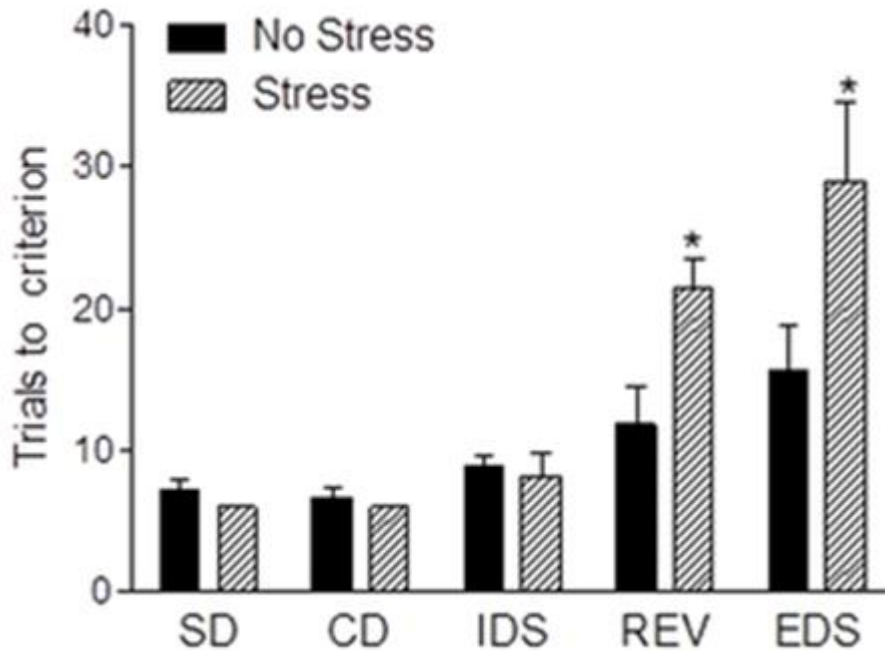


Figure 4: Compared to postpartum females who were unstressed, postpartum females stressed in pregnancy showed impairments on the reversal (REV) and extra dimensional (EDS) phases of the attentional set shifting task as demonstrated by more trials to reach criterion. The number of trials to reach criterion for the simple discrimination (SD), compound discrimination (CD) and intradimensional shift (IDS) did not differ between unstressed and stressed mothers. Bars represent mean + SEM, * $p < 0.05$.