ISDP 2014 Abstracts

EFFECTS OF TRIPHENYLPHOSPHINE AND TRIPROPYLENE GLYCOL METHYL ETHER ON ZEBRAFISH (DANIO RERIO) EMBRYO PHYSIOLOGY AND EARLY POSTNATAL DEVELOPMENT

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In January 2014, an industrial storage tank containing multiple coal processing chemicals ruptured and spilled approximately 37,850 liters of Crude MCHM and polyglycol ethers into the Elk River in West Virginia. This environmental disaster polluted drinking water for more than 300,000 residents, many of whom were pregnant women, infants, and children. These substances cause eye/skin irritation and have been linked to kidney damage and hemoglobin anomalies in rats and humans. What is presently unknown is whether these substances affect physiological, neurological, and behavioral development in young organisms. We examined the effects of tripropylene glycol methyl ether (TPGME, one isomer of polyglycol ethers) and PPh_3 (a substance chemically similar to Crude MCHM) on early physiological development and later behavioral, motor, and social development in zebrafish (*Danio rerio*). Zebrafish provide an excellent model for toxicology studies, as they develop within transparent eggs that provide a clear "window" into embryological development. They are used extensively throughout the biological, biochemical, and developmental sciences to study neurological and behavioral systems, and have recently shown promise as practical screens for chemical toxicology studies. In this study, zebrafish embryos were exposed to 2%, 5%, or 10% solutions of either TPGME or PPh_3 during the embryonic stage (days post-fertilization, dpf: 1–5), a period analogous to the prenatal period in human development. Results showed that both of these substances significantly compromise developmental physiology in embryonic zebrafish when compared to controls. Preliminary tests also reveal that these substances appear to negatively affect developing motor, behavioral, and social systems.

TREADMILL SPEED-DEPENDENT CHANGES IN STEPPING BEHAVIOR IN NEWBORN RATS

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A previous study conducted in our lab found that a moving treadmill belt does not induce stepping behavior in newborn rats. Other research has suggested that animals often require pharmacological or enhanced sensory stimulation to induce stepping on a treadmill (particularly for individuals with spinal damage), before treadmill effects may be seen. The purpose of this experiment was to examine the effect of treadmill speed on stepping behavior in one-day-old rats. Stepping was induced with the serotonergic receptor agonist quipazine. There were four treadmill speed conditions: fast, medium, slow, and non-moving (control). The medium speed (2.5 cm/s) was calculated by measuring the average speed of a P1 rat's limb movements following treatment with quipazine. The slow speed was 30% slower and the fast speed was 30% faster than the medium speed, respectively. During a 30-min test period, subjects were suspended over a treadmill belt following intraperitoneal injection of quipazine (3.0 mg/kg, 50 microliters). Subjects showed treadmill speed-dependent differences in stepping: subjects stepping on a faster moving belt showed significantly more alternated forelimb steps than subjects stepping on a slower moving belt. Subjects in the fast speed condition showed the highest number of steps, whereas subjects in the control condition showed the least amount. Differences were apparent within the first 5 min of the test session. Step area, swing, stance, and inter-limb phase also were calculated. This is the earliest demonstration of treadmill speed affecting locomotor behavior in mammals.

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PARENTAL BEHAVIOR IN JUVENILE MICE (C57BL/6J)

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It was unclear whether naïve juvenile mice, individually housed and exposed to newborns, could display parental behavior like juvenile rats and prairie voles. In Experiment I, we described the behavioral response of naïve male ($n = 10$) and female ($n = 9$) juvenile mice (C57Bl/6J; 20-22 days of age) exposed to newborns (15 min) for the first time. We recorded retrieval behavior, licking, crouching postures, nest building, and exploratory/locomotor activity (climbing, rearing, and time immobile). We found that, at the first exposure, none of the juveniles displayed full parental behavior, although a few females (11%) displayed some behavioral components of parental behavior. None of the juveniles showed behavioral components of parental behavior during a second exposure to newborns. Juvenile females and males spent a lot of time immobile and showed very low levels of rearing, and climbing behavior. In a second experiment we investigated if cohabitation with their mothers and newborn siblings, at the delivery of a second litter, facilitated parental behavior in juvenile females. In contrast to juveniles from single offspring mothers (100% non parental), 21.5% and 34.5% of those from overlapping litters (12-24 hours of continuous exposure to pups), tested as in experiment I, displayed all or some of the behavioral components of parental behavior respectively ($\chi^2 = 10.2$, $p < 0.05$). These findings suggest that juvenile mice are inhibited to display parental behavior, and fluids of parturition and/or few hours of exposure to newborn siblings and mothers can remove this inhibition.

[I+D CSIC, UdelaR to DEO].

CONDITIONED INHIBITION IN INFANT RATS

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Inhibitory conditioning is a very well established phenomenon in associative learning described in humans and in adult animals. Particularly,

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Conditioned Inhibition gathers most of the scientific research in inhibitory learning and still produces interesting theoretical discussions within the field. However, there is no study assessing the existence of inhibitory learning during the early ontogeny of the rat. In this study we assessed the possibility to find Conditioned Inhibition in infant rats (day 9) using a conditioned taste aversion procedure. We tested whether the consumption of saccharin (A) was diminished when paired with a LiCl injection compared to the presentation of saccharin in compound with lemon odor (AX) without any aversive consequence. After this training, retardation and summation tests were carried out to evaluate the inhibitory properties of the lemon odor (X). Results of this study show Conditioned Inhibition in preweanling rats, suggesting that animals at this stage of ontogeny have the capacity to acquire inhibitory learning.

TIMING OF SITTING, STANDING, AND WALKING ONSET AFFECTS THE INFANTS' UNDERSTANDING OF OBJECTS

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As infants start sitting and standing up, they free their hands to access different toys and start manipulating them. While manipulating objects, infants explore object properties and increase their perceptual knowledge of objects. Also, self-locomotion increases infants' opportunities to explore objects. Thus, understanding of objects reflected in advanced manual skills, such as role-differentiated bimanual manipulation (RDBM) of complex objects and tool-use, might be facilitated by advances in sitting, crawling, standing up, and walking skills. In a large sample of 90 infants tested longitudinally from 6 to 14 months, we found that infants that started crawling, standing up, and walking sooner were more advanced than their peers in their RDBM skills. Also, infants that started sitting, crawling, and standing up sooner were more advanced at using tools. We concluded that the development of postural control and locomotion might facilitate the infant's understanding of objects and their properties.
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INDIVIDUAL DIFFERENCES IN SPONTANEOUS EYE BLINKING ARE LINKED TO FRONTAL ASYMMETRY IN HUMAN INFANTS

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The rate of spontaneous eye blinking (SEB), a putative index of dopamine function (DA), is altered by some kinds of cognitive activity and under some pathological conditions. Also, the wide individual differences in SEB rate may reflect individual differences in DA function. However, few researchers have investigated SEB in human infants. We explored whether individual differences in SEB rate are related to frontal asymmetry (FA) in 10-mo-old infants. Researchers of infants have discovered relationships between DA-related genes and FA, and work with adults suggests that DA system function may relate to some aspects of FA. Our design allows us to test for SEB-FA relationships across three frontal regions during various phases of a working memory task. Forty, healthy, term, 10-mo-olds completed a looking version of the A-not-B task. SEB were coded blind to task phase. Phases of the task (show toy, hide toy, reveal toy) were scored separately. EEG data were collected from three frontal regions (frontal pole Fp1, Fp2; medial frontal F3,F4; lateral frontal, F7,F8) at 512Hz. The ANOVA testing F8-F7 asymmetry revealed a significant interaction between SEB group (high, medium, low rate) and Phase. Infants with intermediate SEB rate exhibited right FA during one phase of the task. Observing differences in FA at F8-F7 is consistent with work indicating involvement of this region during tasks requiring vigilance. Additionally, although FA is often considered trait-related, our results show task-related temporal variation in FA. More broadly, results provide converging evidence that SEB in infants reflects some dimensions of DA function.
[NICHD HD049878 to MAB]

ASSESSING SOCIAL BEHAVIOUR FOLLOWING PRENATAL ALCOHOL EXPOSURE IN RATS: WHO WILL PLAY WITH ME?

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In humans and animal models of prenatal alcohol exposure (PAE), aberrant functioning of the prefrontal cortex, which subserves executive function (EF), and its related circuitry underlies many behavioral deficits observed following PAE. Cognitive and social deficits are among the most persistent PAE-related deficits. As EF and social behavior are intricately linked, early aberrant social behavior potentially provides a means of predicting functional outcome on a broader range of EF-related deficits.
Pregnant rat dams were assigned to: 1) Control: *ad libitum* pelleted control diet; 2) Pairfed: restricted access liquid control diet; or 3) PAE: *ad libitum* liquid ethanol diet (36% ethanol- derived calories). A novel social play paradigm assessed behaviour within triads. Each triad consisted of one test animal and two playmates - one same-treatment playmate and one different-treatment playmate. This design allowed a determination of who would play with whom and the use of playmate choices as indicators of behavior anomalies.
In females triads, Control animals reciprocated play less often with PAE than with control playmates. Additionally, although Controls initiated play non-discriminately, the Control females displayed more aggressive behaviour when in triads with PAE than when in triads with Pairfed females. However, in male triads, Control males initiated play with PAE males less than with other controls, whereas in the Pairfed triads this was not the case. In summary, it appears that PAE alters social behaviour such that Controls respond differently to them than to either Pairfed or other Control playmates. Moreover, these alterations appear to be sexually dimorphic.
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IMPAIRED FEAR EXTINCTION RETENTION IN ADOLESCENCE: AGE AT FEAR LEARNING OR AGE AT EXTINCTION?

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Adolescence is a period in which fear inhibition is impaired in both rodents and humans. For example, adolescent rodents exhibit a marked impairment of fear extinction retention relative to both younger (e.g., juvenile) and older (e.g., adult) groups. The present experiments investigated whether the animal's age at the time of fear learning or fear extinction determines if extinction is impaired in adolescence. The results of Experiment 1 showed that adolescent rats exhibited good extinction retention if fear was acquired before adolescence but impaired extinction retention for fear learnt during adolescence. Further, fear acquired in adolescence could be successfully extinguished in adulthood (Experiment 2) but not if extinction occurred up to two weeks after conditioning (but still within the adolescent developmental period; Experiment 3). The age of the fear memory was not the main determinant of the impaired extinction retention in adolescence because fear acquired in late adolescence could be successfully extinguished two weeks later in adulthood, but not if extinction occurred the following day (Experiment 4). Finally, adolescent rats did not show extinction-induced increases in pMAPK expression in the medial prefrontal cortex (mPFC) or the basolateral amygdala (BLA) which were observed in juvenile rats (Experiment 5). This dampened prefrontal and BLA activity following extinction in adolescence occurred even if the fear memory was acquired before adolescence (i.e., when there is no impairment in extinction retention). Taken together, these findings demonstrate that neither the animal's age at the time of fear acquisition or extinction determines whether impaired extinction retention is exhibited during adolescence. Rather, it appears that forming competing fear conditioning and extinction memories *in* adolescence renders this a vulnerable developmental period in which fear is difficult to inhibit. Furthermore, even under conditions which promote good extinction, the neural mechanisms underlying extinction in adolescence differed to those recruited in animals of other ages.
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EARLY LIFE STRESS ACCELERATES NEUROBEHAVIORAL DEVELOPMENT

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In the adult animal, stress predominantly leads to regressive changes in neural circuits, including loss or shrinkage of dendritic arbors and spines and suppressed neurogenesis. In young animals and humans, stress is associated with delayed somatic growth, reduced structural volume of hippocampal and frontal brain regions, and the later development of affective pathology. Based upon these profiles, it is often assumed that the effects of stress on the developing brain are the result of delays or incomplete neurodevelopment. Using a mouse model of early stress, fragmented maternal care, we provide strong evidence at both the neural and behavioral level, that early life stress actually accelerates neurobehavioral development in both males and females. Furthermore, using newly developed computer vision tools to track rodent home cage behavior, we have developed ethologically relevant measures of depression and find that this stress is associated with the development of anxiety and depressive-like behaviors and that female are more severely impacted than males. We can then track the rapid reversal of these symptoms with the newly identified and fast acting anti-depressant, ketamine. Together, these studies demonstrate that stress incurred early in life leads to more rapid, but potentially compromised, neural and behavioral maturation, which may ultimately manifest in the development of affective pathology. Such a model holds the potential to recast how we think about the impact of stress on the developing brain and how early life stress is treated.
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BEHAVIORAL EFFECTS OF MAGNESIUM OXIDE VERSUS MAGNESIUM L-THREONATE DIET IN ADOLECENT SPRAGUE DAWLEY RATS

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Magnesium is an essential mineral that serves as an intracellular cofactor in enzymatic reactions and has an important regulatory role of gating of the N-methyl-D = aspartate receptor which is involved in learning and memory processing. The purpose of this study was to assess the impact of Magnesium Oxide (MgO) versus Magnesium L-Threonate (MgT) during normal early postnatal development on anxiety-related behaviors in rodents. Newly weaned Sprague-Dawley rats, postnatal day (PD) 21, were fed a diet that either contained 0.1% MgO or 0.1% MgT. No significant differences were found for body weights or food intake across the study. Open field (OF) exploration of PD28 or PD35 rats resulted in a Diet X Gender interaction ($F = 9.46$, $p < 0.05$) wherein female rats on MgO spent more time moving around the OF but female rats fed MgT displayed more rearing frequency ($F = 4.11$, $p < 0.05$) and duration ($F = 3.83$, $p < 0.05$). There was a interaction of Diet X Age X Area within the OF Arena ($F = 2.66$, p0.05) wherein rats fed MgT spent more time sitting away from the walls than rats fed MgO, which increased from PD28 to PD35. Preliminary analysis of behavior in an acoustic startle (AS) chamber suggests that rats fed MgT tended to have a faster habituation curve to the startle stimulus and lower velocity of startle than rats fed MgO. Overall, MgT fed rats performed more investigative-like behavior and less anxiety-like behavior in the OF arena than rats fed MgO.
[MJN grant to LAB]

INFANT TEMPERAMENT, ATTENTION, AND FUNCTIONAL CONNECTIVITY, BUT NOT MATERNAL SENSITIVITY, PREDICT INFANT NEGATIVE AFFECT AT 5 MONTHS

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Early developing self-regulation is promoted by biological processes, as well as co-occurring socialization experiences (Kopp, 1989; Sameroff, 2010). We recruited 300 5-month-old infants and their mothers to participate in a longitudinal study of individual differences in the development of self-regulation across infancy and early childhood. During the lab visit, infants and mothers engaged in two positive interaction tasks (toy play, peek-a-boo) and two negative interaction tasks (toy removal, arm restraint). Tasks were conducted in standardized order, with the intent of capturing maternal interaction style during positive interactions and then using the negative interaction tasks to induce distress in the infants. Immediately after the arm restraint task, the experimenter played a Sesame Street video clip for the infants. Independent research assistants coded infant negative affect during the video, infant looking behavior during the video, and maternal sensitivity and positive affect during the positive interaction tasks. Infant EEG was recorded throughout the visit, with a focus on frontal/parietal EEG coherence during the video. Prior to the visit, mothers completed the Infant Behavior Questionnaire-revised. The six predictors (left frontal/parietal coherence, right frontal/parietal coherence, IBQ distress, maternal sensitivity, maternal positive affect, infant attention to video) collectively accounted for 21% of the variance in infant negative affect during the video after the distress inducing tasks. IBQ distress (beta = .16), left hemisphere coherence (beta = -.13), and infant looking behavior (beta = -.38) contributed unique variance. These data imply that maternal interaction style had little impact on the level of negative affectivity in early infancy.
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MATERNAL RELATIONSHIP REPRESENTATIONS AND PRESCHOOLERS' PHYSIOLOGICAL RESPONDING DURING A RELATIONSHIP DISRUPTION

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Previous research has established the importance of physiological regulation in response to stress as a predictor of child outcomes, but no prior study has examined the effect of maternal relationship representations on child physiological regulation. Thirty-seven typically developing preschool-aged children ($M = 42.47$ months, 21 Males) and their mothers completed a Play/No-Play Paradigm (PNPP) in the laboratory. The PNPP mirrored the double/modified still-face paradigm (SFP) used with infants, but involved 2-min episodes of structured play (A), followed by maternal termination of play (B), that followed an A-B-A-B-A format. Heart rate and vagal tone were acquired from the children throughout the paradigm. Each mother also completed a questionnaire measure about romantic partner satisfaction and gave a five-minute speech sample (FMSS) about her child, from which maternal expressed emotion was coded.
Across the PNPP, children's heart rate significantly increased during the 'no-play' episodes and decreased during the 'play' episodes. Vagal tone remained stable across the paradigm. Individual differences in maternal expressed negativity in the FMSS were significantly associated with children's vagal suppression in the first 'no-play' episode and the final 'play' episode of the PNPP. Maternal Positive Relationship Representations, a composite of maternal expressed positivity and romantic partner satisfaction, were significantly associated with augmented vagal tone in children in the second 'no-play' episode and the final 'play' episode. Hence, maternal positive relatonship representations were associated with better child physiological regulation. Negative maternal expressed emotion was associated with reduced physiological regulation in children during a novel preschool adaptation of the SFP.
[Groff Foundation support for undergraduate neuroscience research at Williams College]

NEURAL MIRRORING SYSTEMS IN AUTISM SPECTRUM DISORDERS

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Dysfunction in neural mirroring systems has been proposed to contribute to the social deficits observed in autism spectrum disorder (ASD). Atypical activity in this system, as reflected in attenuation of the EEG mu rhythm, has been demonstrated in several studies; however, normative patterns of activity have been evident in other ASD samples. The etiological and behavioral heterogeneity of ASD likely contributes to these findings. Our work suggests that atypical activity is associated with imitation in both clinical and typical populations, rather than representing a universal impairment while our current work explores differences in neural mirroring systems in genetically defined subgroups.

EXECUTIVE FUNCTIONS CONTRIBUTE TO ACTIVE RELATIVE TO PASSIVE PROCESSING DURING AN EPISODIC MEMORY TASK

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Episodic memory undergoes development throughout middle childhood (Ghetti et al., 2010) and is affected by frontal lobe and executive functioning development (Bjorklund, Dukes, & Brown, 2009). We examined the contributions of active (voluntary attentional control) and passive (absence of attentional resources) processing, as well as executive functions, to the episodic memory performance of 9- to 11-year-old children. Seventy children (33 girls) participated. The episodic memory task involved children recalling computer-presented images that were either clear (passive processing) or fuzzy (active processing). Performance on three executive function tasks was also assessed: a 2-back task (false alarm rate; working memory), a computerized number Stroop (RT mixed; inhibitory control), and a computerized version of the Wisconsin card sort (perseverative errors; set-shifting). The Peabody Picture Vocabulary Test IV was used as a proxy for IQ. Separate regression equations were used to examine recall performance of the fuzzy images and the clear images; predictors were verbal IQ (covariate), and performance on the working memory, inhibitory control, and set-shifting executive function tasks. The predictors accounted for 22% of the variance in clear (passive processing) recall performance, with only set-shifting contributing unique variance. The predictors accounted for 24% of variance in fuzzy (active processing) recall performance, with verbal IQ, set-shifting, and working memory contributing unique variance. Results suggest executive functions differentially contribute to active and passive processing of stimuli and later recall of those images.
[NIH grant HD049878 to MAB]

BDNF HISTONE ACETYLATION IN THE ADULT RAT MPFC IS ALTERED FOLLOWING EXPOSURE TO AVERSIVE MATERNAL CARE

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Early-life stress in the form of caregiver maltreatment has the capacity to alter subsequent neurobiological and behavioral development. Maltreatment early in life can confer susceptibility or resilience to psychiatric disorders, and animal models have demonstrated emotional and cognitive deficits in offspring that experienced early-life stress. The mechanisms behind these phenomena are still unknown, but evidence suggests that epigenetic modifications may play a role. Previously, our lab demonstrated that aversive care during infancy alters methylation of the *brain derived neurotrophic factor* (*bdnf*) gene (Blaze et al., 2013), as well as mRNA expression of epigenetic regulator genes (Blaze and Roth, 2013) in the medial prefrontal cortex (mPFC). While we have well-characterized *bdnf* DNA methylation after our caregiver manipulations, this study aimed to characterize patterns of histone acetylation at *bdnf* promoters. Using a within-litter design, pups were exposed to an adverse (maltreatment condition) or nurturing (cross-foster condition) caregiving environment outside the homecage for 30 minutes each day during the first postnatal week. Remaining pups in a litter were left with the biological mother during each session (providing normal care controls). At PN90, we used chromatin immunoprecipitation (ChIP) and quantitative RT-PCR to measure acetylation of histone 3 lysine 9/14 (H3K9/14, found in actively transcribed promoters) at *bdnf* exons I and IV in the mPFC. Results indicate that in addition to DNA methylation, our caregiver manipulations alter H3K9/14 acetylation in a sex- and *bdnf* exon-specific manner. Future work will examine histone modifications at other genes important in brain development and plasticity, including *reelin*.
[NIGMS 1P20GM103653]

CHRONIC COCAINE EXPOSURE DURING ADOLESCENCE INCREASES IMPULSIVE CHOICE AND ALTERS RESPONSE-REINFORCER COUPLING IN MICE

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Adolescence is characterized by profound changes in the nervous system, increased susceptibility to drugs of abuse, and heightened levels of impulsivity. In the current study, 21 C57BL/6 mice were exposed chronically to 30 mg/kg/day cocaine ($n = 11$) or saline vehicle ($n = 10$) for 14 consecutive days during adolescence. In experiment 1, mice were trained on an impulsive-choice procedure in which they could choose between two levers that resulted in either a small, immediate presentation of milk or a larger presentation of milk after a series of delays (1-71 sec). In experiment 2, responding was placed under seven fixed-ratio (FR) schedules (FR 1-590) and individual response-rate functions were analyzed using a model called Mathematical Principles of Reinforcement. Cocaine-exposed mice were more impulsive (i.e., chose smaller-sooner milk reinforcement more often) than controls. Further, the rate parameter (λ) was higher for cocaine compared to saline mice, suggesting the coupling of responses distal to reinforcement was diminished. These results support the notion that adolescence is a vulnerable period and that pharmacological insults during this epoch have far-reaching effects.

MATERNAL CARE PROGRAMS ESTROUS CYCLE-MEDIATED AFFECTIVE BEHAVIORS IN THE FEMALE RAT

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In women, both depression and anxiety are correlated with menstrual cycle stage. Similarly, in the rodent, levels of locomotive, anxious, and depressive-like behaviors vary across the estrous cycle. In both species, a drop in plasma progesterone precedes the onset of behavioral changes. Progesterone's metabolite allopregnanolone may contribute to these behaviors. Licking/grooming (LG) received by rat dams during the first week of life differentially programs the female hypothalamo-gonadal-pituitary axis, affecting progesterone levels. Low LG offspring show a greater difference in progesterone between proestrus and metestrus than High LG offspring. Given that low parental care is predictive of offspring affective disorders, we hypothesized that Low LG offspring would show increased anxiety, locomotion, and depressive-like behavior, and exhibit greater variability in behaviors between estrous cycle stages relative to High LG offspring. Adult offspring of Low and High LG mothers were tested at proestrus and metestrus on the forced swim test and locomotor activity chamber. Animals were also tested on the elevated plus maze following treatment with finasteride, which blocks conversion to allopregnanolone, or placebo. LG phenotype shaped cycle-related patterns in locomotor activity. Only Low LG offspring showed cycle-dependent depressive-like behavior. Surprisingly, while High LG offspring showed decreased anxiety at metestrus, Low LG animals increased anxious

behavior at proestrus. Finasteride decreased general activity and removed all behavioral differences. Findings from this research link early life experience to estrous cycle-dependent changes in affect, and demonstrate that variations in maternal care mediate allopregnanolone-modulated behaviors.

MATERNAL BEHAVIOR IS MEDIATED BY BRAIN VASOPRESSIN AND CORTICOTROPIN-RELEASING FACTOR – OLD NEUROPEPTIDES WITH NEW FUNCTIONS

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Maternal behavior is the most important pro-social female behavior. Its establishment requires remarkable brain adaptations peripartum like increased activity of the brain oxytocin system. Recently, we characterized the brain vasopressin and corticotropin-releasing factor systems as important mediators of the fine-tuned regulation of maternal care and maternal aggression against an intruder. While vasopressin acts pro-maternal via its V1a and V1b receptors, corticotropin-releasing factor (CRF) facilitates maternal neglect via CRF recptors typ 1 and 2. All of these receptors are expressed in two of the main maternal brain areas, the medial preoptic area and the bed nucleus of the stria terminalis. In either brain region, local manipulations of those receptors lead to modulations of maternal care and/or maternal aggression. Hence, to increase our understanding of how maternal behavior and, consequently, the relationship between mother and infant is regulated provides important insights into possible dys-regulation of maternal attachment as seen, for example, in postpartum mood disorders.
[DFG BO 1958/6-1]

ALCOHOL EXPOSURE ON POSTNATAL DAYS 7-9 DISRUPTS OBJECT-IN-PLACE LEARNING AND TRACE FEAR CONDITIONING IN JUVENILE RATS

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The object-in-place (OiP) task requires subjects to associate an object's identity with its location and therefore combines the task demands of standard novel object recognition (OR) and object location (OL) paradigms. Lesion studies suggest that the OiP task engages the prefrontal cortex (PFC) and the hippocampus (HPC) (Barker et al., 2007; Barker & Warburton, 2011). Both of these regions are sensitive to developmental alcohol exposure (PFC: Whitcher & Klintsova, 2008; HPC: Marino et al., 2004). In the present study, Experiment 1 examined normative rat performance on 2-Object (Ainge & Langston, 2012) and 4-Object (Barker et al., 2007) variants of the OiP task. Experiment 2 sought to determine the effect of neonatal alcohol exposure, during postnatal days (PD) 7-9, on OiP performance on PD26 and on trace fear conditioning (TFC) on PD30-31, which served as a positive control due to its similar reliance on PFC (Gilmartin & Helmstetter, 2010) and HPC (McEchron et al., 1998). We report that normative PD26 rats can perform the 4-Object, but not 2-Object, variant of the OiP task. Additionally, early-life alcohol exposure during PD7-9 disrupted performance on both the 4-Obj variant of OiP and TFC, but not background contextual conditioning during TFC. Our findings suggest that these tasks may rely on similar neural regions and/or mechanisms. Moreover, our OiP task findings extend our lab's report that PD7-9 alcohol exposure fails to disrupt OR and OL performance (Jablonski et al., 2013) by suggesting that combined but not separate processing of OR and OL is disrupted by alcohol.
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SPECIFICITY IN THE BILINGUAL ADVANTAGE FOR MEMORY DURING INFANCY

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Recent studies have demonstrated a link between bilingualism and enhanced memory generalization abilities during infancy (Brito & Barr, 2012; 2014; Brito, Sebastián-Gallés, & Barr, under review). The present study assessed (1) whether this advantage was a global enhancement of memory processes by testing infants on a variety of tasks tapping working memory, cued recall and memory generalization, and (2) how task performance compares across infants exposed to different numbers of languages. In addition to the memory tasks, parent-infant dyads completed a picture-book reading task, to observe emotional responsiveness, and a parental report of productive vocabulary. Our sample included 18 infants in the monolingual group, 18 infants in the bilingual group, 14 infants in the trilingual group, and 14 monolingual infants in the baseline control group (32 male, 32 female; M age = 24.50 months, SD age = .39). Results indicated no difference between language groups on cued recall ($p = .58$), working memory ($p = .85$), emotional responsiveness ($p = .39$), or productive vocabulary ($p = .07$), but a significant difference was found in the memory generalization condition ($F(2, 60) = 3.73$, $p = .031$, $\eta\rho^2 = .14$), with only the bilingual group outperforming the baseline control group. These results support and extend past studies (Brito and Barr, 2012; 2013; Brito et al., under review) and suggest a bilingual advantage specific to memory generalization.
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LONG-TERM RETENTION OF EYEBLINK CONDITIONING IN POSTNATAL DAY 24 AND ADULT RATS

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Eyeblink conditioning is a well-established model for studying the developmental neurobiology of associative learning. In rats, eyeblink conditioning emerges over postnatal days (P) 17-24 when standard peripheral cues (e.g., tones, lights) are used as conditioned stimuli (CS), with acquisition reaching adult-like levels by P24. While there have been numerous advances in identifying neural mechanisms underlying the ontogenetic emergence of eyeblink conditioning, developmental differences in long-term retention have yet to be studied. The present study examined retention of eyeblink conditioning in P24 and adult rats 1, 7, or 28 days after acquisition. Retention was assessed by (1) a CS-alone testing session, and (2) CS-US reacquisition, consistent with a previous study from our laboratory investigating retention in adult rats [Nicholson, Sweet, and Freeman (2003), *Behavioral Neuroscience*, 117, pp. 871-875]. Acquisition and short-term retention (1 day after training) were comparable across ages, but developmental differences were evident with longer retention intervals. There were fewer CRs early in CS-alone testing 7 days after training in P24 relative to adult rats, but CR levels were equivalent across ages during reacquisition testing. In groups tested 28 days after training, however, P24s showed fewer CRs relative to adults during initial blocks of both CS-alone and CS-US testing, consistent with the rapid forgetting observed in younger age groups characteristic of the infantile amnesia phenomenon. The well-characterized neural circuit underlying eyeblink conditioning may provide a good starting point for future studies investigating the neural mechanisms responsible for rapid forgetting in young rats.
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CONTRIBUTIONS OF FRONTAL-PARIETAL COHERENCE AND MATERNAL CAREGIVING TO CHILD EXECUTIVE FUNCTION

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Previous research has demonstrated that early maternal positive affect and infant resting frontal brain activity predict future executive function (EF; Kraybill & Bell, 2013) and that 4.5 year old children exhibit increases in frontal-posterior coherence relative to baseline during executive processing (Bell & Wolfe, 2007). To further enrich our understanding of factors that promote EF development, the present study examines the relationship between maternal caregiving at 36 months, frontal EEG coherence during executive processing at 48 months, and corresponding EF task performance at 48 months. At 36 months, a group of mother-child dyads ($n = 155$) completed age-appropriate puzzles while caregiving behaviors were coded. EEG was recorded while children completed short-term memory and visual attention tasks at ages 36 and 48 months. Hierarchical regression analyses controlled for maternal education (a correlate of socioeconomic status and verbal intelligence), 48-month child verbal ability, and 36-month child EF, which accounted for 4.5%, 1.9%, and 1.6% of 48-month EF task performance, respectively. Maternal caregiving accounted for an additional 2.7% of the variance in 48-month EF and, together, frontal-parietal and inter-frontal coherence accounted for an additional 4.8% of the variance in 48-month EF. The final model accounted for 15.6% of variance in 48-month EF, with maternal caregiving and frontal-parietal coherence being the only unique predictors. These findings suggest that maternal caregiving characteristics such as positive affect and facilitation of infant attention and increases in EEG coherence between frontal and parietal areas during executive processing are linked with higher EF at 48 months of age.
[NIH grant HD049878 to MAB]

GESTATIONAL EXPOSURE TO ABUSE LEVELS OF INHALED TOLUENE DISRUPTS ACQUISITION OF WATER T-MAZE LEARNING BUT NOT IN A SUBSEQUENT REVERSAL TASK

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Toluene is a commonly abused solvent posing significant risk of exposure to females during reproductive years. Toluene inhalation during pregnancy results in exposure level-dependent developmental pathology including low birth weight, structural anomalies, behavioral problems, impaired cognition and memory: a "Fetal Solvent Syndrome". The full impact of prenatal toluene on learning and memory is as yet unknown. We assessed effects of gestational exposure of rats to toluene vapor on acquisition of T-maze performance and their ability to learn a reversal paradigm. High-concentration, brief and repeated exposures mimic typical inhalation patterns in toluene abuse. Timed-pregnant Sprague-Dawley rats were assigned randomly to one of four groups: 0, 8000, 12000, or 16000 parts per million (ppm) toluene for 15 mins/ twice a day from gestational day 8 (GD8) through GD20. On PN42, food-deprived pups were placed in the T-maze and trained to choose a baited arm. After the animal successfully selected the correct arm five times without error, the bait was switched to the other arm. After animals successfully acquired the reversal, the baited arm was again switched. The maximum number of trials was 30 per arm. A Kaplan-Meier Survival Analysis revealed that prenatally exposed animals displayed a "dose-dependent" impairment in acquisition of the task, but did not differ in reversal. The results suggest that toluene abuse in pregnancy can compromise performance in a learning task by offspring, although whether prenatal toluene exposure acts to affect learning, stress or responses to novelty, or memory, is not known.
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LATERALIZED ASYMMETRIES IN THE MANIFESTATION OF 'ACTIVE' AND 'REJECTING' TYPES OF UNIMANUAL MANIPULATIONS DURING INFANCY

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Kimmerle et al. (1995) suggested that between ages 6 and 11 months unimanual manipulation are a significant part of infants' manual repertoire. They found no significant change in the relatively high frequency of various types of manual manipulations from 7 to 11 months and proposed that the skill of unimanual manipulation remains a stable component of the manual repertoire during this period. The current study examined the development of different unimanual actions from 6 to 14 months. Unimanual manipulation patterns for 90 (57 males) normally developing infants were examined monthly from 6 to 14 months of age. Infants were divided according to their hand preference for acquisition (right, left, and no preference, 30 in each group). Handedness for acquiring objects was determined based on monthly observations using a valid assessment of handedness (Michel, Ovrut, & Harkins, 1986). Unimanual manipulation was assessed by simultaneously placing two identical items in the infant's hands for 17 different pairs of toys. The frequency of "active" unimanual actions performed by each hand (shaking, hitting, scraping, mouthing, clacking, picking up, rotating, and taking) were distinguished from the "rejecting" unimanual actions (dropping the object or refusing to grasp it) from video recordings of each monthly visit. Infants who had a hand-use preference for acquiring objects performed more "active" unimanual manipulations during the 6 to 14 month period (but not before) than non-lateralized infants. Infants with a hand-use preference for acquiring objects also performed fewer "rejecting" unimanual actions at all ages. Results are discussed according to the notion of embodied cognition.
[NSF grant DLS 0718045 to GFM and NIH T32HD007376 to Julie Campbell]

HOW HAND-USE PREFERENCES FOR REACHING CASCADE INTO HAND-USE PREFERENCES FOR UNIMANUAL MANIPULATION

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The progressive lateralization theory of handedness (Michel, 2002) predicts that early reaching behaviors during infancy should concatenate into a later preference for unimanual manipulations. To test this theory, unimanual manipulation patterns for 90 normally developing infants were examined monthly from 6 to 14 months of age. Infants were divided according to their hand preference for acquiring objects (30 each of Right, Left and No Preference). Unimanual manipulation was assessed monthly by placing two identical toys in each of the infant's hands using 17 pairs of toys. The frequency of the actions performed by each hand (shaking, hitting, scraping, mouthing, clacking, picking up, rotating, and taking) were analyzed from videos for each monthly visit. The results show that unimanual manipulation is a frequent occurrence during the 6 to 14 month period but declines somewhat by 12-13 months. However, a hand-use preference for unimanual manipulation only begins to appear by 11 months, after the manifestation of a hand-use preference for acquiring objects. By 14 months, infants preferring their right hand for acquisition also prefer their right hand for unimanual manipulation and those preferring their left hand also prefer their left hand for unimanual manipulation. At 11 months, right and left preference groups are different from one another. By 14 months, all three groups differ from one another in their manipulation preference. Identification of a preference by 12 months, but not at earlier months, supports the theory that a preference for unimanual manipulation derives from an earlier preference for acquiring objects.
[NSF grant DLS 0718045 to GFM and NIH T32HD007376 to Julie Campbell]

INFANT GAZE BEHAVIOR AND PHYSIOLOGICAL REGULATION WHEN CONFRONTED WITH THE MATERNAL STILL FACE

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The regulatory function of infant gaze behaviors (gaze at mother, distal scanning, and proximal/focused gaze) during the maternal 'still-face' episodes of the modified/double still-face paradigm (SFP) were examined. The associations between these gaze behaviors and infant biobehavioral

responding (vocal distress, autonomic, and neuroendocrine) and maternal report of temperament were explored. Forty-seven mothers and their healthy, full-term infants (30 males) participated in the SFP when their infants were six months old. The proportion of time infants gazed at mother, scanned, and focused on proximal objects or the self, and duration of vocal distress were coded using Noldus Observational Software. Heart rate and vagal tone were acquired throughout the SFP. Saliva was collected from the infants immediately before (baseline) and 15- and 30-minutes after the SFP and later assayed for salivary cortisol concentration.
Higher proprotion of time spent in focused gaze during the 'still-face' episodes was significantly associated with: less vocal distress; less cardiac reactivity; augmented vagal tone; and maternal reports of less proneness to distress and better self-regulation. More time spent gazing at the 'still-faced' mother was significantly associated with: more vocal distress; increased cardiac reactivity; and vagal suppression. More time spent scanning was significantly associated with: less vocal distress, but vagal suppression; higher basal cortisol levels; and maternal reports of increased proneness to distress. Infants who were able to periodically shift gaze away from the 'still-faced' mother and visually focus on proximal objects or the self showed a robust biobehavioral profile consistent with effective regulation, across multiple levels of measurement.
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GLUCOCORTICOID RECEPTOR EXPRESSION IS ASSOCIATED WITH GENERALIZED FEAR OF NOVELTY IN ADULT MALE SPRAGUE-DAWLEY RATS

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Behavioral inhibition (BI) is characterized by fear of novelty. Several rodent models demonstrated that, like humans, rodents exhibiting BI-like behavior have elevated basal corticosterone, stress-induced hypothalamic-pituitary-adrenal (HPA) axis hypersensitivity, and increased stress-induced hippocampal neuronal activation. Mineralocorticoid and glucocorticoid receptors (MR & GR) in the hippocampus regulate behavior and HPA axis activity and chronic corticosterone exposure impairs HPA axis regulation resulting in elevated serum corticosterone. Augmented amygdalar and hippocampal corticotropin releasing hormone receptor 1 (CRHR1) activity also facilitates fear behavior. We hypothesized that HPA axis regulatory gene expression is associated with behavioral predisposition (BI) that is defined by a generalized fear. BI rats, that approach novelty slowly across different contexts, were predicted to exhibit downregulated hippocampal GR and MR mRNA expression and upregulated hippocampal and amygdalar CRHR1 mRNA expression relative to NON-BI rats. Thirty male rats were classified according to behavior in two exploration arenas with novel objects or a novel conspecific at postnatal day (P) 70 and gene expression was assessed on P135 with quantitative real-time polymerase chain reaction. Increased latencies to approach novelty in both arenas (i.e. BI) were associated with reduced hippocampal GR mRNA expression. Hippocampal GR mRNA expression was positively associated with hippocampal CRHR1 and MR mRNA expression. No temperament differences in hippocampal CRHR1, MR, or amygdalar CRHR1 mRNA expression were observed. The current study supports previous findings of BI-related hippocampal and HPA axis dysregulation in response to stress and adds to our knowledge of potential molecular mechanisms that underlie BI by implicating reduced hippocampal GR availability.

EXPERIMENTALLY-INDUCED ASTHMA SYMPTOMS DURING ADOLESCENCE CAUSE ADULT ANXIETY-LIKE BEHAVIOR AND DECREASED SEROTONIN TRANSPORTER EXPRESSION IN A MOUSE MODEL

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Human and animal studies have shown that physical challenges during adolescence can have significant influences on behavioral and neurological development associated with internalizing disorders such as anxiety and depression. Given the prevalence of asthma during adolescence and increased rates of internalizing disorders in humans with asthma, we used a mouse model to causally test which symptoms of adolescent allergic asthma (lung inflammation or bronchoconstriction) affect adult anxiety- and depression-related behavior and brain function (elevated plus maze open arm time, sucrose preference, and brainstem serotonin transporter mRNA expression). To mimic symptoms of allergic asthma in young Balb/c mice (postnatal days [PND] 5-57), we induced lung inflammation with repeated intranasal administration of house dust mite extract (most common aeroallergen to induce allergies in humans) and bronchoconstriction with aerosolized methacholine (non-selective muscarinic receptor agonist). Three experimental groups were studied: (1) "Lung Inflammation", allergen exposure 3 times/week, (2) "Bronchoconstriction", methacholine exposure once/week, and (3) "Lung Inflammation and Bronchoconstriction", allergen and methacholine exposure on same schedules. Compared to controls, mice that experienced methacholine-induced bronchoconstriction during adolescence displayed a 30-50% decrease in elevated plus maze open arm time 3 days after the final methacholine treatment (PND 60) and a 25-30% decrease in brainstem serotonin transporter mRNA expression 2 weeks later and 3.5 months later (PND 75 and 165). The influence of allergen-induced lung inflammation was not significant. This is the first time that a clinical symptom of adolescent asthma (bronchoconstriction) has been shown to have a potentially long-term causal influence on anxiety-related brain and behavior development.
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NEONATAL EXPOSURE TO ANTIDEPRESSANTS CAUSES INCREASED FEAR LEARNING IN INFANT RATS

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Approximately 10% of pregnant women take selective serotonin reuptake inhibitors (SSRIs) for the treatment of depression. Whilst studies show that these medications can increase the effectiveness of 'talking' therapies and reduce the risk of relapse, it is concerning that SSRIs and their metabolites cross the placental barrier. Animal studies have recently shown that neonatal SSRI exposure causes the offspring to express increased anxiety- and depression-related behaviours in adulthood. In the current experiment we examined whether this adult phenotype is associated with abnormalities in emotion regulation early in life. The results show that infant rats neonatally exposed to Fluoxetine (i.e., Prozac), which is the most commonly prescribed form of SSRI, had higher levels of conditioned freezing to a discrete cue when tested 1 day after training compared to controls. However, both Fluoxetine-treated and control animals exhibited rapid forgetting of this association, showing low levels of CS-elicited fear two weeks later. These results suggest that animals that have been neonatally exposed to SSRIs show an increased propensity to learn fear

associations during infancy. Nevertheless, mechanisms that protect against anxiety at this developmental stage (i.e., rapid forgetting) appear to remain intact. Future experiments will investigate whether this enhanced conditioning is due to associative learning and whether it is seen across the lifespan. The results of these experiments may assist health professionals and mothers to more accurately weigh the benefits of medicating for prenatal depression with the potential costs.
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HOW CHILDREN LEARN TO OPEN CONTAINERS

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Containers are pervasive and common in every culture. We examined how young children learn to open containers as a model system for understanding how children develop the necessary skills to manipulate the tools of their culture.
Opening a container requires critical perceptual-motor skills: Children must recognize important features of the container (e.g., closure type) and perform the necessary opening actions in the appropriate sequence (e.g., twisting actions for screw caps). In Study 1, we asked 12- to 54-month-old children to open screw-cap containers that varied in diameter. Twelve-month-olds displayed a variety of futile actions that failed to open the containers, and 18-month-olds performed the appropriate twisting actions but still failed. By 24 months of age, infants successfully opened the containers $M = 76\%$ of the time. Between 18-30 months of age, the larger and smaller containers proved most difficult to open; children knew the required actions but could not implement them effectively.
How do children learn these skills? In everyday life, mothers often "scaffold" children's activities by providing physical and verbal assistance or instruction. In Study 2, mothers taught their 12- to 54-month-old children to open Tupperware containers. Mothers' scaffolding reflected children's skill at opening and general comprehension level: Mothers of younger children spontaneously exhibited more gestures and modeling of the required pulling action ($M = 1.7$ actions/minute), and mothers of older children used more action words like "pull" and "harder" ($M = 4.6$ words/minute)—replacing physical instruction with verbal as children become more receptive to verbal communication.
[NICHD R37-HD03348 to KEA][IHDSC Seed Award to CTL and KEA]

A CRITICAL ROLE FOR NUCLEUS ACCUMBENS CIRCUITRY IN ISOLATION-INDUCED VOCALIZATIONS IN THE INFANT RAT

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Mammalian infants vocalize when socially isolated. Vocalization guides the return of the caregiver and thereby maintains an environment critical to the infant's survival. Although the role of the periaqueductal gray area (PAG) in these vocalizations is well-established, other aspects of the relevant neural circuitry remain under-studied. Here we report that output from the nucleus accumbens (Acb) is necessary for social isolation-induced vocalizations in infant rats aged postnatal day [P] 12-14. Local inhibition via infusion of the $GABA_A$ agonist muscimol (0.8 μg/side) of the Acb, but not the dorsolateral striatum, blocked isolation-induced vocalizations, an effect that persisted when isolation occurred in a cold (10°C) environment. Candidate neurocircuitry was examined with anterograde and retrograde tract tracers deposited into the Acb and PAG, respectively in pups at P12-14. A small direct projection from the Acb to the ventrolateral (vl) PAG and adjacent mesopontine reticular formation (MRt) was observed. More notable however was that major terminal fields of the Acb within the ventral pallidum, substantia innominata, lateral bed nucleus of stria terminalis, lateral hypothalamus, and the ventral tegmental area/substantia nigra pars reticulata overlapped with dense populations of neurons projecting to the vlPAG and MRN. In conclusion, Acb efferents are critical for social isolation-induced vocalizations in the infant rat and may exert this effect via modulation of basal forebrain and midbrain inputs to the vlPAG and MRN. These findings highlight a possible anatomical macrosystem mediating the mammalian infant response to social separation and, more generally, to the development of social attachment.

THE RELATIONSHIP BETWEEN INFANT TEMPERAMENT AND MATERNAL AND INFANT PHYSIOLOGICAL STRESS

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The hypothalamic-pituitary-adrenal (HPA) axis is immature at birth. This stress regulatory system continues developing across the first years of life, coincident with the development of temperament. The current study examined mother and infant cortisol levels in relation to temperament domains: surgency, effortful control, and negative affect. Mothers reported on infants' temperament ($N = 53$; 5.5-6.9 months) using the Infant Behavior Questionnaire-Revised (IBQ-R). Maternal and infant salivary cortisol was collected at waking and bedtime on three days. Average cortisol slope, indexing diurnal cortisol change, and area under the curve with respect to ground (AUC)g, indexing total cortisol exposure, were calculated. Maternal cortisol was correlated with infant surgency and effortful control; mothers with a flatter diurnal slope had infants who were higher in surgency, $r = -.27$, $p = .05$, suggesting an association between maternal stress dysregulation and higher infant positive affect and activity level, while mothers with smaller AUCg had infants high in effortful control, $r = -.28$, $p < .004$, demonstrating that mothers with lower cortisol exposure had infants higher in self control. Negative affect was only correlated with infant cortisol; infants with larger AUCg, indicating higher cortisol exposure, exhibited more negative affect, $r = .27$, $p = 0.048$. Results suggest that temperament domains vary in their connections with specific maternal and infant cortisol measures. Further research examining the interplay of both systems and the mother's role in infant stress regulation is necessary to elucidate if either temperament or cortisol levels could act as a marker for the development of the other, or if both systems influence each other in a bidirectional manner.

OLD SKILL, NEW SKILL, LEARNING TRUE SKILL

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As the old saying goes, you never forget how to ride a bike. But what really happens to highly practiced skills after they are abandoned? We observed crawling in 27 11- to 12-year-old children and 13 adults who had not crawled in decades. We compared these "rusty" crawlers to two groups of habitual crawlers: 34 infants with 0.1-5.5 months of crawling experience, and a unique group of 5 Turkish adult siblings whose primary form of locomotion was crawling. Infants, children, and adults crawled over a laboratory walkway; Turkish adults were filmed during normal daily activities ($M = 7$ crawling sequences each). Coders scored the timing and placement of steps from video. Infants crawled faster with experience, $p < .05$. But surprisingly, lack of recent experience did not hinder older children: Normalized for body size, children crawled faster, $p < .01$, and

displayed astonishing forms of quadrupedal coordination—running with periods of two, three, and all four limbs in the air. Moreover, both groups of rusty crawlers spontaneously produced a variety of crawling patterns, including gaits unexpected in humans. They paced (moved limbs on same side of body together) in 30.6% of strides and used asymmetrical gaits such as galloping like a horse (12.3% of strides) and bounding like a rabbit (.8% of strides). In contrast, infants and habitual adult crawlers favored symmetrical, trot-like gaits that afford greater stability (71.7% of strides). Children and adults assembled amazing patterns of coordination on the fly. Habitual crawlers were less impressive in speed and flight time, but showed greater stability.
[NICHD R37-HD03348 to KEA]

BOUTS OF STEPS: THE ORGANIZATION OF INFANT EXPLORATION

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Adults primarily walk to reach a new location, but walking in infants serves an exploratory function. What process guides infants' locomotor exploration? We observed 30 13-month-old and 30 19-month-old infants during natural walking in a laboratory playroom. We characterized the bout structure of walking—when infants start and stop walking—to examine how infants use walking to explore and why they choose to initiate walking. Locomotor activity was largely composed of brief spurts of walking. Half of 13-month-olds' bouts and 41% of 19-month-olds' bouts consisted of three or fewer steps—too few to carry infants to a distant goal. Survival analyses of steps per bout indicated that the probability of continuing to walk was independent of the length of the ongoing bout; infants showed no bias towards bouts long enough to carry them across the room to a goal. However, 13-month-olds showed an increased probability of stopping after 1-3 steps, and did not initiate walking more often to compensate for their frequent short bouts. Moreover, most of infants' walking bouts did not end at a discernable goal: In 40% of bouts, infants simply stopped walking in the middle of the floor. We propose that infants' natural walking is not intentionally directed at distant goals; rather, it is a random process that serves exploratory functions. Relations between the bout structure of walking and other measures of walking suggest that random exploration is constrained by walking skill in younger infants, but not in older infants.
[NICHD R37-HD03348 to KEA]

THE DEVELOPMENT OF TOOL USE: PLANNING FOR END-STATE COMFORT

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Tool use requires planning an action sequence: grasping and implementing. Adults demonstrate *end-state comfort*: They plan their grasp so as to implement the tool comfortably and efficiently, even if it requires an uncomfortable initial grasp. Although infants show rudimentary means-end planning, young children fail to show end-state comfort in a variety of tasks. We tested 4-, 8-, and 12-year-old children and adults in four studies to investigate factors underlying children's failure to demonstrate sequential action planning in tool use. In Study 1, participants used a hammer to pound a peg. Four- and 8-year-olds showed less end-state comfort when the handle faced their non-dominant hand— "difficult" condition—than when it faced their dominant hand— "easy" condition; every adult and most 12-year-olds showed consistent end-state planning in both conditions. Four- and 8-year-olds showed substantial intra-individual variability in their initial grasp and implementation of the hammer. In Studies 2 and 3, participants used a spoon to feed themselves or to scoop beans into a bowl, respectively. In both studies, 4-year-olds rarely showed end-state comfort on difficult trials, indicating that Study 1's results were not due to unfamiliarity with the hammer. In Study 4, 4-year-olds were allowed to use only their dominant hand to hammer, resulting in a dramatic increase in end-state planning on difficult trials. Across studies, lack of end-state planning impeded implementation of the tool. Our findings indicate that the lack of end-state comfort in young children may be partly due to task design, not to inability to plan sequential actions.
[NICHD R37-HD03348 to KEA]

HOW MOTHERS TEACH CHILDREN TO OPEN CONTAINERS

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An important step for a child to become a member of society is to learn skills relevant to their culture. Containers are a common artifact across cultures, but the actions required to open them are not directly perceptible—making it a difficult task for young children. Thus, the task of opening containers presents a rich model system for studying how children learn culturally relevant skills in a social context. One way that children might learn these skills is through direct instruction tailored to their ability, i.e. *scaffolding*. This study examined how mothers spontaneously scaffold their children's ability to open containers. Forty-two mothers were asked to teach their children (aged 12-54 months) to open overcap containers—Tupperware that require a pulling action to open. Mothers displayed both physical (pointing to container contents, stabilizing the container, modeling the opening action, etc.) and verbal (encouragement, describing features of the lid, opening action, etc.) scaffolding strategies. Overall maternal scaffolding —both physical and verbal—decreased with children's age. Across age and for both types of scaffolding strategies, mothers of younger children focused more on directing children's attention to the task (pointing to treats inside the containers, shaking the containers, encouragement, and describing treats inside the containers) and simplifying the task (stabilizing the base or loosening the lid) than on providing information about the appropriate actions required for opening. These findings suggest that mothers recognize their children's skill level and vary the amount and type of scaffolding based on children's ability to complete the task and comprehend verbal instruction.
[NICHD R37-HD03348 to KEA; IHDSC Seed Award to CTL and KEA]

STOP, DROP, AND CRAWL: DO INFANTS SELECT APPROPRIATE FORMS OF LOCOMOTION BASED ON THE HEIGHT OF AN OVERHEAD BARRIER?

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Do infants perceive affordances for passage based on the constraints of various locomotor strategies relative to opening size? Furthermore, does experience with a mature locomotor strategy—walking—affect infants' strategy choice when passing under a low barrier that requires crawling? The current study examined whether infants select actions (walking, ducking, crawling) based on the height of an overhead barrier, and whether they modify gait effectively to avoid banging their heads. We tested 12-month-old experienced crawlers, 17-month-old experienced walkers, and a comparison sample of adults on a raised platform with an overhead barrier (the platform prevented infants detouring around the obstacle). An adjustable screen was raised and lowered to create various opening sizes: 130%, 90%, and 70% of standing height which permitted walking, shallow ducking, and deep ducking, respectively and 150% and 90% of crawling height, which required lowering the head and rump while crawling, respectively. In general, infants selected actions appropriate for the barrier height: They walked when the barrier was well above standing height, ducked to accommodate barriers just below head height, and crawled to fit through the openings that were too low for ducking. Interestingly, 17-month-old experienced walkers were less likely to belly crawl under the

lowest height than 12-month-old experienced crawlers and consequently bumped their heads more often. Adults rarely crawled at all except to pass under the lowest height, and they never bumped their heads at any height. These results suggest that as walking experience increases, willingness to revert to a less mature locomotor method decreases.
[NICHD R37-HD03348 to KEA]

INHERITING GUT FEELINGS: PROBIOTICS SAVE RATS FROM THE TRANSGENERATIONAL EFFECTS OF EARLY STRESS ON FEAR-RELATED BEHAVIOURS

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The adverse effects of early life stress (ELS) are pervasive, with mental and physical health consequences for both stressed individuals and their descendants. Previously, work from our lab has demonstrated that maternal separation (MS; a rodent model of ELS) alters the developmental trajectory of fear-related behaviours in two generations of male rats. In the current series of experiments, we show that these effects are attenuated by treatment with the probiotic compound Lacidofil®.
In the first generation, all rats were exposed to MS from P2-14. Throughout MS, either probiotic (Pro) or vehicle (Veh) was administered via the mother's drinking water. Replicating our previous findings, MS-Veh infants exhibited fear relapse and extended fear retention, an adult-like profile that may explain why these animals are prone to anxiety later in life. In contrast, MS-Pro infants exhibited age-appropriate resistance to relapse and infantile amnesia, similar to unstressed rats. Excitingly, probiotic treatment also normalised fear behaviours in the offspring of these animals, regardless of whether treatment was administered to their fathers (i.e., MS-exposed animals) or to the offspring themselves. These findings add to the growing body of research highlighting the importance of the brain-gut axis for mental health and emotional development. Further, they provide preliminary support for the use of probiotics to aid treatment of individuals affected by ELS and protect future generations against the consequences of ELS.
[Petre Foundation Award and UNSW Research Award to CSMC and an Australian Research Council grant (DP120104925) to RR]

EFFECTS OF BIRTH ORDER AND NUMBER OF SIBLINGS ON PERSONALITY AND STRESS RESPONSE

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Siblings have an important effect in the physiological and behavioral development of individuals: younger children develop different behavioral strategies compared with their older brothers. Even in developed societies the presence of older siblings has negative effects through the dilution of resources, especially in younger children with a slower growth rate. The impact of the presence of siblings on personality and stress response in environmental contexts where resources are scarce has been poorly studied. We have tested personality and stress response on 506 adolescents (261 males, 245 females) from a town whose income is below the national average (Ixtenco, Tlaxcala). The study encompassed three stages: first, we collected information about the family structure of each participant; then, we evaluated personality using the Big Five Questionnaire; and finally, we analyzed their coping style (proactive/reactive) in a stress situation (speaking for 3 minutes in front of a video camera). The sample had an age range from 11 to 16 years with a mean of 12.79 years (SD = 0.94); the number of siblings ranged from 0 (only child) to 9 (SD = 1.5). Regarding birth order, 5.9% of subjects are only children, 33.8% are firstborn and 60.3% are lastborn (SD = 1.59). There is a significant difference between the average grade: firstborns have higher average than subsequent children ($n = 307$, $p = 0.004$). The results of stress test indicate that firstborns are more fluent during speaking in front of the camera ($n = 60$, $p = 0.031$). Other behavioral variables associated with stress and personality are being currently analyzed.
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FUNCTIONAL CONNECTIVITY DURING AUDIO-VISUAL SPEECH INTEGRATION: COMPARISONS BETWEEN TD CHILDREN AND CHILDREN WITH ASD

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Children and adolescents with autism spectrum disorder (ASD) show deficits in perception of audio-visual (AV) speech (Irwin et al., 2011). Weaker integration of the auditory and visual speech signals significantly impairs the ability to recover or disambiguate a speaker's message. Little is known about ASD-related differences in the functional networks recruited during perceptual processing. Measures of functional connectivity (EEG coherence) provide information about the synchrony of activation at the scalp level of two underlying brain regions (Thatcher et al., 1987). In typically developing (TD) individuals, functional connectivity involving a fronto-temporo-parietal network (superior temporal sulcus) is linked with AV speech perception (fMRI: Dick et al., 2010). In the present study, EEG was recorded while 6- to 10-year-olds were presented with stimuli (/ba/ versus reduced /ba/ heard as "/a/") designed to be distinguishable auditorily but indistinguishable when visual information from a speaking face is available. Preliminary analyses [based on a subset, $n = 8$ (ASD), 10 (TD)] examine the gamma and beta frequency bands (Doesburg et al., 2008; Schepers et al., 2013). Left frontal-temporal gamma band coherence and right frontal-temporal beta band coherence were consistently higher for TD children (Ms = .44, .31) than children with ASD (Ms = .23, .13). Comparisons of functional connectivity during intact and reduced /ba/ stimuli indicate increased left/right frontal-parietal beta band coherence for TD children during reduced /ba/; whereas children with ASD exhibit no changes in beta band coherence and decreased left temporal-parietal gamma band coherence. These findings indicate potential differences in the functional connectivity related to AV speech integration.
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NEURONAL FIRING PROPERTIES OF THE RED NUCLEUS DURING SLEEP-RELATED TWITCHES AND WAKE MOVEMENTS IN NEWBORN RATS

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Sensory feedback from sleep-related twitches is thought to play an important role in driving activity-dependent development in the infant brain. However, little is known about the neural pathways involved in the generation of myoclonic twitches. The red nucleus (RN), the source of the rubrospinal tract, has been suggested to be involved in the production of twitches during active sleep in adult rats and cats. Here we hypothesized that the RN is also a major source of motor output for twitching in early infancy, a period when twitching is most prominently expressed. We recorded extracellular neural activity in the RN during sleep and wakefulness in unanesthetized, head-fixed rats at 4 and 8 days of age. Neural activity in response to exafferent stimulation of the pup's limbs was also measured. Neurons in the RN fired phasically during periods of twitching and wake movements, as well as in response to peripheral stimulation of the contralateral limbs. Unit activity showed a peak at least 20 ms before the onset of twitches and wake movements. Interestingly, some of the units in the RN also exhibited a peak of activity after twitch onset, suggesting reafferent sensory processing. The temporal relation between limb twitches and neuronal firing in the RN suggests that this area

plays a role in the generation of phasic motor activity during active sleep early in development. The phasic activation of RN neurons before and after twitches suggests that they contribute to the flow of information within a spino-cerebello-rubro-spinal loop.
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PARITY AND INFANT SEX INTERACT TO INFLUENCE MATERNAL CORTISOL IN PREGNANCY AND INFANT SOCIAL BEHAVIOR IN RHESUS MONKEYS

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Hypothalamic-pituitary-adrenal (HPA) axis activity is known to increase during pregnancy, and fetal exposure to high level of glucocorticoids is known to adversely affect subsequent infant neurological development. However, the influence of maternal HPA axis activity on the development of infant social behavior is not well understood. We studied 25 rhesus monkeys (*Macaca mulatta*; 13 primiparous, 12 multiparous) and their offspring (12 males, 13 females) to determine the influences of parity and infant sex on maternal hair cortisol concentrations (HCCs) during pregnancy and infant social behavior. Parity and infant sex significantly interacted to influence maternal HCCs during pregnancy ($F_{(1)} = 11.07$, $p = 0.003$), such that primiparous mothers of males had higher HCCs during pregnancy, while multiparous mothers of females had higher HCCs. The same pattern was observed for the frequency of social behaviors engaged in by infants between 4-6 months: males belonging to primiparous monkeys were more social whereas females belonging to multiparous monkeys were more social. Moreover, we observed a trend for maternal HCCs during pregnancy to positively predict infant social behavior ($R^2 = 0.187$, $p = 0.065$) after controlling for infant sex and parity. These findings suggest that fetal sex may influence chronic maternal HPA axis activity depending on the mother's parity, and that maternal HPA axis activity during pregnancy may program subsequent infant social behavior, which collectively have important implications for studies of the maternal-fetal relationship.
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CONSISTENT FAMILIAL WARMTH PREDICTS CHILD CORTISOL LEVELS

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Research from both animal models and humans has indicated that the quantity of nurturing (maternal care) offspring receive influences their Hypothalamic-Pituitary-Adrenal (HPA) development and function. Using the rodent model, reliability of maternal nurturing has also been shown to be influential in offspring HPA function and behavioral outcomes. Here, we attempt to conceptually translate these rodent findings to human children, and examine the relative contributions of both of these measures of nurturing to child HPA functioning. The present study included 55 children with mild/moderate asthma and caregivers from the NHLBI-funded randomized clinical trial (Childhood Asthma Management Program) who provided data for the analyses. Caregivers completed the Family Environment Scale (FES) at enrollment and annually. Familial warmth was calculated from two subscales of the FES ($Cohesion_{score}$ minus $Conflict_{score}$). Children's baseline and evoked cortisol levels were assessed at year three and utilized in the analyses. ANCOVAs revealed that variability in familial warmth across time, but not the amount of warmth, predicted child basal cortisol levels at year 3 ($F(1,49) = 5.758$, $p = .02$; $F(1,49) = .733$, $p = .396$ respectively). As familial variability in warmth increases, child baseline cortisol also increases. Consistency in family warmth, but not the amount of warmth, predicted child baseline cortisol levels, suggesting that predictability in family behaviors influence the development of child HPA function. These findings confirm previous empirical work investigating maternal care reliability in rodents and emphasize the need to look beyond a single time point or single measurement in order to obtain an understanding of contributions of the family environment to child biological regulation.
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CAREGIVER MALTREATMENT AND ITS EFFECTS ON CNS DNA METHYLATION AND FEAR-RELATED BEHAVIORS IN ADOLESCENCE

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Quality of maternal care is one of a myriad of factors contributing to the development of behavior and, when disrupted, possibly the later development of psychiatric disorders. Environmentally-driven epigenetic changes may be an important mechanism underlying these behavioral outcomes. One such change, DNA methylation, typically results in transcriptional repression via the addition of methyl groups to cytosines. Previous work from our laboratory has shown within the adolescent medial prefrontal cortex gene-specific alterations in methylation levels in response to early-life stress (brief and repeated exposures to caregiver maltreatment). Our goal here was to examine whether there are both gene-specific and genome-wide changes in other regions of the adolescent brain. Infant male and female Long Evans rats were subjected to either nurturing care (from their biological mother or foster dam) or maltreatment from a foster dam for 30 minutes daily from postnatal day (PN) 1 to PN7. We then investigated methylation of the *brain-derived neurotrophic factor* (*bdnf*) gene in the hippocampus (dorsal vs. ventral) and amygdala (homogenate of central, lateral, and basolateral nuclei) of these rats once they reached adolescence (PN30, at baseline conditions). Results indicate significantly higher levels of methylated *bdnf* DNA in the ventral hippocampus and amygdala of female maltreated-rats. We are currently investigating global methylation and hydroxymethylation levels in these rats to determine if there are also differences in these two forms of cytosines across the genome in maltreated-rats. We are also examining adolescent fear behaviors in a separate cohort to assess the possibility of deficits in acquisition or extinction of conditioned fear in maltreated-rats.
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CHANGES IN THE FUNCTIONAL ORGANIZATION OF THE NEOCORTEX FOLLOWING LESIONS TO VISUAL CORTEX EARLY IN DEVELOPMENT

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To understand the extent of recovery of the neocortex following insult, our laboratory has previously used the short-tailed opossum (*Monodelphis*

domestica) as a model organism to investigate cortical reorganization. Opossums are born extremely early in development, which has allowed us to investigate the effects of lesioning the caudal pole of cortex before thalamocortical afferents have innervated cortex (postnatal day 4, P4). When examined in adulthood, these lesions result in a rostrally-shifted, compressed representation of somatosensory and auditory cortex and a small, caudally-located visual area. Thalamocortical projections also shifted rostrally such that inputs from the major sensory nuclei correctly projected to their cortical targets.

The studies presented here demonstrate how plastic changes to the neocortex differ when cortex is bilaterally lesioned after thalamocortical axons have innervated the neocortex (P12 in opossums). Functional organization of the neocortex was accessed in adulthood using electrophysiological recording techniques. P12 lesioned animals do not show a compression or a rostral shift of sensory areas. Additionally, only a small portion of cortex in which neurons responded to visual stimulation was found at the caudal-most portion of remaining cortex in some cases. Somatosensory cortex had a normal topography, with body and forepaw representations located medially and the face representation located laterally within S1. These results indicate that the extent of cross-modal plasticity following cortical insult is severely restricted once thalamocortical afferents have innervated the cortical subplate. Importantly, the persistence of a visual cortex is highly dependent on when cortical injury occurs relative to the development of thalamocortical axons.

RELATIONSHIPS AMONG MATERNAL DEPRESSION, HEART RATE, AND HEART RATE VARIABILITY DURING PREGNANCY AND FULL-TERM BIRTH WEIGHT

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Many studies of maternal depression during pregnancy suggest that depression is associated with low birth weight. However, in those studies maternal depression is typically comorbid with other risk factors, including premature birth. In the research presented here, maternal depression was examined in low-risk pregnancies resulting in full-term births, and a different relationship is found. Data from four different cohorts were combined in which maternal heart rate (HR) and heart rate variability (HRV) was collected during a baseline period of rest. 227 pregnant women in their 36th week of pregnancy completed self reports of depressive symptoms, CES-D (Radloff, 1977). Maternal HR and HRV data were adjusted for differences between cohorts. Birth weight was adjusted for gestational age at birth and sex. A *positive* correlation between CES-D scores and birth weight was found ($p = .015$). Maternal HR was significantly higher in depressed pregnant women ($p < .001$) and high frequency HRV was significantly lower ($p = .004$). Birth weight was compared across maternal HR and HRV tertiles; women gave birth to heavier babies if they had higher HR ($p = .014$) and lower HRV($p < .001$). We hypothesize that elevated HR in depressed pregnant women is a potential mechanism for increased birth weight found in their newborns. Higher HR may indicate increased cardiac output and increased blood flow (oxygen and nutrients) to the fetus. This work suggests that a potential curvilinear relationship between maternal depression during pregnancy and birth weight warrants further exploration.

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EMBRYONIC ALCOHOL EXPOSURE IMPAIRS THE DOPAMINERGIC SYSTEM AND SOCIAL BEHAVIOURAL RESPONSES IN ADULT ZEBRAFISH

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The zebrafish is a powerful neurobehavioural genetics tool with which complex human brain disorders including alcohol abuse and fetal alcohol spectrum disorders may be modeled and investigated. Zebrafish innately form social groups called shoals. In the present work we build on our past research, which demonstrated that, a single exposure (24 hours post-fertilization) to low doses of alcohol (0, 0.25, 0.50, 0.75 and 1% v/v) for a short duration (2 hours) lead to impaired group forming, or shoaling in adult zebrafish. Using a similar dosing regime, in the current experiment we exposed zebrafish embryos to two concentrations of alcohol (0.5% and 1% v/v) for two hours, after 24 hours post fertilization. Once fish were approximately between the ages of 8 to 12 months we tested their response to an animated shoal to access shoaling. Using high-pressure liquid chromatography (HPLC) we measured dopamine and its metabolite DOPAC (3,4-dihydroxyphenylacetic acid). We found that embryonic alcohol exposure lead to exposed fish swimming at farther distances from the stimulus compared to untreated controls and that exposed fish had lower levels of dopamine and DOPAC in response to the stimulus presentation compared to controls. These findings suggest that zebrafish may become a useful translational tool for the analysis of the mechanisms behind the changes seen in fetal alcohol spectrum disorders.

THE EFFECTS OF EARLY SOCIAL EXPERIENCES ON THE DEVELOPMENT OF MIRROR NEURONS IN MONKEYS

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Our studies in monkeys and humans have investigated the issue of how mirror neurons (MN) emerge during development and which experiences could be critical for their formation. Electroencephalographic findings in newborn macaques showed that a mirror mechanism operates in the early stages of postnatal development and that early adverse social experiences affect its developmental trajectory. This mechanism therefore could be used as a marker of social skills in postnatal development with critical implications for psychopathologies. These studies provide an original account of basic aspects of social cognition, and offer new insights on the interactions between brain plasticity and early experience.

DYNAMIC NEUROPSYCHOLOGICAL EMOTION REGULATION PROFILES AMONG YOUTH, 10-22 YEARS OLD

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The ability of the prefrontal cortex (PFC) to appropriately modulate the amygdala(AMY) and ventral striatum (VS), key regions for affective, motivational and reward processing, is critical for normative emotional regulation (ER). Successful ER also depends on the hypothalamus-pituitary-adrenal (HPA) axis functioning during stress, as measured by cortisol[1]. Cortisol directly influences neural structures via glucocorticoid (GC) receptors[1], with chronically high concentrations of GCs dampening PFC and increasing AMY function[1]. Finally, adolescence is a critical period for both the development of AMY-PFC functioning/connectivity[2,6,7], and plasticity and possible recalibration of HPA functioning[13], making it an important window to assess changing relationships between ER and underlying neurobiology. Prior work in this area has relied heavily on ER paradigms using static, adult faces[8]. This developmental fMRI study ($N = 60$ neurotypical female youth, evenly distributed between 10-22 years of age) utilized a peer-matched, dynamic video affect-labeling paradigm. For measures of pubertal development and HPA response, we assayed salivary measures of testosterone, dehydroepiandrosterone-sulfate and cortisol. Because high levels of stress are known to adversely impact brain

development[1,2]and neuroendocrine functioning[3,4], all of which can have detrimental effects on behavioral outcomes[3], we also asked participants to report on adverse childhood experiences (ACEs). Preliminary results indicate that the dynamic peer affect-labeling paradigm reliability recruits AMY, ventral striatum (VS), and both ventromedial and lateral PFC. The results of this study will have important implications for future studies of high-risk youth in which we will examine whether cortisol reactivity (and recovery) during the affect labeling paradigm mediates the relationship between ACES and behavioral or neural ER profiles.
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FRENCH FRIES: HEALTHY OR UNHEALTHY? PREDICTORS OF CHILDREN'S ABILITY TO EVALUATIVELY CATEGORIZE FOODS

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Children begin to synthesize health-related knowledge about food from an early age. By the time they are 4 years old, they begin to cross-classify foods into taxonomic, script, and evaluative categories. The goal of the present study was to determine whether factors, such as children's BMI, food neophobia, and maternal feeding styles, predict children's ability to evaluatively categorize foods. Sixty six 3-8 year old children participated in a task in which they were asked to categorize a series of pictures of food as healthy or unhealthy. Additionally, mothers completed a series of questionnaires to assess their perceptions of their children's eating habits and their approaches to feeding her child. Results indicated that for girls, parental feeding styles that involved less restriction and pressure predicted more accurate evaluative categorization of the foods over and above the baseline predictor of age. For boys, neither levels of neophobia, nor maternal restriction or pressure produced significant increases in the proportion of variance explained by age. These findings suggest that while the use of controlling feeding strategies may not predict boys' evaluative categorization abilities, the use of these strategies with girls may be negatively associated with their ability to learn about the healthfulness of foods.

POSTNATAL PATTERNED VISUAL EXPERIENCE AFFECTS PERCEPTUAL DISCRIMINATION AND SOCIAL INTERACTION IN ZEBRAFISH LARVAE

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In recent years, zebrafish (*Danio rerio*) have emerged as a powerful animal model for studying the developmental mechanisms of early neural, physiological, behavioral, and social organization in vertebrates. Genetically regulated influences on species-typical developmental trajectories in this model organism are well documented. However, systematic explorations of how emergent sensory capacities and ontogenetic experiences affect developing neural, behavioral, and social systems are less prevalent in the literature. Reliable behavioral measures for studying the broad range of early adaptive capacities in zebrafish are still emerging, particularly during the larval and juvenile stages of development. Zebrafish visual capacities are known to demonstrate high degrees of plasticity during the embryonic, larval, juvenile and pre-adult developmental stages (days post fertilization, dpf: 1–42). In this study, we examined how altered visual experience affects early patterns of perceptual discrimination and social interaction in larvae and juveniles. Experimental subjects were exposed to three different types of enhanced patterned visual stimulation during the early stages of development (dpf: 5–30). Results suggest that young zebrafish can discriminate between distinct visual stimuli, and these early perceptual abilities are affected by the type of patterned visual stimulation encountered during development. Preliminary tests also reveal that early visual experience differentially affects perceptual preferences and social interaction patterns during early development, depending on the type of enhanced visual experience encountered. Zebrafish show excellent promise as a reliable animal model for exploring how dynamic variations encountered in early environments affect perceptual system functioning, behavioral adaptation, and neurobiological plasticity in organized systems.

DEVELOPMENT OF HAND-USE PREFERENCE FOR TOOL-USE IN INFANCY

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Tool-use is a complex manual skill that children master during the first two years of their life. According to the cascade theory of handedness development (Michel, 2002), hand-use preference for a manual skill becomes more prominent as the skill is being mastered. The goal of the current study was to understand the development of the tool-use skill, and hand-use preference for tool-use in a sample of sixty infants (20 right-handers, 20 left-handers, and 20 without a stable hand-use preference for object acquisition) tested monthly from 10 to 14 months. Both unsuccessful tool-use attempts and successful tool-use actions were recorded. The multilevel data analysis showed that, as infants developed, left-handers decreased the number of tool-use attempts while other infants slightly increased it. The number of tool-use actions increased significantly, and handedness for attempted tool-use became more lateralized with age in all infants. That is, infants with right hand-use preference or no preference for acquisition became more right-handed while attempting tool-use, whereas infants with left-hand preference for acquisition became more left-handed while attempting tool-use. In contrast, all infants preferred the right hand more with age for successful tool-use. We conclude that the skill of tool-use continues to develop during the second year of the child's life, and further change in hand-use preference for tool-use is expected during that period.
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FEEDBACK-BASED LEARNING IN THE ADOLESCENT STRIATUM

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Characteristic peaks in striatal sensitivity to feedback and reinforcement have been linked to heightened reward sensitivity in adolescents. However, less attention has been given to the role that heightened striatal sensitivity has in feedback-based learning. This talk will highlight recent fMRI research examining this question in a sample of adolescents and adults. Applying a reinforcement learning model to the data revealed ontogenetic differences in feedback-based learning and striato-hippocampal activation, with greater neural sensitivity in adolescents versus adults. These data suggest that unique neurodevelopmental changes during adolescence may facilitate the heightened learning that occurs during this significant developmental window.

SALIVARY ALPHA-AMYLASE AND BEHAVIORAL REACTIVITY TO STRESS IN INFANTS EXPOSED TO INTIMATE PARTNER VIOLENCE

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Research indicates that prenatal and postnatal exposure to stress may affect biobehavioral functioning in infancy. Two components of biobehavioral functioning are sympathetic nervous system reactivity and behavioral distress, but the two systems are not necessarily coordinated (e.g., Spinrad et al., 2009) and may serve distinct functions. The current study examined whether infants who were exposed to a specific type of stressful event, maternal exposure to intimate partner violence (IPV), prenatally and/or postnatally display distinct patterns of salivary alpha-amylase (sAA) and

behavioral reactivity compared to non-exposed infants when undergoing a laboratory stress task. Subjects were 182 mothers and their 12-month-old infants. sAA samples were taken before and after infants participated in an arm-restraint task designed to elicit frustration. Behavioral coding of the same task provided measures of infant emotion reactivity and regulation. Latent profile analysis was used to create profiles of infants' sAA and behavioral responses; a four-class solution emerged. Postnatal IPV and mother-reported internalizing symptoms predicted infant membership in a group characterized by high emotion reactivity, high emotion regulation, and moderate sAA reactivity. Nurturing parenting was associated with a profile characterized by moderate emotion reactivity, low emotion regulation, and moderate sAA reactivity. Thus, IPV and parenting influence infants' patterns of behavioral distress but are dissociated from their sAA responses. Further, the results indicate that sAA reactivity is not necessarily a marker of behavioral distress.

EARLY BRAINSTEM DYSFUNCTION AND 4-MONTH ATTENTION REGULATION ARE RELATED TO LATER DIAGNOSIS OF ASD IN NICU INFANTS

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We compared neonatal ABR abnormality, later regulation of attention [4-month arousal-modulated-attention (AMA) in visual preferences], and subsequent outcome risk [2-3 month performance (Bayley Infant Neurobehavioral Screener, BINS; Mullens Scales of Early Learning, MSEL)] in three groups: 1. ASD-diagnosed [two samples: previously reported $n = 28$; recent $n = 26$)], 2. Younger siblings of ASD (baby-sibs); NICU $= 22$; non-NICU $= 15$); and 3. overall NICU sample ($n > 2000$). Infants also were characterized by CNS injury before hospital discharge [ABRs in NICU; 4-month AMA: looking functions derived from pairs of 1-,3- and 8-Hz on/off illuminations presented under higher and lower arousal conditions]. More abnormal ABRs [invariably normalize] were related to ASD diagnosis in NICU infants (91% vs 25%). Both ASD sub-samples had lower gestational age, higher proportions of abnormal ABRs, but did not differ on any neonatal behaviors. At 4-months (adjusted), ASDs showed more looking at higher rates than rest of NICU population (effect size $r = 0.244$), and a small but significant increase in abnormal scores on the BINS (effect size $r = 0.278$). Baby sibs (NICU vs. non-NICU) did not differ from overall sample on proportion abnormal neonatal ABRs, arousal regulation (effect size $r = 0.025$), or increased early risk (BINS abnormality effect size $r = 0.021$). Disruption in early brainstem development appears associated with ASD. Lack of baby sibs' effects may be due to being too subtle at this age and/or small sample size. However, lower gestation at birth, higher proportions of initial abnormal ABRs, along with poorer arousal regulation after the neonatal period remain linked to ASD with increased sample sizes.
[Autism Speaks grant #7598 to JMG]

NEONATE RATS CRAWL TOWARDS VANILLA OR ETHANOL ODOR AFTER PRENATAL EXPOSURE TO THESE STIMULI: THE ROLE OF THE OPIOID SYSTEM

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Rat fetuses can perceive chemosensory stimuli derived from their mother's diet, and they may learn about those stimuli. In previous studies we have observed that prenatal exposure to ethanol the last days of gestation (GD17-20) increases acceptance of ethanol flavor in infant and adolescent rats. Whereas these results were not found after prenatal exposure to vanilla, cineole or anise, which suggests that the pharmacological properties of ethanol, mediated by the opioid system, underlie the effects observed with this drug. However, considering that other studies report enhanced acceptance of non-ethanol flavors experienced prenatally when subjects were tested before infancy, we explore the possibility of observing similar results if testing neonate rats exposed to vanilla during GD 17-20. Results of Experiment 1 show that rat neonates exposed prenatally to vanilla or ethanol ¿crawl¿ for a longer distance towards the experienced odor than control pups. In Experiment 2, ethanol or vanilla were administered prenatally with antagonists of kappa or mu opioid receptors. Blocking mu, but not kappa receptors, reduced responding to vanilla odor in pups exposed to vanilla. While the response to ethanol in neonates exposed prenatally to this drug was affected by both antagonists. Results confirm that exposure to a non-ethanol odor enhances neonatal response to it and that kappa opioid receptor system plays an important role in this effect. Results also suggest that with ethanol exposure the prenatal opioid system is wholly involved, which could explain the longer retention of the enhance response to ethanol after prenatal experience with the drug.

ACETALDEHYDE SEQUESTERING WITH D-PENICILLAMINE DECREASES OPERANT RESPONSE TO ETHANOL IN 5 DAY-OLD RATS EXPOSED PRENATALLY TO ETHANOL

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Ethanol exposure during the last days of gestation increases ethanol acceptance in neonate, in infant and in adolescent rats. Recent studies with neonate rats evidenced the crucial effect of acetaldehyde, the first metabolite of ethanol, in ethanol reinforcement at this early age. In this study we investigated the role of acetaldehyde on the reinforcing properties of ethanol exposure during the prenatal stage. Therefore, we administered dams during gestational days 17-20 with water, ethanol or ethanol + D-Penicillamine (DP) and tested the offspring on postnatal day 5 in an operant conditioning test. The test consisted in a 15-minute operant training phase (intraoral ethanol 3% as the reinforcer) followed by a 6-minute extinction phase. The results show that in the training phase, pups exposed prenatally to ethanol respond more to ethanol comparing to water-exposed pups and than pups exposed to ethanol and DP, while the latter group did not differ from the water control group. Similarly, on the extinction phase, only ethanol exposed pups showed an increased response on the first minutes, compared to pups from all remaining groups. In conclusion, results show that eliminating acetaldehyde after prenatal ethanol exposure reduces the postnatal positive response to ethanol, highlighting the important role of acetaldehyde on ethanol reinforcing properties.

DEVELOPMENTAL SSRI EXPOSURE DECREASES SEROTONIN AND 5-HIAA LEVELS IN THE PFC, BUT NOT THE HIPPOCAMPUS, OF PRENATALLY STRESSED JUVENILE OFFSPRING

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Selective serotonin reuptake inhibitor (SSRI) medications are the most frequently used antidepressant for maternal mood disorders during pregnancy and postpartum period. SSRIs cross the placental barrier suggesting impact on fetal development. Likewise, serotonin plays a significant role in stress regulation and fetal development. Investigation of SSRIs and their effects on the serotonergic system is crucial in understanding development. The aim of this study was to determine effects of the SSRI medication, fluoxetine, on maternal and offspring serotonergic

systems. Rat dams were subjected to gestational stress and fluoxetine (5mg/kg/day) or vehicle treatment via osmotic mini-pumps. At weaning, brains of dam, male and female offspring were collected. Half-brains were used to assess levels of serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in the hippocampus and prefrontal cortex (PFC). Results show developmental fluoxetine exposure markedly decreased 5-HT ($p = .06$) and 5-HIAA levels ($p = .02$) in the PFC of prenatally-stressed offspring ($p = .02$), with no significant differences between groups in 5-HT or 5-HIAA levels in the hippocampus. In dams, the ratio of 5-HIAA/5-HT, an indication of 5-HT metabolism, was significantly lower in the PFC of fluoxetine-treated dams ($p = .036$). Furthermore, in the hippocampus there was a significant interaction effect of stress/fluoxetine ($p = .006$) with Prenatal-Stress/Vehicle dams having significantly higher 5-HT metabolism compared to Control/Vehicle and Prenatal-Stress/Fluoxetine dams. Further work will investigate the effect of fluoxetine exposure on neuronal plasticity in the hippocampus and PFC. Understanding the impact of developmental SSRI exposure on serotoninergic systems and neurodevelopmental processes will enhance understanding of the benefits and risks of these medications.

EFFECTS OF NEONATAL FLUOXETINE EXPOSURE ON AFFECTIVE AND SOCIAL BEHAVIOR IN HIGH AND LOW VOCALIZING RATS

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Although current medical practice is to continue psychotropic medication of pregnant women with depression despite concerns about its behavioral teratology, there are few animal studies focused on long-term behavioral effects of prenatal antidepressant exposure. In addition, studies have not looked at individual differences in baseline affective state as a source of response variability. In this study, fluoxetine, a selective serotonin reuptake inhibitor (SSRI), was administered to rat pups from postnatal day 2 to 7 to model exposure to antidepressants in the human third trimester. Four behavioral measures were conducted from the neonatal to adult age periods in Low and High lines selectively bred for their rate of ultrasonic vocalizations after brief maternal separation. Neonatal fluoxetine administration decreased distress calls in both lines, but to a greater extent in High line rats than Low line. Neonatal fluoxetine also impaired motor coordination in neonates. There was no neonatal fluoxetine-related reduction in anxiety when tested in older subjects. However, neonatal fluoxetine altered social behavior in both juvenile and adult subjects. As expected, High line subjects displayed more anxiety behavior than Low line subjects at all three test ages. These results suggest that there are long-term behavioral consequences of antidepressant use during late pregnancy, and that baseline maternal affect may be an important indicator of sensitivity to these adverse effects in neonates.

EARLY LIFE STRESS IN MALE RATS LEADS TO ALTERED INSULIN/IGF1 SIGNALLING AND IMPAIRED MITOCHONDRIAL FUNCTION

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Numerous epidemiological studies have shown that affective disorders are strongly comorbid with metabolic disorders, and exposure to chronic psychological stress can lead to altered metabolic states in preclinical models. We used the rodent models of maternal separation (MS) and chronic unpredictable stress (CUS) to examine effects of early and adult stress, respectively, on serum metabolite levels, insulin/IGF1 signaling, and muscle mitochondrial content. MS induced a significant elevation in circulating triglyceride levels, a decline in circulating IGF1 levels, decreased phosphorylation of Akt in the muscle, and increased transcription of FOXO-responsive genes in the liver and muscle. Taken together, these results suggest a deficit in peripheral lipid clearance and decline in signaling through the insulin/IGF1 pathway. Furthermore, MS caused an overall reduction in mitochondrial mass as indicated by mitochondrial DNA content in the muscle, along with transcriptional downregulation of several genes involved in the regulation of mitochondrial biogenesis and function. Since mitochondria have been shown to be important in maintaining synaptic plasticity and modulating neuronal survival, we also checked whether mitochondrial content and function were affected in the hippocampus and medial prefrontal cortex. We observed robust but distinct transcriptional regulation of mitochondria-associated genes in these two brain regions. Animals that were exposed to CUS in adulthood were identical to controls in insulin/IGF1 signaling and mitochondrial function in the muscle. These results indicate that adverse experience in early life can evoke persistent changes in peripheral metabolic pathways and establish mitochondrial abnormalities, which may enhance predisposition for the development of metabolic dysfunction in adulthood.

HOW CHILDREN AND ADULTS ALTER THEIR WALKING PATTERNS TO MEET TIMING CONTRAINTS

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Walking requires adapting to keep pace with task constraints. When children are between 5- and 7-years old, their walking approximates adult walking in the absence of constraints. To examine how children and adults adapt their walking to meet timing constraints, 57 5- to 7-year olds and 20 adults walked to two audio metronome paces (slow and fast). Both children and adults modified their walking when faced with timing constraints. However, children demonstrated more difficulty actually meeting constraints. At the slow pace, children had more trouble matching the metronome compared to adults, which was associated with altered footfall patterns ($p < .01$). The youngest children's walking patterns deviated most from the slow metronome pace ($p < .001$), but practice did not improve their ability to meet the constraint ($p > .05$). Five-year old children were the only group that did not display carryover effects of walking to the metronome paces. Findings are discussed in relation to what contributes to the development of adaptation in children.

SENSORY, MOTOR AND COGNITIVE DEVELOPMENT IN THE 5XFAD MOUSE MODEL OF FAMILIAL ALZHEIMER'S DISEASE

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The 5xFAD mouse model of familial Alzheimer's disease (AD), shows amyloid beta plaques at 2 months of age and cognitive deficits at 4-6 months of age. Because of the early onset of pathology, we examined the neurodevelopmental profile of 5xFAD mice and their wildtype (WT: C57BL6xSJL) littermates. A neurodevelopmental test battery assessing sensory-motor, motor activity, and memory development was performed on 62 pups (both sexes, approximately half 5xFAD and half WT) from 2 to 24 days of age. Early in development, the 5xFAD pups performed better on the righting reflex than WT pups, and later in development, WT females showed increased activity in the open field compared to 5xFAD mice. There were no significant genotype or sex differences detected for physiological milestones such as eye opening, pinnae detachment, acoustic startle, grasp reflex or loss of cross extensor reflexes. Both genotypes and sexes had similar performance in novel object recognition, homing and forelimb grip strength. As expected, males weighed more than females over the course of development and the growth rate did not differ between 5xFAD and WT mice. In our studies of the development of 3xTg-AD mice, we found deficits in sensory development and novel object recognition, but advanced development of motor reflexes. Unlike the 3xTg-AD mice, the 5xFAD mice showed no neurodevelopmental deficits and may therefore act as an appropriate model to study the progression of the disease.

INVESTIGATION OF THE ANTIDEPRESSANT PROPERTIES OF

PRENATAL CHOLINE SUPPLEMENTATION IN A GENETIC RAT MODEL

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Major depressive disorder is one of the most common mental disorders in the United States with several consistent behavioral features in both humans and animal models, such as anhedonia, memory deficits, and stress-coping difficulties. There are also well-established neural markers, which include increased stress reactivity and decreased neural plasticity. These behavioral and neural markers of depression are linked with dysfunction in the expression and functioning of the neurotrophin, brain-derived neurotrophic factor (BDNF). To counteract the behavioral and neural insults of depression, the nutrient choline may act as a dietary alternative or augmentation to conventional antidepressant drug therapies. Supplemental choline in the maternal diet has enduring benefits on neural function—including increasing BDNF expression in adulthood, resulting in counter-depressant cognitive, physiological, and neurochemical changes in the offspring. In the present study, we sought to investigate whether supplements in dietary choline during prenatal development may counteract the behavioral and neural defects in a genetic rat model; rats in this model had a monoallelic deletion in the BDNF gene (BDNF+/−). A battery of behavioral tests assayed anhedonia and memory. The findings were that rats all BDNF+/− rats displayed anhedonia, but prenatal choline supplementation prevented memory deficits and differential responses to stress seen in standard-fed BDNF+/− rats. Effects on neural plasticity are still under investigation but the evidence gathered so far expands our understanding of the extent to which choline-induced increases in BDNF buffers against depressive-like symptoms.

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MATERNAL ANTIDEPRESSANT USE ALTERS HIPPOCAMPAL NEUROGENESIS THROUGHOUT DEVELOPMENT IN MALE AND FEMALE OFFSPRING

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Postpartum depression (PPD) disrupts healthy maternal care and consequently represents a form of early life adversity for developing offspring. Antidepressants such as fluoxetine are commonly prescribed for treating PPD. However, fluoxetine can remain active in breast milk, raising serious concerns for breastfeeding mothers. Unfortunately, little is known regarding the consequences of developmental exposure to antidepressants and whether it differentially affects males and females. In this study, we utilized a rodent model of PPD in which dams were treated with high levels of corticosterone to induce a depressive-like phenotype as well as concurrent fluoxetine to model treatment of PPD. We then examined hippocampal neurogenesis in the post-weaning, adolescent, and adult male and female offspring. Preliminary findings reveal that maternal postpartum corticosterone diminished expression of doublecortin, an endogenous marker of immature neurons, in the ventral hippocampus of adult male but not female offspring. Maternal postpartum corticosterone tended to diminish doublecortin expression only in the presence of concurrent maternal postpartum fluoxetine in the dorsal hippocampus of both male and female offspring. We predict that maternal postpartum corticosterone and fluoxetine will also affect post-weaning and adolescent offspring and that males and females may be differentially sensitive to these effects. This study will provide a better understanding of how maternal corticosterone and its pharmacological treatment impact development of the hippocampus.

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18–24 MONTHS HANDEDNESS PREDICTS 36 MONTHS EXPRESSIVE LANGUAGE SKILLS

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Previous longitudinal work found that a consistent right hand preference for object acquisition in early infancy (6 to 14 months) predicted advanced language ability at 24 months. Here we are reporting on handedness trajectories for role-differentiated bimanual manipulation (RDBM) when children were toddlers (18 to 24 months) and language outcomes at 36 months. To date, 44 children have completed the 36-month language assessment as measured by the Preschool Language Scales, 5th edition (PLS-5; Zimmerman et al., 2011). The PLS-5 measures expressive and receptive language separately on two subscales. Latent class growth analysis on toddler handedness scores revealed four classes in the sample: consistent left hand preference (8.5%), mixed left hand preference (14.5%), mixed right hand preference (24%), and consistent right hand preference (53%). Planned *t*-tests compared the combined consistent hand preference group (61.5%) and the combined mixed hand preference group (38.5%) on 36-month expressive and receptive language skills. Children with a consistent hand preference trajectory as toddlers had higher PLS-5 Expressive Communication scores ($M = 109.44$, $SE = 3.34$) compared to children who exhibited a mixed hand preference trajectory as toddlers ($M = 93.83$, $SE = 3.56$; $p = .003$, $d = 1.16$). By contrast, PLS-5 Auditory Comprehension scores did not differ by toddler handedness trajectory ($p = .121$, $d = 0.49$). The preliminary findings suggest that consistency in handedness trajectory continues to be linked with language development through 36 months of age.

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ADOLESCENTS' INTERNALIZING AND EXTERNALIZING PSYCHOPATHOLOGY PREDICT THEIR AFFECT-SPECIFIC HPA AND HPG AXES REACTIVITY: DUAL-AXES COUPLING

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We examined adolescent psychopathology-neuroendocrine associations within a developmental framework that acknowledged the interdependence of stress (HPA) and gonadal (HPG) hormone systems in the regulation of responses to everyday affective contexts. 51 youths ($M = 13.47$, $SD = .60$ years), who had been recruited as preschoolers with varying levels of behavior problems, completed an anxiety induction in the lab and an anger induction at home. Salivary samples were collected to evaluate HPA (cortisol/DHEA ratio) and HPG (testosterone) responses to both affect inductions. Preschool-age internalizing and externalizing problems did not predict adolescent hormonal responses, whereas concurrent problem-hormone relations were evident. Dual axes coupling during anxiety induction was normative, whereas youths with comorbid internalizing and externalizing problems exhibited selective activation of the HPA axis. Decoupling of the stress and gonadal axes was normative during anger induction, whereas adolescents with high externalizing problems marshaled dual axes co-activation. This is some of the first evidence suggesting affective context determines whether dual axes coupling is reflective of normative or problematic functioning in adolescence.

FAMILY NURTURE INTERVENTION IMPROVES MATERNAL CAREGIVING BEHAVIOR IN THE NICU

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The Family Nurture Intervention (FNI) is an intervention model recently used in a randomized, controlled trial study conducted in the neonatal intensive care unit (NICU) at Columbia. The intervention is designed to facilitate affective communication and co-regulation between mothers and their premature infants. Our hypothesis is that FNI will help re-establish physiological and psychological co-regulation between mother and infant, which is initiated in utero and interrupted by premature birth. FNI begins shortly after birth, continues until discharge and involves mutual calming sessions that include scent-cloth exchange, vocalizations, eye contact, holding, and family-based support sessions. The current analyses assessed the impact of FNI upon quality of maternal caregiving behavior (MCB). MCB was coded during single sessions (~30 min) involving holding and feeding interactions in the NICU prior to discharge at 36 weeks gestational age (GA). Seventy mothers and their premature infants met inclusion criteria for this study (FNI, $n = 37$; standard care (SC, [$n = 33$]), which included premature birth between 26 and 34 weeks GA, weight >third percentile for GA, and no congenital defects.
Relative to mothers in the SC condition, those in the FNI group showed significantly higher quality MCB, which remained significant when controlling for birth order, maternal depression, and maternal anxiety. A significant condition-by-twin status interaction showed that FNI had greater effects on MCB of mothers of singletons ($n = 50$). This is the first study to demonstrate that in-unit MCB can be enhanced by hospital-based intervention. Implications for sustained benefits to both mothers and infants are discussed.
[This research was supported by the Columbia University Nurture Science Program and the Einhorn Family Charitable Trust. Author M. M. Myers received salary support from the New York State Office of Mental Health]

LOCOMOTOR EXPLORATION IN INFANTS (AND THEIR MOTHERS)

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Traditionally, researchers study infant locomotion by collecting standard gait measures (e.g., step length and speed) as infants walk continuously along a straight, uniform path. Consequently, the traditional story about the development of locomotion concerns improvements in gait. We took a different approach. We investigated developmental changes in infant locomotion when movements are not restricted to a straight continuous path—natural locomotion as infants freely explore a space and interact with toys, furniture, and caregivers. Here, we report preliminary data from 22 13- and 19-month-old infants and their mothers. Dyads were videotaped together for ten minutes in a laboratory playroom. We tracked infants' and mothers' location in the room by digitizing their left heel location frame-by-frame from video. On average, younger infants traveled a smaller total distance (39.0 m vs. 67.5 m), covered a smaller area of the room (8.4 m^2vs. 12.8 m^2), and moved less during each walking bout (128.4 cm vs. 170.1 cm). Rather than walking in a straight line, infants in both age groups produced a wide range of curved paths (bout displacement/distance was <.9 for 57% of bouts). Mothers traveled larger distances than their infants (64.0 m and 76.0 m, for mothers of 13- and 19-month-olds, respectively) but covered similar areas of the room (9.2 m^2vs. 11.1 m^2) compared with infants. Findings indicate that natural locomotion differs in important ways from standard gait measures. Natural infant walking is locomotor exploration and mothers' movements are influenced by their infants' exploration.
[NICHD R37-HD03348 to KEA]

ELEVATED PRENATAL PROGESTERONE ALTERS PERCEPTUAL AUDITORY LEARNING IN BOBWHITE QUAIL (COLINUS VIRGINIANUS) EMBRYOS AND NEONATES

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Recent studies have established that elevated yolk hormones of maternal origin affect offspring phenotype. To test the effects of elevated yolk progesterone on prenatal and postnatal auditory learning, three experimental groups were formed: bobwhite quail chicks hatched from eggs with artificially elevated progesterone (P), chicks hatched from an oil-vehicle control group (V), and chicks hatched from a non-manipulated control group (C). In two experiments of prenatal and postnatal auditory learning, individual chicks were either passively exposed to an individual maternal assembly call for 24-hrs prior to hatching, or for 24-hrs after hatching, respectively. Chicks were then tested individually for their preference between the familiarized maternal call and a novel call at 24 and 48-hrs following hatching. Analysis of yolk hormone levels using high performance liquid chromatography with tandem mass spectroscopy found that progesterone levels were significantly elevated in P-treated eggs and were also present in the egg yolk longer into prenatal development than the two control groups. Chicks from the P group failed to demonstrate a preference for the familiar bobwhite maternal assembly call at 24 or 48-hrs after hatch, while chicks from the C and V groups demonstrated a significant preference for the familiarized call. Chicks from the P group showed an enhanced preference for a familiar maternal call presented for 24-hrs after hatch compared to the two control groups. The results of these experiments suggest that elevated maternal yolk hormone levels in pre-incubated bobwhite quail eggs can influence auditory perceptual learning in embryos and neonates.
[NSF grant BCS 1057898 to RL]

EYETRACKING, CLOSED CIRCUIT HDTV, AND IMITATION: PARSING THE VIDEO DEFICIT

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Tests of children's imitation learning from video reveals a "video deficit effect", wherein children fail to transfer what they learn across dimensions (i.e. 2D-3D or 3D-2D). Understanding the source of this generalization decrement is becoming important as technology and online learning increasingly pervade children's lives. Competing theories suggest that physical or social discrepancies between 2D and 3D demonstrations may control this effect, as may the diminished representational flexibility of children. However, discriminating between these competing accounts remains difficult using current methods. Towards this end, we combined eyetracking with a High Definition Closed Circuit Television setup, which, along with careful psychophysical controls, enables us to independently vary social and physical factors related to learning. Our question: is it the absence of social information, or is it a physical limitation of the 2-D medium itself that places an upper limit on how effective social learning can be in video demonstrations? We collected eye-tracking data during demonstration and assessed imitation performance of 18 and 24 month olds (in live, CCTV, and yoked video groups) using a puppet task established in the literature as appropriate for 6-24 month old infants. Preliminary data suggest that the VDE may result from a perceptual impoverishment due to the 2D screen, rather than the lack of social interaction. These data speak to how to improve video learning for young children, and to the limitations of screen media more generally.

DEVELOPMENT OF FEAR CONDITIONED RESPONSES IN CHILDREN

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Fear conditioning paradigms can offer insight into neurobiological ontogeny of anxiety. This presentation will include research that examined fear conditioning and extinction using fear-potentiated startle and skin conductance response in children. The results of these studies indicate significant interaction effects of age and anxiety. Children under 10 years of age show poor discrimination between reinforced conditioned stimuli (danger cues) and non-reinforced stimuli (safety cues), and anxiety increases fear responses during conditioning. Fear-potentiated startle to the safety cue significantly predicts child anxiety levels, suggesting that impaired safety signal learning may be a risk factor for anxiety disorders in adulthood.

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CORTISOL RESPONSE TO FAMILY INTERACTION AS A BIOMARKER FOR ADJUSTMENT DURING LATE ADOLESCENCE

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There is a growing body of literature that aims to demonstrate associations between function of the hypothalamic-pituitary-adrenal (HPA) axis and psychological outcomes during late adolescence/emerging adulthood. A separate literature links family functioning and late adolescent outcomes. The present study integrates these two lines of research, investigating the interacting contributions of HPA axis and family functioning to the emergence of internalizing and externalizing symptoms during late adolescence. Late adolescents (N = 101) between the ages of 17 and 19 were assessed at three time points across their first college year; during the summer before college, and once during the fall and spring semesters, respectively. During the summer assessment two parents accompanied the late adolescent child, and all family members provided four saliva samples each at 20-minute intervals. Later assessments of late adolescents included measures of internalizing and externalizing symptoms. Family functioning moderated the relationship between cortisol response and anxiety during the fall and spring semesters. The highest-risk group was late adolescents with increasing cortisol who came from families that were observed to be distant and negative. The approach taken by this study provides a first step toward understanding how interrelationships among elements of physiology and family functioning contribute to later symptomology.

PRIOR MATERNAL STRESS ALTERS THE TRAJECTORY OF FEAR DEVELOPMENT IN SUBSEQUENT INFANT RAT OFFSPRING AND HAS SPECIFIC EFFECTS ON SUBSEQUENT MATERNAL BEHAVIOUR

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Earlier work showed that maternal separation (MS) affects maternal behaviour, and more recent work has shown that MS causes an early emergence of adult-like fear retention and fear inhibition in the infant offspring. Little is known about whether either of these effects are transmitted to subsequent generations. In the first stage of this research, female rats were bred and then exposed to maternal separation (MS) or standard-rearing (SR). Females were then bred again but were not separated from that subsequent litter. Pups from subsequent litters (MS-2 and SR-2) were fear conditioned at postnatal day 17 and tested for fear retention, or were extinguished and tested for the renewal effect. The results show that MS-2 infant rats exhibited more adult-like fear behaviour (better retention of fear and more relapse following extinction) despite never being exposed to any direct environmental stressors themselves. We also examined various aspects of maternal behaviour directed towards these subsequent litters. Four behaviours were examined: arched-back nursing (ABN), licking-grooming (LG), time off nest, and pup retrieval. Preliminary evidence indicates that dams of MS-2 litters do not differ systematically from SR-2 dams on measures of ABN, LG, or time off nest. However, dams of MS-2 litters retrieved their offspring faster compared to dams of SR-2 litters. Taken together, these findings demonstrate the potent and long-lasting impact of maternal trauma, and indicates that a mother's previous experiences can be a key mediator of offspring fear development. Further, the findings suggest that prior maternal stress may have specific influences on subsequent maternal behaviour.

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THE ASSOCIATION OF PARENTAL CHRONIC PHYSIOLOGICAL STRESS WITH CHILDREN'S EXECUTIVE FUNCTIONING

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The current study investigated the relation of parental chronic physiological stress to young children's executive functioning (EF) performance. EF is highly related to early school achievement, emotion regulation and social competence (Blair & Razza, 2007). We collected hair cortisol samples from children and parents as a biomarker of chronic hypothalamic-pituitary-adrenocortical (HPA) activity and measured children's and parents' EF using the NIH Toolbox Flanker Inhibitory Control and Attention Test. This behavioral measure of EF requires focus on a particular stimulus while inhibiting attention to stimuli flanking it. In our preliminary sample ($N = 31$, 16 males, $M = 4.17$ years), a conditional process model ($F(4, 26) = 6.59$, $p = <.001$) predicting child EF from parental hair cortisol, age, and gender yielded a main effect of age ($p = <.001$, 95% CIs:0.56, 1.95), demonstrating that older children performed better on EF. There was a significant child gender x parent hair cortisol interaction ($p = .01$, 95% CIs: 4.91, 32.10), revealing a conditional effect of parent's hair cortisol on child's flanker scores for boys ($p = <.001$, 95% CIs: 8.29, 28.95) but not for girls. Thus, if parents had higher chronic physiological stress, male children showed better EF ability. Parents' EF and children's own chronic stress were not significantly related to children's EF. Results indicate the importance of considering gender because of potential gender-specific pathways in predicting EF. Follow-up studies should explore possible mechanisms underlying the link between parental chronic stress and boys' EF performance, such as differences in parental expectations for child's performance.

SOMATIC AND NEUROENDOCRINE CHANGES IN RESPONSE TO CHRONIC CORTICOSTERONE IN ADOLESCENT MALE AND FEMALE RATS

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The stress response activated by the hypothalamic-pituitary-adrenal (HPA) axis is known to lead to neurobehavioral and metabolic changes. Exposure to chronic stress can promote the development of physiological and behavioral dysfunctions, including alterations in feeding behavior and body weight, effects further modified by the sex of the individual. The profound changes in physical growth and sexual maturity that occur during puberty triggered by the hypothalamic-pituitary-gonadal (HPG) axis are intimately related to the functioning of the HPA axis. Due to this close connection between the two axes, and their respective roles in reproductive function and metabolism, we hypothesize that chronic CORT exposure may affect males and females differentially during adolescence. In this experiment, we explored the effects of a 28-day exposure to CORT (150 or 300 μg/ml) dissolved in drinking water on the physiology and neuroendocrine function

of adolescent male and female rats. We used this approach as a non-invasive method of altering plasma CORT levels. We found that CORT-treated males and females showed significantly reduced weight gain, food intake, water intake, and adrenal gland weight than control animals, with males showing a greater response than females. Additionally, CORT-treated males, but not CORT-treated females, demonstrated a significant decrease in plasma ACTH concentration, thymus gland weight, and plasma testosterone concentration. These data indicate that chronic CORT administered non-invasively to rats through their drinking water results in numerous somatic and neuroendocrine changes in a sex-dependent manner.

BABY IN A BIND: TRADITIONAL CRADLING PRACTICES AND INFANT MOTOR DEVELOPMENT

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Prominent theories of infant development are built on the premise that freedom to move is essential (e.g., Gibson, 1988; Piaget, 1954). Accordingly, cross-cultural and experimental studies show that exercise facilitates infant motor skill and restricted movement inhibits skill acquisition. However, previous work on restricted movement is limited to children reared under conditions of social deprivation. We examined a traditional childrearing practice in Central Asia that restricts infant movement without depriving infants of loving caregivers—the gahvora cradle. We report preliminary data from $N = 119$ infants (0-24 months of age) in Tajikistan on: the cradling process (mothers were video-recorded placing infants into the gahvora); extent of restriction (based on 24-hour time diaries); and effects of gahvora use on motor development (demonstrated motor skills during spontaneous activity). We found that cradling is a 7-step, highly organized process. Restrictive components included: swaddling infants' legs, arms, and torsos; binding the limbs to the gahvora; and using blankets and opaque coverings that obstructed visual surrounds. Restrictive components remained constant across age. Cradle use decreased with infants' age, from 17 hours/day at birth to 6 hours/day at 24 months of age. Unrestricted time on the ground increased with age from 7 hours/day at birth to 15 hours/day at 24 months. More gahvora use predicted less mature motor skills, statistically controlling for infants' age. Cradle use initially delays motor development relative to Western norms, but by 2 years of age, most Tajik infants walk. Possibly decreases in cradle use and increases in ground time mitigate effects of cradling.
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MATERNAL BEHAVIOR INFLUENCES THE DEVELOPMENT OF A REFLEXIVE ACTION PATTERN IN THE NEWBORN RAT

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In neonatal rats, the leg extension response (LER) is a spinal-mediated coordinated action pattern characterized by bilateral hyperextension of the hindlimbs that occurs in the context of maternal-infant interactions. The LER occurs in response to maternal anogenital licking (AGL) of the pup's perineum. Certain cues influence the mother to perform AGL on the pup, one of which is olfaction. Past research has examined the role of olfaction in these interactions and has found that intranasal application of zinc sulfate (ZnSO4) to the dam induces hyponosmia, thereby reducing the incidence of AGL. The purpose of this experiment was to examine the effect of reduced AGL on the expression and postnatal development of the LER. Pregnant dams received an intranasal application of air (control), distilled water (control), or ZnSO4 on the day before birth and every other day thereafter until postnatal day 9 (P9). The LER was experimentally evoked in pups, using a vibrotactile device, at P1, P5, or P10. Pups born to ZnSO4-treated dams showed significantly shorter bilateral LER durations and significantly smaller ankle angles than pups born to control dams. Effects were apparent until P10. These results suggest that variations in maternal behavior affect the expression of early motor behavior, including reflexive action patterns.
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PRENATAL MATERNAL STRESS AND BDNF METHYLATION IN NEWBORNS

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The *BDNF* gene codes for a neuronal growth factor involved in neural development, cell differentiation, and synaptic plasticity. BDNF protein is present in the brain and periphery and is important for placental and fetal development. Rodent studies show that early life stress, including prenatal stress, induces altered *BDNF* methylation. To date there have been no studies examining prenatal psychosocial stress exposure on *BDNF* methylation in humans. This study examined the effect of prenatal exposure to maternal stress on *BDNF* methylation at CpG sites distributed across the *BDNF* gene region. Participants were mothers and newborns in the eastern Democratic Republic of Congo, a region with extreme conflict and violence to women. Umbilical cord blood, placental tissue, and maternal venous blood were collected after birth, and maternal interviews assessed culturally relevant chronic and traumatic war-related stressors. Prenatal stress exposure showed broad effects in cord blood and placental tissue but not maternal blood, suggesting an intergenerational effect of stress exposure. Prenatal stress associations with methylation differed in cord blood vs. placenta. Most significant associations were observed at CpG sites situated in transcription factor binding sites. Results suggest prenatal exposure to maternal stress predicts *BDNF* methylation, especially in evolutionarily conserved and functionally relevant regions of the *BDNF* gene.
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EFFECTS OF PERINATAL STRESS AND FLUOXETINE IN ADULT MICE

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We have found that late prenatal/early postnatal exposure to fluoxetine has long-term behavioural effects, in mice, when they are assessed in adulthood. These behavioural effects appear limited, but some have the potential to be beneficial. Chronic maternal stress during the early developmental period results in sustained, etrimental consequences for the offspring, as adults. This presentation will discuss findings that show that some of the long-term, stress - related consequences can be reversed by fluoxetine administration, when administered to the mother during the perinatal period.
[NSERC PGS-D and AIHS doctoral scholarships to VK and NSERC Discovery and CIHR operating grants to RHD]

WEANLING GUINEA PIG PERFORMANCE ON A SPECIES-RELE-

VANT MEMORY FOR LOCATION TASK

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Early cognitive testing of adult guinea pigs *Cavia porcellus* revealed that this species is capable of performance at least equal to that of rats and mice, but only with methods that use species-relevant tasks and stimuli. In this study we test the hypothesis that weanling guinea pigs also possess memory for location and can learn simple associations by hiding bits of romaine lettuce in colored tunnel blankets. Similar blankets, constructed of Polar Tek fleece (Monsanto), serve as burrows in their home cages. The apparatus for testing was a cage sized arena with either one (training) or two (testing) tunnel blankets of contrasting colors. All blankets were rubbed with lettuce prior to testing in order to mask the location. Weanling guinea pig pups were placed in the arena and given two trials per day on a single blanket until they successfully located the lettuce on two consecutive days. Pups were then tested with two trials per day for 5 days per condition with two days rest in between. The conditions were: (a) one blanket location, (b) lettuce in one of two blankets, (c) the second of two blankets, (d) lettuce switched back to the original location, but with the opposite blanket color. All seven weanlings were able to locate the lettuce successfully for most conditions, and savings was apparent on successive testing days. These results suggest that weanling guinea pigs have memory for location and the ability to learn simple associations.
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SOCIAL PREFERENCES FOR FAMILIAR INDIVIDUALS WITHIN BROWN-HEADED COWBIRDS (*MOLOTHRUS ATER)* ACROSS CHANGING GROUP CONDITIONS

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Social interaction is an important component in the development and expression of species-typical behavior. Nonetheless, across many species little is known about the within-group interaction patterns that shape an individual's social experience. Many vertebrates inhabit loosely structured groups where both group size and composition fluctuate over short timescales. In such groups the ability to recognize and preferentially associate with familiar individuals may construct the social networks necessary for group cohesion. In a series of two studies we explored the strength and persistence of social preferences in brown-headed cowbirds across a series of introductions between groups of novel and familiar conspecifics. Across introductions females maintained significant preferences to approach familiar conspecifics, and the individual variation in the strength of those preferences remained consistent. Male preferences changed across the introductions. During the first introduction males showed a significant preference to approach familiar conspecifics, but increased their approaches towards novel conspecifics in subsequent introductions. These studies suggest that within cowbird flocks female social networks may be important in maintaining group cohesion, whereas male social networks may be important in the integration of novel conspecifics into the group.

SERTRALINE (AN SSRI ANTIDEPRESSANT) DURING PREGNANCY IN AN ANIMAL MODEL OF MATERNAL STRESS AND DEPRESSION: EFFECTS ON LITTER CHARACTERISTICS, MATERNAL CARE, AND MATERNAL DEPRESSIVE-LIKE BEHAVIOR

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Depression is twice as prevalent in women as in men and the highest risk for women to develop a depressive episode is during her reproductive years. However, less research has been done to study the effects of antidepressants in females. In males, antidepressants often increase neurogenesis in the dentate gyrus. This study aims to investigate the effects of sertraline (Zoloft®, a selective serotonin reuptake inhibitor, SSRI) given to pregnant rats on depressive-like behavior, maternal care, and hippocampal neurogenesis levels. First, all animals received corticosterone or vehicle (40mg/kg, s.c.) for three weeks to induce 'stress/depressive-like' behavior. After 16 days of treatment with CORT or vehicle, animals were then treated with sertraline or vehicle (water) (20 mg/kg, p.o.). Following 21 days of CORT or oil treatment, rats were mated until pregnant. One group receiving sertraline was discontinued from treatment on gestational day 16, and another group continued sertraline treatment throughout pregnancy. This was done to assess the effects of discontinuing antidepressant treatment during pregnancy. Maternal care was observed from postpartum day (PD) 2-8 and Forced Swim testing was conducted before and after pregnancy to assess the effects of sertraline on depressive-like behavior. One day after the pups were weaned (PD 21), animals were sacrificed via perfusion to investigate neurogenesis levels in the dentate gyrus. We hypothesize that corticosterone administration will alter forced swim test behavior, maternal care, and hippocampal neurogenesis levels, and this will be modified by successive treatment with sertraline. This study will help us to further our understanding of the association between maternal stress, depressive-like behavior and antidepressant treatment, and the consequences for the well-being of the mother.

A COMPARATIVE INTERPRETATION OF THE FETAL RESPONSE TO SPEECH

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The objective of this study was to compare two current interpretations of the fetal cardiac response to repetitive speech in fetuses tracked from 28 to 38 weeks gestational age (GA). Study methods allowed for interpretations using Richard's and colleagues four phases of attention (automatic interrupt, stimulus orienting, sustained attention, and attention termination) and the behavioral learning process, habituation. Using a longitudinal single-subject design, all mothers recited a short passage (twice a day, repeated 3 times) from 28 to 34 weeks GA and their fetuses were tested at 28, 32, 33, 34 weeks GA. Following discontinuation of recitation at 34 weeks GA, testing continued at 36 and 38 weeks GA. Experimental subjects were tested with a recording of a female stranger speaking the assigned passage and control subjects tested with a novel passage. Habituation of the cardiac response to the passage was demonstrated rarely and never beyond 34 weeks GA. The cardiac response transitioned from phase 1 (automatic interrupt) to phase 2 (stimulus orienting) in both experimental and control groups from 28 to 38 weeks GA. Only experimental subjects demonstrated a transition in the cardiac response from phase 1 to phase 3 (sustained attention); occurring between 34 to 38 weeks GA. This comparison between interpretations of the fetal cardiac response to speech, has provided evidence for the longitudinal application of Richards and colleagues' phases of visual attention to auditory attention, however, further investigation into the role a behavioral learning process other than habituation (eg. respondent or operant conditioning) is needed.
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THE INFLUENCE OF MATERNAL EDUCATION ON LIFETIME VULNERABILITIES FOR CHRONIC STRESS AND HEIGHTENED PHYSIOLOGICAL REACTIONS TO STRESSORS

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We examined parental education as predictors of vulnerability to biological and perceived chronic stressors into adulthood. Measures included hair cortisol (hCORT) and cardiovascular parameters as indicators of chronic stress and overall health. The community subjective social status ladder was included to examine relationships between maternal education and assessments of social standing in adult offspring. Participants (N = 107;

ages 18-30; M =22.23, SD = 3.01; 50.4% female) were recruited from an urban public university and residents of surrounding low-income areas in Boston, MA. Maternal and paternal education were positively associated with change in sympathetic nervous system (SNS) recovery after a cognitive challenge task (rM = .22, $p < .05$, rP = .45, $p < .001$). Maternal education also predicted SNS resting and reactivity changes before and after the cognitive challenge (lower maternal education, higher resting SNS) (SymREST: $F(2, 90) = 3.46$, $p < .05$); and increased SNS reactivity during a cognitive stress task (SymTASK: $F(2, 89) = 2.06$, $p < .05$). Maternal education was negatively associated with hCORT (B =-.215, $t(1, 104) = 2.24$, $p < .05$) (higher maternal education, lower hCORT). Additionally, maternal education predicted one's present subjective status (higher maternal education, higher reported status on the SSS ladder) ($F(2, 106) = 3.20$, $p < .05$). These findings support work that finds parental education is a unique predictor that may influence vulnerabilities across biological and perceived domains in adulthood and call for analysis of underlying developmental mechanisms. [Supported by NIMHD 5P20MD002290 and Center for Clinical and Translational Sciences at University of Massachusetts Medical School]

DIFFERENT ESTIMATES OF INFANT HAND PREFERENCES RESULT FROM DIFFERENT HAND PREFERENCE ASSESSMENTS

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A general consensus has arisen that restricts reliable handedness assessment to the period beyond infancy. However, some evidence indicates that infant hand-use preferences for acquisition are stable across assessments for infants as early as eight to 14 months of age. The development of these preferences can be studied using a variety of assessments and classification criteria, and this variation in methodology may contribute to differences in the apparent stability of hand preference. This project compares two procedures for assessing infant hand-use preferences and classifying them into groups. One hundred and fifty infants were assessed in two tasks; one using nine presentations of objects, the other using 32 presentations. Monthly classifications of hand preference for each task were then determined by either a commonly used proportional decision criterion (one hand used 50% more often than the other) or a criterion based on a conventional alpha probability of 0.05 that the use of the two hands diverge by chance. While both tasks reveal a large proportion of the infants having no hand preference, the nine presentation task shows greater fluctuation from month to month than does the 32 presentation task. Using a group based trajectory model, the nine presentation task was not able to distinguish more than two latent classes (one showing no preference, the other trending toward right-hand preference). However, when a 32 presentation task was used, an additional latent class of left-preference infants was distinguished. These differences would create confusion about the stability of infant hand-use preference and its development.
[NSF Development and Learning Sciences judged this research to be non-competitive and Julie Campbell was supported by NIH T32HD007376]

ASSESSMENT OF EMOTIONAL EXPERSSION (AMEE)

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One key symptom of Autism Spectrum Disorders (ASD) is difficulty recognizing and processing emotions (Gliga, et al, 2014). This presentation will explore some early stage findings for an assessment of emotion perception that does not rely on verbal ability and thus could be a nonverbal screening tool for ASD. Typically developing children and adults participated in a matching task in which they were required to match the emotion of different schematics and human faces. The first study measured how typically-developing children and adults detect emotional expressions. The results of that initial assessment are being used in the development of assessment tool for emotion processing disorders. The focus of this presentation will be the data from our adult and typically developing children and the creation of an app to run the task on an Android operating system.

The first assessments were done with a population of college students. The task was presented over 4 tiers of increasing difficulty. The college students learned the task across all four difficulty levels ($F(3,976) = 36.33$, $P < .001$).

Following the college students typically developing children were tested. The children showed age differences ($F(4, 6) = 16.63$, $p < .001$), indicating the task can differentiate between the emotion recognition of three, four and five year olds.

The next steps include putting the task onto an easy to use app format and testing it with a population of young children with ASD.

EFFECTS OF OVERWEIGHT STATUS ON INFANTS' MOTOR MILESTONES

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This study examined effects of body dimensions, ethnicity, and socioeconomic status on infant motor development. Overweight status in infancy is a growing public health concern, and low income is a risk factor, but little is known about effects of overweight status and SES on motor development—in particular onset ages for independent mobility (belly crawling, hands-knees crawling, cruising, and walking). We report preliminary data on motor milestones in 36 infants from predominantly middle-class families and 28 infants from predominantly low-income, immigrant families at 10, 13, and 19 months of age. The middle-class infants showed expected age-related changes in onset ages: On average, belly crawling at 6.4 months, hands-knees crawling at 8.2 months, and cruising at 9.8 months. In contrast, of the low-SES infants who achieved each milestone, onset ages for belly crawling, hands-knees crawling, and cruising were not staggered and occurred at approximately the same age (8.3–8.8 months). Although onset age for walking was similar between groups (12.40 months), 60% of low-SES infants had not achieved hands-knees crawling at 10 months, whereas all of the middle class infants had. Moreover, in low-SES infants, overweight status was associated with delays in onset ages for locomotor milestones: Babies with larger WHO weight-for-length percentiles began crawling on belly and hands-knees, cruising, and walking at older ages. The overweight effect in motor milestones did not exist in middle class infants for hands-knees crawling, cruising, or walking. Results suggest that interactions between overweight status and sociocultural factors may contribute to delays in independent mobility.
[NICHD R37-HD03348 to KEA]

EARLY LIFE STRESS IMPAIRMENT OF SELECT HIPPOCAMPUS DEPENDENT FUNCTIONS IS SEX AND AGE SPECIFIC

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Acute traumatic events as well as prolonged stress during early life have been shown to lead to increased risk of affective pathology and have been associated with cognitive impairments in both humans and animal models. The emergence of some forms of pathology appears to be sensitive to developmental status, with a significant increase in risk for affective disorders during adolescence. Expression of pathology may be linked to disturbance in the development and functioning of basic learning systems, which may be related to comorbidity with cognitive impairments. However, the effect of early life stress on the development of learning during development is not well understood. To study the effects of early life stress (ELS) on hippocampal function during development we used maternal bedding restriction from postnatal day 4 to 11. We then tested control and

ELS female and male mice on various hippocampus-dependent tasks across multiple developmental time-points. Our results show that compared to controls, ELS males, but not females, have impairments in the novel object location task. However, no impairment were observed for either sex in the novel object identity task, suggesting that early life stress may have profound and sex selective effects on hippocampal development and functioning. These studies identify sex differences in the impact of early life stress on hippocampal function and may provide a system in which to study factors that confer risk or resilience to early life stress.

DOES DIRECTION OR CONSISTENCY OF HAND PREFERENCE PREDICT TODDLER STACKING ABILITY?

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The Modified Progressive Theory of Handedness suggests that a hand preference results from a history of cascading manual asymmetries for a variety of actions throughout infancy. An infant who consistently uses one hand across multiple action types could gain proficiency using their preferred hand and perform more expertly on challenging manual tasks. This project will test the relation between early handedness consistency will predict performance on a challenging manual task (stacking). Forty-two infants (17 females) were assessed for a hand preference from 6-14 months of age (object acquisition) and subsequently from 18–24 months of age (role-differentiated bimanual manipulation) across 16 monthly visits. Toddlers were also administered an age-appropriate, stacking task from 18-24 months, which comprised small blocks, large blocks, wood rings, and seriated cups. Using a multilevel Poisson longitudinal model, stacking increased linearly across the 18–24 month ages. Preliminary results also indicate that consistent toddlers ($n = 22$) stacked more items than inconsistent toddlers ($n = 20$) initially; however there were no differences in how toddlers increased their ability. Although a consistent hand preference affects initial stacking achievement, both groups increase stacking ability in similar ways. With the addition of more toddlers, further analysis will specify if the direction of handedness (right or left) and consistency interact to predict toddler stacking ability.

AN ATTENTIONAL BUT NOT RACIAL BIAS UNDERLIES THE OTHER-RACE EFFECT IN INFANCY

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Over the first postnatal year, infants' ability to discriminate faces from unfamiliar races decreases, reflecting the origins of the *Other-Race Effect*. Despite several examples of this pattern, the underlying mechanism remains unclear. We propose that this seeming loss of function results from a developing selective attention bias to frequently experienced own-race faces. Selective attention enhances online visual acuity, contrast sensitivity, and early visual processing. Increased selective attention to own-race faces would yield more effective online processing of those faces versus other-race faces. Therefore, biasing attention to a face, either naturally or experimentally, would improve online visual processing and subsequent discrimination of that face, *regardless of race*. In the present study we used a classic spatial cueing paradigm to experimentally bias 9 month-old infants' selective attention to own- versus other-race faces and examined their subsequent discrimination of those faces. Results showed that infants discriminated *both* own- and other-race faces when they were the focus of the attention bias but failed to discriminate *either* own- or other-race faces when those faces appeared outside the attention bias. These data are consistent with an attentional bias mechanism underlying the ORE that exploits the benefits of attentional enhancement of vision, allowing for a powerful yet efficient way to increase the gain to daily relevant faces while retaining flexibility if other-race faces become relevant.
[NIGMS-NIH grant P20 GM103645 to DA; James S. McDonnell Foundation Scholar Award in Understanding Human Cognition to DA]

EARLY DEVELOPMENT OF FUNCTIONAL BRAIN CONNECTIVITY IN INFANTS' RECOGNITION OF FAMILIAR FACES

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Whereas adult studies have revealed that face recognition is dependent on the coordination of activity among networks of brain cell assemblies, research on the neural correlates of face recognition in infancy has largely focused on localized responses at individual recording sites. The present work examined functional connectivity in the infant brain that subserves the recognition of familiar faces. To investigate functional connectivity and its development in early infancy, we examined EEG coherence between spatially separate scalp recording sites in infants 3 and 6 months of age. Infants viewed images of their mothers' faces and of appearance-matched female strangers while EEG was recorded. Coherence was examined across frequency bands between posterior temporal sites, and between longer-range occipital to frontal connections. Analyses revealed functional connections in the developing infant brain that differentiate familiar and unfamiliar faces. The topography of those connections corresponds closely to those reported for adults. Although posterior alpha coherence differentiated faces across ages, theta and beta effects were observed only at 6 months, indicating a critical age span of network development in face processing. Theta coherence differentiated faces between posterior temporal sites, and beta coherence did so between occipital-frontal sites. These findings provide new insight into the formation of functional connections in the human brain, and the utilization of those connections in face recognition over the early months of life.
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OBSTACLE AVOIDANCE IN 9-MONTH-OLD INFANTS' REACH TO GRASP

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Adults have a propensity when reaching for objects to circumnavigate other objects in the vicinity even when those objects are not on the direct path to the target. Lesion studies with neurological patients suggest that the dorsal stream vision-for-action pathway is involved in guiding the automated avoidance seen in healthy subjects. The present work examined 30 9-month-old infants in a reach-to-grasp task involving multiple objects. Research on infants and young children has suggested that the dorsal stream may have a more protracted developmental time course than other visual system structures. Infants were outfitted with motion-analysis sensors on their wrists and presented with a visually salient target object when either no other objects were present in their manual workspace or when a simple, smaller, and less salient object was placed in the workspace at one of four positions around the target object. None of the four positions of the second object occluded infants' direct reach toward the target. Reach movements were compared between target-only and target-with-other conditions. Target-only reaches were straighter than when a second object was present in the manual workspace, and target-only reaches also took less time to complete. By 9 months, infants' non-target accommodation in reach-to-grasp movements has begun to resemble that of adults when multiple objects are present in the workspace. To the extent that the behavioral

propensity to accommodate extra objects in the workspace is guided by dorsal stream activity, the development of such function appears likely underway by 9 months of age.
[This work was supported by the Intramural Division of the NICHD/NIH.]

INDIVIDUAL DIFFERENCES IN CAREGIVER REDIRECTION AND INFANT GAZE SHIFTING IN A NATURALISTIC SOCIAL SETTING

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At-risk populations demonstrate specific patterns of caregiver behavior. For instance, maternal depression has been associated with decreased *sensitivity*, in which caregivers share joint focus with infants' current focus of attention, and increased *redirectiveness* or intrusiveness, in which caregivers try to shift infants' focus. Differences in sensitivity and redirectiveness, both in connection with and separate from depression, predict later cognitive and behavioral outcomes in children (e.g., language comprehension, hyperactivity). However, the proximal mechanisms underlying these relationships are not well understood. We posit that increased redirectiveness coupled with lower sensitivity might disrupt focused joint attention in the context of early social interactions and impair learning. To investigate this, we identified individual differences in redirective and sensitive behaviors within a non-clinical parent sample during a 15-min naturalistic dyadic interaction with their 5-month-old infants, and explored whether infants' visual responses (contingent gaze shifts) to caregivers' actions varied with parent behavior. Parents' overall responsiveness and sensitive and redirective responding were coded. Caregivers with extreme patterns of sensitivity and redirectiveness ($n = 12$) were subsequently selected for analysis. Infants of caregivers exhibiting high redirectiveness and low sensitivity ($n = 6$) were more likely to shift gaze in response to their caregivers' overall responsiveness than infants with highly sensitive and minimally redirective caregivers ($t(10) = -2.66$, $p = 0.02$). This result held when controlling for gaze shifts that occurred contingently on redirections ($t(10) = -3.29$, $p = 0.01$), suggesting that infants had generalized gaze-shifting to non-redirective parental responses. Future work will assess how variation in gaze shifting during naturalistic social interactions predicts development outcomes.
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ACUTE MODERATE-TO-VIGOROUS PHYSICAL ACTIVITY RESULTS IN SPECIFIC EXECUTIVE FUNCTION GAINS IN PRESCHOOLERS

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Although there is a well-established relationship between physical activity and cognitive function in adults, few studies have examined this relationship in very young children approaching the transition to formal schooling. In a sample of preschool children ($N = 81$) randomly assigned to an active or passive videogame condition, we examined whether acute moderate-to-vigorous (MVPA) physical activity resulted in improved task performance across six executive function domains (information processing, memory, inhibitory control, task switching, ideation fluency, and task persistence) following each activity condition. Increased physical activity (MVPA condition) resulted in domain specific executive function gains. Specifically, preschoolers persisted longer in a frustrating task and were able to recall more words following the active (vs. the passive) activity condition. Results demonstrate that short bouts of MVPA may be insufficient to produce global executive function gains in very young children, but may be helpful for enhancing performance in specific school-relevant domains. Therefore, these findings suggest the importance of re-establishing and maintaining MVPA opportunities in school settings.

CONTRIBUTIONS OF THE HOME ENVIRONMENT TO EARLY DISPARITIES IN LANGUAGE DEVELOPMENT

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Infants who perceptually tune to the phonemes of their native language at an earlier age, thereby losing the ability to discriminate non-native phonemes, develop stronger language skills later in childhood. We hypothesized that socioeconomic disparities, which have been associated with differences in the quality and quantity of language in the home, would account for individual differences in phonetic discrimination. Eighty-eight infants were assessed on measures of phonetic discrimination at 9-months and the quality of the home environment (HOME) at 15-months. Receptive and expressive language abilities were assessed at both ages. Although socioeconomic disparities in phonetic discrimination were not found, socioeconomic disparities were significantly associated with HOME scores ($p < .001$). After adjustment for SES, HOME scores accounted for unique variance in phonetic discrimination ($R^2 = .14$, $p < .001$), suggesting that infants from warmer, linguistically richer home environments at 15-months were more tuned to their native language and therefore less able to discriminate non-native contrasts at 9-months relative to infants whose home environments were less responsive. This association persisted when controlling for 9-month language abilities ($p = .002$), rendering it less likely that infants with better language abilities were simply engendering higher quality home interactions. Finally, a trend was found between phonetic discrimination skills at 9-months and language ability at 15-months, potentially supporting previous evidence that early speech discrimination may predict later language abilities. These findings suggest that nurturing home environments may be more critical than SES in contributing to language perception, with implications for language development more broadly.
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DYSREGULATION OF THE CORTISOL DIURNAL RHYTHM SOON AFTER ADOPTION PREDICTS ADHD SYMPTOMS YEARS LATER IN KINDERGARTEN FOR INTERNATIONALLY-ADOPTED CHILDREN

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Internationally adopted children are at increased risk for attention problems and Attention Deficit Hyperactivity Disorder (ADHD; Wiik et al., 2011). Presumably this reflects the impact of early life stress. Chronic stress is associated with alterations in the cortisol diurnal rhythm: lower early morning peak levels and flatter slope. The present study examined whether internationally-adopted children with this diurnal pattern would, years later, exhibit more attention problems. Eighty children (45% female) reared in orphanages/foster homes overseas for 6-36 months ($M = 19.4$ mos.) before US adoption and 35 non-adopted (NA; 54% female) comparison children completed four assessments over a two-year period, followed by a kindergarten classroom assessment. Morning cortisol levels and diurnal slope were aggregated across participants' first four assessments. Kindergarten teachers completed the ADHD subscale of the MacArthur Health and Behavior Questionnaire (Essex et al., 2002).
Preliminary analyses were conducted with 47 adopted and 22 NA children (remaining participant data still being processed will be re-analyzed for conference presentation). Preliminary results indicate that, compared with NAs, adopted children showed greater ADHD symptoms, $F(1,65) = 7.69$, $p < .01$. Adopted children were also more likely to show the cortisol pattern of low morning levels and flatter slope than were NAs, $\chi^2(1, N = 67)$

$= 5.09$, $p < .05$. Notably, this pattern predicted more ADHD symptoms in kindergarten, $F(1, 63) = 4.65$, $p < .05$. A formal test of mediation will be conducted within the final sample. Results suggest that early life stress is associated with an altered diurnal pattern of cortisol production and an increased risk for attention problems.
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EFFECTS OF IN UTERO ANTIDEPRESSANT (SERTRALINE) EXPOSURE ON OFFSPRING OUTCOME IN AN ANIMAL MODEL OF MATERNAL STRESS AND DEPRESSION

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Exposure to maternal medication *in utero* may have a crucial impact on the maturation of the undeveloped brain of the fetus. Currently, many women suffering from depression are successfully treated with antidepressants. However, the impact of antidepressants and the cessation of antidepressant administration during pregnancy on the developing fetus are still unclear. In the present study, we sought to investigate the impact of sertraline (a selective serotonin reuptake inhibitor; SSRI), given during pregnancy on the offspring's biological and behavioral development. First, female rats were treated with either chronic vehicle (oil) or corticosterone (CORT, 40mg/kg, s.c.) for 21 days to induce depressive like-behavior. Following 16 days of CORT administration, animals were further divided into either daily sertraline administration (20mg/kg, oral gavage) or vehicle (water). After cessation of the CORT or oil treatment, the female rats were mated with males until pregnant. One group of sertraline treated animals were continually treated with sertraline for 21 days (until parturition), while the other group discontinued sertraline treatment on gestational day (GD) 16. Offspring of each sex were sacrificed either on postnatal (PD) day 1, PD 30 or as adults after being tested in a battery of behavioral tests starting at PD90. We hypothesize that offspring of sertraline treated dams will show alterations in neurochemical profiles and differences in behavioral tests depending on the length of *in utero* sertraline exposure. Results from this study may help elucidate some of the potential impacts of exposure of antidepressants during pregnancy.

MODELING HIV-1-ASSOCIATED NEUROCOGNITIVE DISORDERS AND THE THERAPEUTIC EFFECTS OF THE PHYTOESTROGEN METABOLITE S-EQUOL IN THE HIV-1 TRANSGENIC RAT

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Combination antiretroviral therapy (cART) has been highly successful in reducing mother-to-child-transmission (MTCT) of AIDS in the U.S.; however, globally, MTCT is presently responsible for >1000 new HIV-1 infections each day. Longitudinal studies are critical for systematically addressing pediatric HIV-1/AIDS, and are also fundamental to our understanding of chronic HIV-1-associated neurological disorders (HAND). For the first experiment, ovariectomized Fischer HIV-1 transgenic (Tg) rats (ns = 41-43) were tested for prepulse inhibition (PPI) of the auditory startle response (ASR) bimonthly (2-8 months), using auditory and visual prepulses, and were also tested on a series of operant tasks tapping attention and executive function. Deficits were revealed in the development of perceptual sharpening, sustained attention, and core components of executive function. Daily oral treatments of the phytoestrogen metabolite S-equol (0.2 mg), administered to the animals at 6-8 months of age, improved the performance of the HIV-1 Tg animals on the sustained attention task. Assessment of neuronal networks with diOlistic labeling of pyramidal neurons in the PFC suggested that S-equol also ameliorated synaptodendritic alterations in the HIV-1 Tg animals. In experiment 2, treatment with S-equol. begun at 2-3 months of age, significantly protected against deficits in sustained attention and had enduring effects on performance one month after the treatment ended. In summary, the HIV-1 Tg rats displayed impaired performance in preattentive processing, attention, and executive function, prior to clinical signs of wasting. S-equol was effective in both ameliorating and preventing attentional deficits, suggesting that the microbiota-gut-brain axis may be an important venue for potential therapeutics for HAND.
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HEART RATE-MOVEMENT COUPLING IN HUMAN INFANTS: EFFECTS OF SLEEP POSITION

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The prone sleep position is a risk factor for Sudden Infant Death Syndrome (SIDS); however, it is unclear why prone sleeping increases SIDS risk. The ability of the autonomic nervous system to coordinate changes in heart rate (HR) with body movements (MOV), HR-MOV coupling, develops early and increases throughout gestation. Factors that decrease coupling between HR and MOV might place infants at greater risk. This study tested the hypothesis that coupling would decrease in the prone sleep position. The fidelity of HR-MOV coupling in newborn and one month old infants was assessed by quantifying cross correlation between HR and MOV. A continuous MOV signal was derived from low pass filtering of ECG. Preliminary analyses indicated that cross correlation (CC) between HR and MOV was not significantly different between newborn and one-month old infants. In addition, there was no significant effect of sleep state on CC. However, there were significant differences in CC as a function of sleep position, although differences were not in the direction predicted (prone, $n = 73$, $CC = 0.663 \pm 0.016$ vs supine, $N = 95$, 0.615 ± 0.014, $p = 0.03$). Three alternative interpretations for these unexpected effects are considered. One, for reasons unknown, higher coupling rather than lower may be a marker of risk. Second, higher HR-MOV coupling in the prone position may be an indicator of adaptation to the risk position. Three, HR-MOV coupling may be unrelated to risk. We are exploring these alternatives by examining other physiological signals and measuring coupling in other risk groups, e.g. preterm infants and/or affected by adverse exposures prenatally.
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PRENATAL AUDITORY ENRICHMENT MODIFIES PERCEPTUAL NARROWING IN BOBWHITE QUAIL NEONATES

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We explored whether enriched prenatal auditory experience could interfere with the emergence of a species-typical auditory preference in bobwhite quail neonates. Embryos received passive prenatal exposure to a soundtrack of (a) the maternal call of three species of quail (bobwhite, Japanese, and scaled quail mix), (b) only the Japanese maternal call, or (c) only the bobwhite maternal call for 10 min/hr for 24 hr per day during the last week of prenatal development. A control group received no enriched prenatal auditory experience. Following hatching, chicks from all groups were tested at either 24 hr or 48 hr for their preference between the bobwhite maternal call and the Japanese maternal call. Results indicated that prenatal auditory enrichment can interfere with the emergence of chicks' species-typical preference for the bobwhite maternal call, at least temporarily. Chicks receiving prenatal exposure to the quail mix and chicks receiving prenatal exposure to the Japanese maternal call both failed to prefer the bobwhite maternal call at 24 hr following hatching, but at 48 hr (after receiving two days of postnatal experience with broodmates) did prefer the species-typical bobwhite call. In contrast, chicks receiving prenatal exposure to the bobwhite maternal call and control chicks significantly preferred the bobwhite maternal call at both ages. Our results

suggest that diverse prenatal auditory experience can disrupt the typical postnatal emergence of a species-specific preference for the bobwhite maternal call in bobwhite chicks. Further, our results suggest that the course of perceptual narrowing during early development is guided by species-typical experience.
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MODELING VICARIOUS EXPERIENCE IN ADOLESCENT MICE

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This talk will summarize an experimental approach for measuring vicarious experience in laboratory mice. The paradigm entails allowing 'witnesses' to observe 'targets' undergoing a series of tone-shock parings, followed by an assessment of how the witness mice respond to tone-playbacks. Individuals form the gregarious C57BL6/6J (B6) strain exhibit heart rate deceleration in response to conspecific distress and freeze to social distress-associated tones, whereas mice from the less social BALB/cJ strain are much less responsive. This genetic background difference can be reproduced when playback of distress vocalizations is paired with a tone, indicating that the ascending auditory pathway is a major system engaged by conspecific distress. More recent studies demonstrate that the social environment during the first 3 weeks post-weaning are critical for this vicarious freezing phenotype: B6 mice deprived of adolescent social interaction do not maintain a vicarious freezing phenotype 24 hours post-conditioning. A longitudinal study of sociability and tone induced-vicarious freezing in 6 strains demonstrated substantial heritability of both phenotypes, but no genetic relationship between them, suggesting that sociability does not necessarily predict vicarious responding to others' distress. Along with the work of others, our findings bear similarities to the construct of empathy, whereby individuals adopt an emotional state more appropriate to another's situation than one's own. The utility of such animal behavior modeling approaches, however, ultimately relies on the ability to demonstrate consistency across species in brain responses to others' distress. The talk concludes with our recent attempts to map metabolic and inducible transcription factor responses in the mouse brain as they experience social distress.

DEVELOPMENTAL SSRI EXPOSURE AND NEUROENDOCRINE OUTCOMES

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Selective serotonin reuptake inhibitor (SSRI) medications are the most common antidepressant treatment used for maternal mood disorders during pregnancy and the postpartum period. Up to 10% of pregnant women are prescribed SSRIs. These medications cross the placenta raising questions about their role in fetal development. In turn, serotonin itself plays an important role in stress regulation and sexual differentiation of the brain. Recent clinical work shows that prenatal SSRI medications act to 'program' the developing HPA system. Preclinical research also points to a long-term effect of perinatal exposure to SSRIs on neuroendocrine outcomes related to both the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) systems. Not surprisingly, these effects markedly differ in male and female offspring. The present talk will highlight emerging evidence from preclinical studies investigating the impact of perinatal SSRI exposure on the developing HPA and HPG systems, investigating both neuroendocrine effects and behavioural outcomes. Overall, this research demonstrates that perinatal exposure to SSRI medications has a long-term impact on neuroendocrine and behavioral outcomes. In some cases exposure to SSRIs during development may act to protect against the effects of maternal stress. However, much more work is needed before we can fully understand the risks and benefits of developmental exposure to SSRIs.

DEVELOPMENTAL PROGRAMMING OF NEUROENDOCRINE AND MESOLIMBIC DOPAMINE PATHWAYS BY VARIATION IN MATERNAL CARE

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The hypothalamic medial preoptic area (MPOA) and the ventral tegmental area (VTA) both critically regulate maternal behavior, and are developmentally shaped by experience of maternal care. Variation in MPOA hormone receptor levels emerge by postnatal day 6 among female rats reared by low or high licking and grooming (LG) dams, and epigenetic regulation of estrogen receptor-alpha (ERα) is established prior to weaning. Variation in VTA dopamine cell levels are likewise evident by PN6 and may result from differences in transcription factors regulating dopamine neuron differentiation and maintenance. Furthermore, over-expression of ERα enhances both dopamine cell levels and maternal behaviors.
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WHAT THE MOTHER DOES AT NIGHT TIME TELLS US ABOUT MATERNAL SELF-STRESS REGULATION

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Maternal stress has been a major cause of problems in child development and the mother's ability to regulate her own stress response system is therefore important for the development of her offspring. Here, in the rat we show that maternal care related behaviors, specifically licking of the pups (LG) and arched-back nursing (ABN) at sleep time selectively predicts maternal ability to regulate her own hypothalamic-pituitary-adrenal (HPA) axis. In a study involving 19 dams and their litters maintained on a 12/12 light/dark cycle, we observed maternal care behavior during 2 sessions in the dark periods (DARK) and 2 lightt sessions (LIGHT) during PND 3-12 and obtained the following two measures of maternal self-stress regulation shortly after the pups were weaned: resting level of circulating CORT ($CORT_B$) and a rapid CORT response to a 1-min swim stress after a 5 min delay ($CORT_E$). We found that the frequency of ABN during LIGHT period is positively correlated with $CORT_B$ ($Rs = 0.580$, $p = 0.0086$) and negatively correlated with $CORT_E$ ($Rs = -0.530$, $p = 0.0186$) and the ABN during DARK period showed no statistically significant correlation with the two maternal CORT measures ($CORT_B$: $Rs = 0.060$, $p = 0.930$; $CORT_E$: $Rs = -0.130$, $p = 0.590$). Similar but marginally significant findings were found for DARK versus LIGHT LG behavior. In both cases, the mother's ability to maintain a low resting CORT level and to mount a rapid CORT response to environmental challenge are selectively predicted by a low frequency of ABN and LG during LIGHT period. These findings indicate that maternal care behavior observed during the animals day and night times have distinctive functional implications, with night time (LIGHT period) behavior alone serving as an effective marker for maternal HPA regulation. These patterns of association may suggest that poor maternal self-stress regulation contributes to an inappropriate level of maternal care behavior–-engaging too much active care when it is time to sleep, and the inappropriate level of maternal care behavior may also reciprocally contribute to poor maternal self-stress regulation. A practical implication of these findings might be that staying up when its time to sleep may have negative consequences for a mother's ability to regulate her own stress response system.

MOLECULAR AND TRANSGENERATIONAL BEHAVIORAL EFFECTS OF PRENATAL ETHANOL EXPOSURE

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We investigated ethanol-related neurophysiological and behavioral consequences of moderate PEE, during infancy and adolescence, and their transgenerational transmission. Pregnant Sprague Dawley rats received 1g/kg Ethanol (E) or water (W) via gavage once daily, or remained undisturbed (C) from gestational days 17-20. Some offspring (F1) were tested for voluntary alcohol consumption (PND 14) and some males for sensitivity to acute ethanol-induced sedation-hypnosis at 3.5g/kg or 4.5g/kg doses (PND 42) using the loss of righting reflex (LORR) test. A third set matured and produced a non-exposed F2 generation. At PND 42, brains were collected in a subset of undisturbed males (F1 and F2) to measure the expression of gamma aminobutyric acid (GABA) type-A receptor α1 and α4 and ≏ subunits proteins in several brain areas since the GABAergic system mediates ethanol-induced sedation-hypnosis.
Transgenerationally, PEE significantly increased infant ethanol consumption and attenuated LORR duration at 3.5g/kg but not 4.5g/kg dose, although the difference between F1 E and C consumption only approached significance. Both F1 E and W expressed higher α1 in the cerebral cortex than control while only W were higher than C but indifferent from E in F2, whereas α4 expression was unaffected. F1 E expressed higher ≏ in the cerebral cortex compared to C but not W, while no effect was observed in F2.
These results reveal that PEE may influence the behavior of multiple generations and although GABAA receptor subunit expression was altered in the F1, further investigation is needed to understand the underlying mechanisms of the transgenerational effect.
[The Developmental Exposure to Alcohol Research Center (DEARC) supported this project]

ADVERSE EARLY LIFE EXPERIENCE EVOKES DYSREGULATED EXPRESSION OF HISTONE MODIFYING ENZYMES ACROSS THE LIFE-SPAN IN THE RAT MEDIAL PREFRONTAL CORTEX

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Early life adverse experience has been demonstrated to exert a lasting influence on emotionality and cognitive behavior, often persisting long after the initial exposure to stress into aged life. Studies have demonstrated epigenetic regulation of gene expression as a powerful mechanism evoked by early experience to exert transcriptional control on neuronal function and behavior across a life span. In this study we have examined the expression of genes encoding histone modifying enzymes including histone acetyltransferases (HATs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and histone demethylases (KDMs) in the medial prefrontal cortex, a region strongly implicated in the top down control of emotional and cognitive behavior, at different time points across life. Further we have also asked how the age-dependent expression of histone modifying enzymes is altered in response to the early stress of maternal separation. We observe that early stress experience is associated with altered transcriptional regulation of genes encoding histone modifying enzymes suggesting the possibility that epigenetic machinery may itself be a target of downstream effects of early life adverse experience. We also observed a persistent decline in expression of certain genes like MLL1, 2, SMYD3 which regulate poised chromatin marks like H3K4 dimethylation. This transcriptional dysregulation is not accompanied by global changes in histone acetylation or histone methylation levels in early life or adulthood, possibly indicating that many of these histone modifying enzymes may regulate gene expression in a target specific rather than a global manner.

DIFFERENTIAL EXPRESSION OF TRYPTOPHAN HYDROXYLASE-2 AND FOS-FAMILY PROTEIN IN THE MATERNAL AND NON-MATERNAL BRAIN

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The peripartum period includes numerous neurochemical changes in the female mammalian brain including switching from aversion to attraction to pups. Serotonin pathways influence maternal behavior, but its molecular regulation is unknown. We found that tryptophan hydroxylase-2 (TPH2, enzyme for brain serotonin synthesis) is associated with differences in maternal behavior among virgin and postpartum females exposed to pups. FosB proteins (markers of chronic stimulation) may regulate the plasticity of TPH2. Similar to chronic drug and sex behavior, we hypothesize that differential FosB protein expression may be the "molecular switch" from aversion to attraction to pups in sensitized virgins and postpartum females. In fact, we found that early postpartum females express more ΔFosB protein in the nucleus accumbens core than do virgin females. Increased ΔFosB expression in the nucleus accumbens core of postpartum females may sensitize accumbens D1 cells to dopamine, enhancing rewarding feelings associated with maternal behavior. We also measured FosB protein expression in PPD 7 females that had pups removed on the day of parturition or remained with pups until PPD 7. We also examined virgin females that were repeatedly exposed to pups with some virgins showing maternal behavior while others did not. Our results suggest that TPH2 and FosB proteins may be important components of the molecular mechanisms involved in the onset of maternal behavior.
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SCOPOLAMINE IMPAIRS OBJECT-IN-CONTEXT AND OBJECT-PLACE-CONTEXT MEMORY IN DEVELOPING RATS

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The object-in-context (OiC) and object-place-context (OPC) tasks are variants of the novelty-preference paradigm that assess contextual recognition memory that is associative and incidental. The OiC task measures associative object-context memory (Dix & Aggleton, 1999), while the OPC task evaluates "episodic-like memory" for an object, the context in which it appeared, and its spatial location within the context (Eacott & Norman, 2004). Our previous work has demonstrated that in rats, these tasks have different ontogenetic profiles; the OiC task emerges before postnatal day (PD) 17, whereas the OPC task is observed on PD 31 but not PD 26. The present study further investigated the ontogeny of these tasks by examining the role of cholinergic systems in OiC and OPC recognition during development. Rats aged 26 d (for OiC task) and 31 d (for OPC task) were administered 0.5 mg/kg scopolamine hydrobromide, a cholinergic muscarinic antagonist, or saline injection prior to training/testing. Scopolamine impaired OiC and OPC memory in developing rats relative to saline controls. These findings suggest that cholinergic function may be critical in contextual recognition memory during development. Further experiments are underway to determine whether these cognitive deficits are specific to the contextual memory modality, and to ensure that the observed behavioral impairments were not due to peripheral cholinergic blockade.
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MOTHER-INFANT SYNCHRONY MODERATES THE RELATION BETWEEN EARLY ASSOCIATIVE LEARNING AND JOINT ATTENTION

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Contingency detection (Tarabulsy et al., 1996) and mother-infant synchrony (Feldman, 2007) are fundamental aspects of social development. However, little is known about how these factors interact to influence the development of social behavior. Recent findings suggest that heterogeneity in early associative learning (i.e., contingency detection) predicts quality of social behavior across infancy (Reeb-Sutherland et al., 2012). Here, we expand upon these initial findings by examining whether synchrony in mother-infant interactions moderates the relation between early associative learning and joint attention, a measure of early social cognition. The sample consisted of 51 infants (29 males). At 1 month, associative learning was assessed in sleeping infants using an eyeblink conditioning (EBC) paradigm. At 5 months, a 2-minute period of mother-infant social engagement was coded and a synchrony score was computed. At 12 months, the Early Social Communication Scale (ESCS) was administered and responding to (RJA) and initiating joint attention (IJA) were assessed. A significant two-way interaction between EBC and synchrony was found for IJA ($\beta = 2.07$, $SE = 1.002$, $\Delta R^2 = .069$, $p = .045$), but not for RJA. Follow-up analyses revealed a significant effect for high synchrony in which high EBC performance was related to increased IJA ($\beta = .613$, $SE = .242$, $p = .015$). No relation between synchrony and IJA was found for low EBC performance. The current results suggest that infants who have enhanced contingency detection early in life have enhanced joint attention later in life but only when they experience heightened synchrony with their mother. These findings reveal a novel mechanism through which the interaction between fundamental contingency detection and mother-infant synchrony impact the quality of social behavior development.
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THE EFFECT OF MATERNAL SMOKING ON FETAL MOUTH MOVEMENTS AND TOUCH BEHAVIOUR: A COMPARISON BETWEEN FETUSES OF NON-SMOKING MOTHERS AND SMOKING MOTHERS

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Although cigarette smoking is known to harm fetal development in terms of pre- and postnatal growth and increased risk of fetal mortality, morbidity as well as cognitive and neurological development, the effect of smoking on the development of specific fetal movement (fetal mouth-movement and fetal touch behaviour) during the second and third trimester of pregnancy is unknown.
A longitudinal cohort study recruited twenty fetuses (16 non-smoking mothers: 8 girls, 8 boys and 4 smoking mothers: 2 girls, 2 boys; n = 80 scans). Mothers were scanned four times at 24, 28, 32 and 36 weeks. Rates of fetal movements were coded from 4-d scans and analysed using a poisson log-linear mixed -model. Fetuses of smoking mothers showed a significantly higher rate of mouth movements compared to fetuses of non-smoking mothers, after controlling for maternal stress and depression. As pregnancy progressed these differences between the smoking and non-smoking groups widened. Differences between the two groups in the rate of self-touch, remained constant as pregnancy progressed and was borderline significant (p = 0.07). Furthermore, maternal self-reported stress was significantly related to fetal mouth movements and fetal touch behaviour; whereas maternal depression was only related to fetal facial movements.
This exploratory study indicates that fetal movement rates differ significantly between maternal smokers and non-smokers. A larger study is needed to investigate specific effects, including the interaction of maternal stress and maternal smoking.

COMPARED EEG ACTIVITY BETWEEN CHAMPIONS OF THE LOGIC OLYMPICS AND UNDERGRADUATE REGULAR STUDENTS DURING LOGICAL REASONING EXERCISES

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Electroencephalogram (EEG) is a non-invasive, economical and reliable method that allows us to represent electrical brain activity with high temporal resolution. The EEG signal portraits cerebral changes on frequency bands associated to cognitive tasks. Based on the Neural Efficiency Hypothesis, which suggests that there is a lower cortical activation in subjects with higher levels of intelligence, in this work we registered the brain electrical activity of 20 regular undergraduate students and 18 champions of the International Logic Olympiad. EEG activity was recorded over 14 scalp locations during rest (3 minutes) and during performance on 14 visually presented logical tasks (8-13 minutes). The latency and the accuracy of the responses were also measured. We found a significant difference between latencies: regular students responded faster than champions; however, as we expected, there was a lower accuracy of the responses in regular students. Preliminary EEG analysis shows an increase in posterior alpha power during the resting period in champions and undergraduate students. Additionally there was also a decrease in lower alpha and an increase in beta activity in anterior brain areas of regular students during the solving of logical reasoning. This change is probably associated with higher attentional demands. We did not find this last pattern of activity in the registers of three champions; however, we are still analyzing the EEG data from 15 champions.

DIFFERENCES IN EXECUTIVE FUNCTIONS OF ADOLESCENTS AND THEIR RELATION WITH BIRTH ORDER

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Within siblings birth order is a condition associated with physiological and behavioral differences. It has been reported that firstborns tend to behave more thoughtful, obedient and with better organization in achieving their goals; in contrast, in lastborn prevails a more spontaneous, risky and defiant behavior. These features are known as executive functions and depend on the frontal lobes functioning. Frontal lobes maturation begins in childhood and continues into adolescence and early adulthood, their development is related also with increased abilities in abstract reasoning, attentional shifting, response inhibition and processing speed. This is one of the reasons to consider that adolescence is a developmental period characterized by impulsivity, low regulation and control, suboptimal decisions. In this research, we tested two groups, one of 24 firstborn adolescents and another of 24 lastborn, using a Battery Performance of Executive Functions and Frontal lobes (BANFE). We found significant differences; firstborns scored higher in tasks related to orbito-medial area, this brain region is responsible for functions such as inhibitory control, risk avoidance and monitoring rules. Behavioral findings correspond with those described in the literature however, no difference in their performance in tasks involving cognitive flexibility and shifting was obtained. Future studies should consider larger samples.

REAL-TIME MEASUREMENT OF COVERT ATTENTION DURING FREE-LOOKING IN INFANTS

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From birth onward, visual foraging is a major way that infants gather information about their world. To understand this important behavior, we must discover how spontaneous gaze shifts are related to the dynamic allocation of attention to fixated *and* non-fixated spatial locations before the shift. Here we describe a new method for the *real-time* measurement of covert attention in young infants during *free-looking*. Objects in different

locations flickering at different rates are used to drive steady-state visual evoked potentials (SSVEPs) that are detectable in EEG recorded from the scalp. As we have previously described (Robertson et al, 2012), changes in the amplitude of the SSVEP driven by a flickering object reflect changes in attention allocated to the object in 12-week-olds. We have now developed efficient point-by-point computational routines (including artifact detection) in LabVIEW (National Instruments), which allow changes in the relative amplitudes of SSVEPs driven by multiple fixated and non-fixated objects to be tracked in real-time. This real-time information can be used to trigger stimulus events when specified conditions of covert attention, gaze, and timing are met. Such events can be used to probe the dynamics of visual foraging (e.g., facilitation and inhibition of return) in individual infants during free-looking. The computational routines will be described and illustrative raw and processed data from individual infants will be presented.
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MATERNAL AND PATERNAL POTENTIATION OF VOCALIZATIONS BY PRAIRIE VOLE PUPS

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After a brief reunion with their dam, re-isolated rat pups emit ultrasonic vocalizations (USV) at increased rates, compared to an initial isolation (maternal potentiation). We are testing the hypothesis that the magnitude of potentiation is a marker of the extent of co-regulation between the infant and its caretaker. Rat pups reared with their dam and sire demonstrate potentiation to the sire as well as to unfamiliar males, in contrast to the fear shown in response to the unfamiliar males by dam only-reared pups. Here we investigate for the first time maternal and paternal potentiation in the prairie vole, Microtus ochrogaster, a species recognized for monogamous pair-bonding and bi-parental rearing of pups. Prairie vole pups are known to emit USV when placed in isolation. Our preliminary work indicates that isolated, 8-11 day-old prairie vole pups also show USV potentiation following reunions with either anesthetized dam or sire, but not after contact with a group of anesthetized littermates. Pups reduce vocalizations during reunions with all three types of companions (contact quieting). Handled control pups show neither quieting nor potentiation. A differential potentiation response to the dam and sire in prairie voles when compared to other species may provide insight into the role of active parenting in the psychobiological processes that lead to co-regulation between parent and infant.
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MILK INTUBATION REDUCES NIPPLE ATTACHMENT BY DEPRIVED MOUSE PUPS

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Nipple attachment by rat pups 12-days-old or younger is unaffected by nutritional status: attachment latencies of deprived and non-deprived pups are nearly identical (e.g. Hall et al., 1975). In contrast, mouse pups (*Mus musculus*, C57BL/6) 4- to 12- days of age given access to the nipples of their anesthetized mother following deprivation, attach more quickly and more frequently than do non-deprived pups. Furthermore, after interacting with a female that provides maternal care but no milk, mouse pups exhibit satiety-like behaviors, as measured by increased attachment latencies and decreased attachment frequencies. In the current experiment we used an oral gavage of milk to isolate the potential nutritive influences on nipple attachment. On PND 8 or 12, pups were deprived of their mother for two hours. Deprived pups were intubated with milk (4% body weight) or received the intubation procedure but no milk. Following intubation, pups were tested in an apparatus that presented overhead the ventrum of the pups' anesthetized mother, allowing the pups to use natural postures to locate and attach to a nipple. Pups that received milk showed decreased frequencies and increased latencies to attach compared to pups in the intubation only groups. These results suggest mouse pup nipple attachment may be under the control of internal sensory mechanisms. Isolating the specific internal mechanism, nutrition, gut-fill, post-absorptive cues, or hydration, requires further investigation.
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PRENATAL CHOLINE SUPPLEMENTATION MAY PROTECT THE FETUS FROM SOME OF THE ADVERSE OUTCOMES OF PRENATAL STRESS

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Background: Prenatal maternal stress increases risk for offspring neuropsychiatric illness. However, there are no prevention strategies, at least in part because mechanisms have been difficult to elucidate. Using both human and murine models, we (a) examine the relationship between prenatal stress and cerebral inhibition development (b) develop a biologically-based model, and (c) use this model to test, in both animal and human studies, the preventive promise of perinatal choline supplementation.
Methods: Pregnant murine models receive either psychosocial or mock-infection (poly(I:C)); in humans prenatal stress is inferred by anxiety disorder or Interleukin 6 levels. P50 sensory gating (animal and human) and prepulse inhibition (animal) assess offspring cerebral inhibition. Perinatal choline supplementation is evaluated in animal and human studies. CHRNA7/Chrna7 genotypes are used to assess stimulation of the $\alpha 7$ nicotinic receptor as a potential mechanism.
Results: In both animals and humans, elevated prenatal stress is associated with impaired development of cerebral inhibition. In murine models, choline supplementation protects against the effects of stress; Human infants exposed to perinatal phosphatidylcholine supplementation also demonstrate improved cerebral inhibition development. CHNRA7/Chrna7 genotype predicts response to supplementation.
Discussion: Elevated prenatal stress leads to impaired cerebral inhibition development. Perinatal choline supplementation protects against that impact. The interaction between choline supplementation and genotype supports $\alpha 7$ nicotinic receptor stimulation as the mechanism. Dietary supplementation has the potential to improve neurodevelopment decreasing risk for later onset of mental illness.
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PROSPECTIVE MOTOR DEVELOPMENT THROUGH 18-MONTHS POST-BIRTH AFTER IN UTERO ANTIDEPRESSANT EXPOSURE

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Fetal exposure to serotonin reuptake inhibitor antidepressants (SRIs) alter serotonin levels in the fetus and may contribute to alterations in neurobehavioral development. This study presents motor development data

from a larger prospective longitudinal study of 243 pregnant women and their infants followed from the second trimester of pregnancy through 18-months post- delivery. Women were categorized into one of 3 groups based on standardized measures of current psychiatric diagnoses and treatment during pregnancy: No depression or SRI treatment (NoEXP), Depression without SRI treatment (DEP) and SRI treatment for depression (SRI). Their infants were examined with standardized measures of neurobehavioral and motor development at post-birth Weeks 1, 2 and 4, and Months 6 and 18. Generalized linear models were used to examine group differences in scores at each age, differences in relative motor performance on standardized scores across ages (repeated measures, longitudinal design), and relationships from early to later ages. Infants in the SRI group had less optimal quality of movement throughout the first month post-birth as well as less optimal postural control at 6 months post-birth compared to those in the NoEXP and the DEP groups. There were no significant differences between groups at 18-months post birth on measures of gross and fine-motor performance on the Peabody Motor Scales. However, there was a significant relationship between newborn quality of movement and 6-month postural control and 18-month motor scores. These data will be discussed in the context of maternal depression severity, dose-response measures, and covariates.

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ONTOGENY OF OBJECT-IN-CONTEXT RECOGNITION MEMORY RETENTION

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The novel object paradigm is useful for examining interactions among forebrain memory systems (Langston & Wood, 2010, Hippocampus, 20, 1139-53). One variant of this paradigm is Object-in-context (OiC) recognition. This task utilizes rats' preference for exploring novel objects in relation to the context they are encountered in (Dix & Aggleton 1999, Behav. Brain Res., 99, 191-200). Rats that explore a set of identical objects in one context (Objects AA in Context 1), followed by another set of identical objects in a second context (Objects BB in Context 2), show a preference for Object B when tested with Objects A and B in Context 1 (reflecting the novel mismatch of Object B in Context 1).We have previously reported that OiC recognition ontogenetically emerges before postnatal day (PD) 17, and performance in the OiC task does not increase with age from PD17 to PD31 (Ramsaran, Westbrook, & Stanton, 2013, Dev. Psychobiol., 55, p.784). The current study examines retention of OiC memory in developing rats, on PD 17 and 26 after a short (5-min) and long (24-hr) delay. The results show that both PD17 and PD26 rats are able retain conjunctive associations of objects and contexts after 5-min but not after 24-hr. These results suggest that the short-term and long-term memory capabilities for OiC recognition do not differ between preweanling and juvenile rats. Implications for the ontogeny of brain memory systems are discussed.

INFANT EXPLORATORY LEARNING AND THE DEVELOPMENT OF LOWER EXTREMITY COORDINATION: INFLUENCE OF PREMATURITY

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Infants born preterm with very low birth weight are at increased risk for developing spastic cerebral palsy, which is characterized by walking limitations due to excessive in-phase hip-knee joint coordination. Previous research demonstrates that full-term infants will exhibit less in-phase hip-knee coordination when specific leg actions are reinforced using an overhead infant mobile. The purpose of this study is to determine the ability of 3-4 month old preterm infants to: (1) learn through discovery, the contingency between leg action and mobile activation, and (2) demonstrate less in-phase hip-knee coordination when leg actions are reinforced with mobile activation. On two consecutive days, 14 full-term infants and 12 preterm infants participated in a task in which an overhead infant mobile rotated and played music when the infant moved either foot vertically across a virtual threshold. The full-term group, but not the preterm group, increased the percentage of mobile activation to meet performance criteria on Day 2. Neither group met learning criteria. However, both full-term and preterm groups contained infants that learned the contingency. Infants who learned the contingency, but not infants who did not learn the contingency, demonstrated less in-phase hip-knee joint coordination when interacting with the mobile on Day 2 as compared to spontaneous kicking on Day 1. Thus, some preterm infants may be able to generate less in-phase hip-knee coordination when participating in contingent learning paradigms. These results provide the initial scientific support for the development of early therapeutic interventions to reinforce more typical hip-knee coordination patterns of preterm infants.

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SOMATOTOPIC ORGANIZATION OF THE CEREBELLUM IN EARLY DEVELOPMENT

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Many areas of the brain are somatotopically organized, containing specific areas that process information for a specific part of the body. In most somatotopically organized brain areas, like the somatosensory cortex (S1), representation of adjacent body regions are contiguous in their neural representation. Somatotopy in the cerebellum, however, is "fractured," with the representation of body parts broken up into many small areas, distributed throughout the cerebellum. How and why this fractured somatotopy develops is unknown. In week-old rats, we have observed significant increases in complex and simple spike activity in the cerebellar cortex following twitches during active sleep and have hypothesized that such spontaneous activity contributes to activity-dependent neural development, including somatotopic organization**.** Therefore, we examined somatotopic organization in 8- and 12-day-old rats, a period in which the cerebellum is undergoing extensive structural and functional changes. For each pup, we provided unilateral stimulation of the whiskers and measured c-Fos expression in cerebellar cortex and deep cerebellar nuclei**.** Using this paradigm we observed somatotopic labeling of c-Fos at both ages and are currently measuring the distribution, location within the cerebellar microcircuitry, and density of c-Fos expression to examine age-related differences. Knowledge of cerebellar somatotopy at these young ages will allow for functionally precise electrophysiological recordings of cerebellar activity and for precise manipulations within this sensorimotor circuit.

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CYCLE-BASED EVALUATION OF NEONATAL SLEEP ARCHITECTURE INDICATES DIFFERING SLEEP PRESSURES THAN WINDOW-BASED OBSERVATION

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Neonatal sleep architecture is predominantly reported as a behavioral state proportion within convenience observation windows. Because sleep's biologically relevant unit is cycles, we explored neonatal sleep cycle organization during the first two days after birth.

Uninterrupted cycles of active/quiet sleep lasting $\geq$30 minutes from circumcised male ($n = 14$; 5 ± 3.4hr latency after circumcision), time-matched uncircumcised male ($n = 6$), and female ($n = 34$) neonates were

analyzed for infant state using pressure-sensitive, nonintrusive Motility Monitoring System-recorded respiratory and movement patterns. Males and females did not differ on: maternal age, parity, labor anesthetic, delivery or feeding method, data-relevant infant age, or number of qualifying cycles. 5-minute APGAR scores were all >7. Circumcised and uncircumcised males presented similar sleep-variable trends.
5-minute Apgar scores were related to TS% ($r = .490$, $p = .003$), and inversely to QS% ($r = -.522$, $p = .002$), for females, but not males. Males' cycle duration increased and quiet sleep (QS%) decreased from first-to-second measure, while females were unchanged ($p = .007$; $p = .032$). Males' increasing active sleep (AS%) trend marginally differed from females' decreasing trend ($p = .055$). Transitional sleep (TS%) increased only among males ($p = .009$). TS% was inversely related to QS% for males ($r = -.637$, $p = .002$) and females ($r = -.602$, $p < .001$) at later measures, but not to AS%. Males' cycle duration (increasing) and QS% (decreasing) differed from females, at a similar time when trends in others' work indicate overall QS rebound among circumcised males. The female-specific TS%-Apgar relation and AS% maintenance deserve further investigation. Here we highlight the need for a nontraditional, cyclic perspective in defining neonatal sleep architecture.
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Original project by E. B. Thoman]

MODIFYING DEPRESSION/ANXIETY-LIKE BEHAVIORS BY ENVIRONMENTAL MANIPULATIONS DURING PERI-ADOLESCENCE IN WISTAR KYOTO RATS

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Therapeutic potential of environmental intervention during peri-adolescence was studied using the WKY rat model of depression/anxiety-like genetic predisposition. Environmental enrichment (EE) was used to counteract genetic vulnerabilities towards depression/anxiety-like profiles by exposing WKY rats and their control strain Wistar to different environments during peri-adolescence, a sensitive time frame for emotional development. Rats were raised in EE from weaning to early adulthood (PND 60) and subsequently underwent behavioral tests. We also examined the role of social environment; rats of both strains were housed in large groups of 6-8 housemates from weaning until young adulthood. We further attempted to model a real-life situation such as an after school program by introducing rats of both strains to brief (2hr), daily exposures to an EE cage for a period of 10 days (PND 35-45). In early adulthood we performed behavioral tests as well as MRI with DTI analysis to assess differences in brain structure between those exposed to brief EE and controls. Overall WKY rats benefited greatly from exposure to all forms of EE and depression/anxiety-like indices were greatly reduced. Wistar rats were also influenced by the manipulations but with less pronounced results related more to changes in locomotion/exploration or anxiety like behavior and no effects on depression-like symptoms. The environmental manipulations modified the psychopathological-like phenotype of the WKY genetic rat model of depression and anxiety.

BRAINSTEM GABA RATHER THAN SEROTONIN CONTRIBUTES TO DELAYED AROUSAL TO HYPOXIA AFTER PRENATAL ALCOHOL EXPOSURE

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Prenatal alcohol exposure (PAE) increases the risk for the SIDS. Arousal to hypoxia is modulated by both medullary GABAergic and serotonergic (5-HT) mechanisms and is impaired after PAE. We hypothesized that PAE related impairment of arousal is also mediated by both GABAergic and 5-HT mechanisms. Pregnant dams received an ethanol (ETOH), iso-caloric pair fed (PF) or a standard chow (CHOW) diet. Artificial cerebrospinal fluid (ACSF) or nipecotic acid (NIP), a GABA reuptake inhibitor, was microinjected into the medullary raphe of P15 and P21 pups from each diet group. After recovery, pups were exposed to 4 episodes of hypoxia and the time to arousal (latency) was determined. Brainstem GABA and 5-HT was measured (HPLC) in pups from both ages from each diet group. A separate cohort of P15 pups from each group was exposed to 90 minutes of 10% oxygen and rapidly perfused to allow the double labelling of the early gene *c-Fos* and TPOH. At P21, after ACSF injections, ETOH pups had longer arousal latencies than the PF and CHOW pups combined (CON group) ($P < 0.001$) and higher brainstem levels of GABA ($P = 0.013$), but not 5-HT. NIP injected CON pups had arousal latencies similar to those in ACSF injected ETOH pups while NIP injected ETOH pups had no further increase in arousal latency. There were no differences in the number of 5-HT neurons or in the number of activated (FOS^+) 5-HT neurons during hypoxia. Our data suggest that GABAergic, rather than 5-HT mechanisms are important in PAE related arousal impairment.
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THE ROLE OF ACTIVE SLEEP IN POSTNATAL CEREBELLAR DEVELOPMENT

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The cerebellum is a critical sensorimotor structure that undergoes substantial postnatal development in rats. In 6-day-old rats, we have shown that Purkinje cell activity is state dependent, as seen by increased firing rates for both complex and simple spikes during active sleep. In the present study extracellular Purkinje cell activity was recorded in unanesthetized 4-, 8-, and 12-day-old rats as they cycled between sleep and wake. This age range spans a period when extensive functional and structural changes occur within the cerebellar cortex and deep cerebellar nuclei. We observed age-related changes in firing rate for both complex and simple spikes, as well as the emergence of a mature complex spike waveform by 12 days of age. State-dependent unit activity during active sleep peaked at 8 days of age, whereas 12-day-old rats showed an increase in the proportion of units exhibiting wake-related activity. At all three ages, units showed strong twitch-dependent activity suggesting that proprioceptive feedback from twitches plays an important role in the emerging sensorimotor integration function of the cerebellum throughout development. Finally, to test the importance of active sleep on cerebellar development, 8- and 12-day-old rats were sleep-deprived and expression of three plasticity-related genes (Arc/Arg3.1, Mapk3, and Cacna1a), important for Purkinje cell development, was measured. Following sleep deprivation, gene expression only differed at 8 days of age, suggesting that this age is a pivotal time point for the early phase of plasticity within the cerebellar system that is dependent on active sleep and twitching.
[This work is supported by NIH grant HD63071 awarded to MSB]

NEURODEVELOPMENTAL CONTRIBUTIONS TO SHIFTING AFFECTIVE-MOTIVATIONAL AND REGULATORY INTERACTIONS ACROSS ADOLESCENCE

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Adolescence is a phase of the lifespan associated with shifts in emotional and motivated behavior, paired with regulatory capacity that is continually being refined with experience. Our research probes the contributions of brain development (and associated changes in brain function) to shaping

interactions between affective-motivational and regulatory processes across development. This talk will feature research that probes human behavior and brain function while processing affective-motivationally relevant information with varying regulatory demands. Results emphasize how linear and nonlinear neurodevelopmental trajectories shape neural circuitry-level interactions across distal brain structures in childhood, adolescence, and early adulthood, which putatively shape affective and motivated behavior.

RAPID EMERGENCE OF AN ALTERED 5HT2A RESPONSIVITY AND PERTURBED IEG PATTERN FOLLOWING EARLY ADVERSE EXPERIENCE

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Rodent models of early life stress like Maternal Separation (MS) have been used to investigate the neural basis of early stress-mediated alterations in emotional behaviour. Animals with a history of MS show increased anxiety-like behaviour and a blunted hypothalamic-pituitary-adrenal (HPA) axis response to stress in adult life, as well as changes in gene expression in key cortical regions like the medial prefrontal cortex (mPFC). Also, MS animals exhibit potentiated responses when administered the 5-HT2A receptor agonist, DOI (1-(2, 5-dimethoxy-4-iodophenyl)-2-aminopropane) as evidenced by enhanced head twitch responses and by cortical induction of the immediate early gene (IEG) Arc mRNA. In the present study we addressed when the altered $5HT_{2A}$ responsivity emerges after the cessation of MS. We observed that MS animals showed an enhancement in the head twitch response upon administration of DOI at P21. We then assessed the pattern of DOI-induced neuronal activation at P21 by using the IEG c-fos as a marker. We noted both reduced baseline and DOI induced c-fos positive cell number in the mPFC of animals with a MS history, suggesting impaired DOI mediated induction of the IEG c-fos. Experiments are currently underway to probe the epigenetic and signaling mechanisms underlying this differential 5HT2A responsivity that arises during postnatal life soon after the cessation of MS. We are also in the process of addressing whether common epigenetic and signalling mechanisms may underlie the rapid emergence of altered 5HT2A responsivity following distinct models (postnatal fluoxetine treatment and maternal influenza infection) of perturbed early life experience.
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THE IMPACT OF DELAY ON VISUOSPATIAL WORKING MEMORY PERFORMANCE DURING TODDLERHOOD

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Visuospatial working memory (VSWM) refers to one's ability to retain and manipulate visual and spatial information for a short time. Age-related improvements in VSWM have been measured between the ages of 7.5 to 12 months, with 12-month-olds performing significantly better after longer delays than 7.5-month-olds on the AB task (Diamond, 1990). This progression has since been expanded to explore working memory development in children over 3 years (Ewing-Cobbs, Prasad, Landry, Kramer, & Leon, 2004). However, little has been done to investigate age-related differences or the impact of delay on VSWM in 1.5- to 3-year-olds. We tested 307 18-, 24-, and 30-month-olds using a VSWM object occlusion task, *Hide the Pots* (HTP; Bernier, Carlson & Whipple, 2010). A ball was hidden underneath 1 of 3 colored cups. Following either a 2s or 10s delay the child was allowed to retrieve the ball. There were 3 test trials and scores ranged from 0 to 3. At 24 months, performance was significantly better on the 2s versus 10s delay; $t(74.95) = 6.88$, $p < 0.01$, $M_{2s} = 2.48$, $SD_{2s} = 0.74$, $M_{10s} = 1.38$, $SD_{10s} = 1.03$. There were significant age-related increases in HTP performance between 18 and 24 months with a 2s delay: $t(212) = -5.05$, $p < 0.01$, $M_{18} = 1.91$, $SD_{18} = 0.89$, $M_{24} = 2.48$, $SD_{24} = 0.74$. Preliminary results show no significant age-related differences in performance between 18 and 30 months with a 10s delay ($M_{18} = 1.17$, $SD_{18} = 0.72$, $M_{24} = 1.38$, $SD_{24} = 1.03$, $M_{30} = 1.77$, $SD_{30} = 0.83$). These results reveal both age-related changes and uncover potential limits to VSWM during toddlerhood.
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RELATIONS BETWEEN MATERNAL AND INFANT PHYSIOLOGICAL STRESS: THE IMPORTANCE OF INFANT SLEEP

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Infants have immature physiological stress systems (Gunnar & Donzella, 2002). Early life stress is associated with disruption of children's daily salivary cortisol rhythms (Fisher et al., 2006) as well as risks for poorer medical and mental health later in life (Anda et al., 2010; Lupien et al., 2000). Therefore it is important to understand how maternal stress shapes infants' developing stress systems. 88 mother-infant dyads participated in a home visit when the infant was 6 months. Mothers collected salivary cortisol from themselves and their infants on 3 days at waking and bedtime. For both mother and infant, area under the curve (AUC) was calculated to assess cumulative diurnal cortisol exposure. Maternal hair cortisol indexed chronic physiological stress over the past 3 months. Finally, mothers reported on parenting stress and infant sleep duration. Infant AUC was not predicted by maternal salivary measures but instead was positively related to maternal hair cortisol ($r = .39$, $p < .01$). This suggests that maternal chronic physiological stress is a better predictor of infant cortisol exposure. Duration of nighttime infant sleep explained 14% of the variance in maternal hair cortisol ($R^2 = .14$, $F(1,74) = 13.13$, $p < .01$), while maternal perceived stress did not relate to maternal hair cortisol. Mediation analyses revealed that infant sleep indirectly influenced infant AUC through its effect on maternal hair cortisol ($ab = -.0024$, 95% CI [-.0069, -.0006]), while there was no direct effect of infant sleep on infant AUC. These results demonstrate that maternal sleep deprivation due to infant sleep difficulties takes a physiological toll and this maternal chronic stress is a risk factor for infants' cumulative cortisol exposure.

MU RHYTHM SUPPRESSION IN TERM AND PRETERM INFANTS

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Infants born prematurely are at increased risk for long-term cognitive delays or impairments relative to healthy term infants. Surprisingly, medical course during the immediate post-natal period does not provide good predictive power as to which infants will experience long-term impairments. Furthermore, behavioral assessments at young ages also do not provide sufficient sensitivity to determine the children at highest risk. Therefore, we assessed preterm infants (<29 weeks gestational age) with an uncomplicated course at 3 & 6 months corrected age relative to healthy full-term infants. We measured the responsiveness of the sensorimotor resting mu rhythm using MEG during an imitation and resting task. The infants also underwent developmental assessment using the Bayley Scales of Infant Development III (BSID-III). The preterm infants at 3 months corrected-age had significantly lower cognitive scores on the BSID-III relative to the term infants. A pattern of mu rhythm suppression was observed across groups with greatest mu power during rest and mu rhythm

suppression during infant squeeze conditions. Furthermore, the frequency of the second harmonic of the mu rhythm was significantly greater in preterm infants relative to term infants. In light of our previous results indicating a linear increase in mu rhythm frequency in healthy term infants 0-12 months, the current results suggest that the preterm infants' sensorimotor mu rhythm may be more developmentally advanced relative to term infants. This result is contrary to our hypothesis that mu rhythm development would be delayed in preterm relative to term infants and may be associated with their preterm birth.
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LABORATORY RATS GONE WILD: MOTHERHOOD IN A NATURAL CONTEXT

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Experiences shape the behavior of an organism throughout its lifetime, allowing for adaptable and flexible responses to changing environments. Experience-driven behavioral modifications are mediated by epigenetic mechanisms that program patterns of gene expression within the brain. To identify molecular genetic and epigenetic mechanisms that drive change, standard laboratory models in which rodents gain single, somewhat one-dimensional, experiences are used. For example, the experience of becoming a mother results in a sustained sensitivity toward pups, which functions to ensure the mother will successfully handle the demands of motherhood. Yet, how demanding is motherhood under standard laboratory conditions? My laboratory has initiated an innovative research program to tackle this issue. By comparing the experience of motherhood in a standard rat cage with an outdoor, semi-natural environment roughly 3000X its size, we are exploring how complex experiences shape behavior. Continuous video monitoring has allowed us to link adaptable behavioral responses of new mothers to molecular alterations within the brain that drive plasticity across neural circuits involved in the regulation of maternal behavior.

FEAR OF NOVELTY IS REDUCED BY EARLY POSTNATAL EXPOSURE TO NOVEL STIMULI IN BOBWHITE QUAIL

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Neophobia, the fear of novelty, is a behavioral trait found across a number of animal species, including humans. Although sometimes adaptive, the sorts of behavior associated with this fear can often prevent the exploration of different environments, thereby limiting access to potentially beneficial resources. The present study assessed how a brief period of early postnatal exposure to one novel stimulus could potentially generalize and serve to decrease fear of novelty when tested in the presence of markedly different novel stimuli. 390 bobwhite quail neonates were allocated to different postnatal exposure conditions (24 hours of exposure to a novel aversive auditory tone, a novel aversive visual stimulus, or no exposure) then tested within one of two apparatuses. Measures of exploration were assessed as indicators of general fearfulness. Results revealed that 24 hours of postnatal exposure to a novel visual stimulus effectively reduced the expression of fearful behaviors in chicks (compared to naïve chicks) when subsequently tested in the presence of a novel auditory tone. Chicks receiving postnatal exposure to a novel auditory tone also subsequently demonstrated decreased levels of fearfulness when tested in the presence of the novel visual stimulus. These results indicate that exposing chicks to one type of novel stimulus during early development can influence how they behave in the presence of novel stimuli with markedly different properties. More generally, our results suggest that experience with novel stimuli can moderate whether and to what extent neophobia will develop during early development.
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DIFFERENCES IN RECRUITMENT OF FLEXOR AND EXTENSOR MUSCLES DURING STEPPING IN CHICK EMBRYOS

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Repetitive flexor and extensor muscle activity produces stepping. However, during late embryogenesis, many sequences of stepping appear to be produced by flexor activity alone, suggesting excitatory drive may have a flexor bias. The objective of this study is to determine if there is a difference in flexor and extensor muscle recruitment during stepping.
Embryonic day 20, we recorded ankle flexor and extensor muscle activity bilaterally. Differences in recruitment were tested by comparing within subject the total number (burst count) of flexor and extensor bursts during stepping ($N = 6$). Left and right ankle muscles were analyzed separately to account for potential effects of asymmetric posture. A flexor recruitment bias was defined as a flexor burst count $\geq$ 1.5X extensor burst count. Recruitment failures within sequences of rhythmic bursting (i.e., burst deletions) were also analyzed.
Preliminary results indicate an overall greater recruitment of ankle flexor muscles during stepping. Results for the left ankle indicated that 2 of 6 embryos demonstrated a flexor bias; flexor bursts outnumbered extensor bursts, and rhythmic flexor activity exhibited fewer burst deletions than extensor activity in all embryos. In the right ankle, 3 embryos demonstrated a flexor recruitment bias. However, right ankle extensor bursts outnumbered flexor bursts in 3 embryos, and extensor burst deletions were more prevalent in 3 embryos, while flexor deletions were more prevalent in 3 embryos.
Our data provide preliminary evidence that excitatory drive may have a flexor bias during stepping in late embryogenesis. Findings also may be relevant to other behaviors.
[NIH HD053367 to NSB]

HISTAMINERGIC MODULATION OF LOCOMOTOR ACTIVITY UNDER CONDITIONS OF SUBTHRESHOLD NEUROTRANSMITTER STIMULATION IN THE NEONATAL RAT, AN IN VITRO AND IN VIVO STUDY

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It is well known that various neurotransmitters are in involved in the initiation and modulation of locomotor activity, notably including glutamate, GABA, serotonin, and dopamine. However, comparatively little is know about the role of histamine in modulating locomotor circuit function. Here we examine the effect of histaminergic modulation of locomotor activity in the newborn rat, under conditions of subthreshold neurotransmitter stimulation. In an in vitro experiment, we show that the H_1 receptor agonist pyridylethylamine (20 micromolar) induces fictive locomotion (as recorded from left and right ventral roots at the lumbar level) in the isolated spinal cord of the newborn rat, when combined with subthreshold levels of neurotransmitter stimulation (5-HT and NMDA). Next we show that hindlimb air-stepping behavior is induced in vivo when pyridylethylamine (1.22 mg/kg) is administered intraperitoneally in combination with a subthreshold concentration of the serotonergic receptor agonist (1.0 mg/kg quipazine), but not when administered alone. These results reveal a previously unknown role of histamine on spinal locomotor function.
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THE IMPACT OF SLEEP DEPRIVATION AND CHRONIC STRESS ON INFANT FRONTAL GAMMA ACTIVITY IN SOCIAL AND NONSOCIAL CONTEXTS

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EEG gamma activity predicts cognitive and language function, yet little is known about environmental influences on gamma. We examined gamma (25-48 Hz) as a function of sleep, chronic stress, and recording context. Using a within-subjects design, we recorded EEG from 12-month-old infants (N = 63) in four conditions. In two face-to-face conditions, an experimenter directed the infant's attention to pictures (joint attention) or played peek-a-boo (social engagement). In two nonsocial conditions, the experimenter was behind a curtain and the infant viewed pictures while the experimenter stayed silent (nonsocial) or commented on the pictures (language-only). Infant hair cortisol indexed chronic physiological stress, and mothers reported duration of infants' nighttime sleep. Sleep duration related to greater increase in left frontal polar (LFP) gamma power during both face-to-face conditions compared to both nonsocial conditions, $r = .41$, $p = .004$, as well as greater increase in right frontal (RFP) gamma power during joint attention compared to the nonsocial baseline, $r = .33$, $p = .04$. These findings suggest that sleep-deprived infants show less neural activation to social stimulation. A general linear model associated higher infant hair cortisol with lower LFP-RFP gamma coherence, $F(1,28) = 7.82$, $p = .009$. There was a condition x cortisol interaction, $F(1,28) = 6.56$, $p = .016$, such that hair cortisol predicted reduced prefrontal functional connectivity during the two face-to-face conditions and the language-only condition, but was unrelated to connectivity during the nonsocial baseline. Results indicate the sensitivity of high-frequency neural activity to sleep deprivation and chronic stress, particularly during social interactions, and underscore the importance of obtaining measures of functional brain activity across multiple contexts.

RESPIRATION PATTERN RECOGNITION FOR AUTOMATED CLASSIFICATION OF SLEEP STATES IN NEWBORN INFANTS

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Changes in newborn sleep state are accompanied by alterations in many key physiological measurements, such as heart rate variability, respiratory patterns, oxygen consumption and cerebral blood flow. As a result, sleep state patterns are often used as dependent or outcome variables used to monitor effects of exposure or interventions; for instance, state distribution changes with diet, drugs, sleeping position, and physical stimulation. Despite its importance, the automated assignment of state in the immature neonate remains difficult and unreliable. We developed an automated pattern recognition methodology based on respiration data and applied the algorithm to identify sleep states in 48 newborn infants. Respiration, along with behavioral coding, was collected during a ten-minute baseline period. Sleep states (active, quiet, indeterminate and awake) were identified based on benchmarking the variance of the instantaneous breathing ratio (IBR) and moving average for every sixty-second epoch against threshold values that were determined using a separate sample of 1,074 newborn infants collected in other ongoing studies. The performance of the automated system was compared to manual sleep codes. Results showed an overall agreement of 95% (median value) between the automated methodology and the human expert for these 48 studies. Given that sleep state has pervasive influences on cardiorespiratory, neuroelectric, and behavioral activity, this automated technique may offer a feasible and standard approach for analysis of existing data sets and future large cohort studies.
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MOTION AND MULTISENSORY REDUNDANCY DRIVE THE DEVELOPMENTAL SHIFT OF INFANT SELECTIVE ATTENTION TO THE MOUTH OF A TALKER

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Lewkowicz & Hansen-Tift (2012) found a shift in human infants' selective attention from the eyes of a talker at 4 months of age to the mouth of the talker at 8 and 10 months. Importantly this shift is correlated with the emergence of babbling and a growing interest in speech. Because of this, attention to the mouth can provide infants with redundant and, thus, maximally salient audiovisual cues which can, in turn, facilitate speech and language acquisition. Here, we investigated the separate role of mouth movement and vocalization cues in the attentional shift from the eyes to the mouth of a talker. Using an eye-tracker, we measured selective attention in 4-, 8-, and 10-month-old infants when they were exposed to a static/silent face (Experiment 1), a static/talking face (Experiment 2), and a silently talking face (Experiment 3). In Experiments 1 and 2 all age groups attended more to the eyes whereas in Experiment 3, 4- and 8-month-olds looked more at the eyes while 10-month-olds, looked more at the mouth. Overall, these findings show that the eyes-to-mouth attentional shift observed in infants between 4 and 8 months of age is mediated by three interacting factors. These are an emerging interest in speech, dynamic visual speech cues, and the redundancy of audiovisual speech.
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DIFFERENTIAL PROCESSING OF SENSORY REAFFERENCE FROM SELF-GENERATED MOVEMENTS

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Effective motor control requires that we distinguish sensations arising from self-generated movements (i.e., reafference) from those arising from other-generated movements (i.e., exafference). To do this, reafferent signals are compared to motor copies (or corollary discharges). Recent findings in newborn rats demonstrate that myoclonic twitches, limb movements exclusive to REM sleep, produce sensory responses throughout the brain, whereas wake-related limb movements do not. Here, while recording from the hindlimb region of primary motor cortex (M1), we show the same differential processing of twitch- and wake-related reafference and demonstrate that this effect is not due to a global suppression of sensory feedback during wakefulness. Next, we tested the hypothesis that twitches are processed as if they are unexpected. To achieve this, we evoked self-generated movements using manipulations that differ with respect to their expectancy and, therefore, their presumed recruitment of corollary discharge. Only unexpected movements triggered M1 activity, thus supporting the notion that twitches, uniquely among all known self-generated movements, are processed as if they lack corollary discharge. This unique feature is necessary if twitches are to drive activity-dependent development of the sensorimotor system.
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NEONATAL NEUROMETABONOMIC SIGNATURES PREDICT ADULT CORPUS CALLOSUM VOLUME AND ANXIETY RESPONSES FOLLOWING INTRAUTERINE ASPHYXIA AND/OR C-SECTION DELIVERY

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Parturition is a critical process in mammalian development. Intrauterine disturbances such as perinatal asphyxia, and Caesarean Section (C-section) delivery can harm the immature central nervous system and disrupt normal trajectories of early brain development, increasing the risk for symptoms of neural dysfunction later in life. Identifying presymptomatic signs of disrupted neurodevelopmental trajectories early on can lead to more effective methods of intervention. Most neonatal brain injury, or lack of brain injury in C-section, influences the metabolic system, which can be observed through spectral-based neuroimaging of brain metabolic profiles, leading to the identification of biomarkers for neurodevelopmental dysfunction. Following exposure to intrauterine asphyxia and/or C-section delivery, we combined metabonomics with high-resolution 7-tesla proton magnetic resonance spectroscopic (7T H MRS) imaging to acquire and analyze time-dependent, dynamic neurometabolic responses of neonatal rat striatum. Profiles of neurometabolites significantly differed for asphyxiated and non-asphyxiated infant rats. Regression analysis revealed that glutamate + glutamine to creatine + phosphocreatine (Glu + Gln/Cr + PCr), and glycophosphocholine + phosphocholine to Cr + PCr (GPC + Pch/Cr + Pcr) ratios significantly predicted adult corpus callosum volume. Correlation analyses revealed that inositol to Cr + PCr (Ins/Cr + PCr) and taurine to Cr + PCr (Tau/Cr + PCr) ratios were significantly correlated with anxiety responses to novel object and an unknown intruder. These findings suggest early identification of metabolic biosignatures following a birth insult can lead to early diagnosis of neurodevelopmental dysfunction.

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NEONATAL PAIN EXPOSURE AND EARLY BRAIN MATURATION PREDICTS NEURODEVELOPMENT AT 18 MONTHS IN CHILDREN BORN VERY PRETERM

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Background: Infants born very preterm are exposed to frequent invasive procedures during neonatal intensive care, which are associated with altered brain development. Our aim was to examine whether procedural stress/pain, together with changes in brain microstructure over time, are associated with cognitive, language and motor outcomes at 18 months corrected age (CA) in children born very preterm.
Methods: 120 infants born $\leq$32 weeks gestational age had serial diffusion tensor imaging scans near birth and again at term-equivalent age. Fractional anisotropy (FA) as an index of brain maturation was measured in 7 functionally related brain region groups. FA change was calculated as the difference between the first and second scans divided by the time between scans. Procedural stress/pain was operationalized as the number of invasive procedures from birth to term-equivalent age, adjusted for neonatal clinical factors related to prematurity. Outcome was assessed at 18 months CA using the Bayley-III.
Results: The interaction between the number of invasive procedures (adjusted for clinical confounders) and FA change of the white matter was associated with cognitive outcome (adjusted $R^2 = 0.17$, $p = 0.04$). The interaction between the number of invasive procedures and FA change of the gray matter was associated with motor outcome (adjusted $R^2 = 0.32$, $p = 0.04$).
Interpretation: Greater exposure to invasive procedures and slower brain maturation predicted poorer neurodevelopmental outcomes at 18 months CA. The extent to which stress and pain management strategies are neuroprotective needs to be evaluated in order to optimize outcomes in children born very preterm.

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ORGANIZATIONAL EFFECTS OF MATERNAL TESTOSTERONE ON FETAL NEUROBEHAVIOR

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The architecture and neural scaffolding of the human brain is erected in the brief 266 days of gestation, making the fetus particularly vulnerable to organizing influences of the intrauterine environment. Dramatic alterations in the maternal endocrine state serve to maintain the pregnancy and facilitate maturation of the fetal nervous system, directly reaching the fetus via the placenta and indirectly effecting changes to placental function. Although it is recognized that maternal sex steroids target the fetal brain, the organizational role of maternal testosterone is not well understood. The association of maternal testosterone and fetal neurobehavior was examined in a longitudinal cohort of 177 maternal-fetal dyads measured at 24, 30, and 36 weeks gestation. Testosterone was measured in maternal saliva and fetal neurobehavior was assessed by measures of fetal heart rate, movement, and their integration, collected by fetal actocardiograph. Results revealed that maternal testosterone levels significantly increased across the second half of gestation and exhibited within-individual stability across gestational periods. Maternal testosterone did not vary by fetal sex. By near term, at 36 weeks gestation, fetuses of women with higher testosterone presented with a higher heart rate and showed less integration of somatic and cardiac systems, indexed by lower coupling and longer latencies between movement excursions and cardiac accelerations. These preliminary findings suggest that maternal prenatal testosterone exerts accumulating organizational effects on maturation of the fetal nervous system.

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SELECTIVE SOCIAL BUFFERING OF BEHAVIORAL AND ENDOCRINE RESPONSES AND FOS INDUCTION IN THE PRELIMBIC CORTEX OF INFANTS EXPOSED TO A NOVEL ENVIRONMENT

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In mammals, the presence of the mother can reduce or "buffer" stress responses of her young in threatening conditions. We compared the stress buffering effects of the mother, a familiar littermate, and an unfamiliar adult male in guinea pig pups exposed to a novel environment. Three classes of response were examined: short latency active behaviors (i.e., vocalizations), slower developing passive, depressive-like behaviors driven by inflammatory mechanisms, and hypothalamic-pituitary-adrenal (HPA) activity. We also examined Fos induction in the prelimbic cortex, a prefrontal region hypothesized to mediate stress-buffering effects. Only the mother significantly reduced all classes of response, and depressive-like responses were reduced only by the mother. Contrary to expectations, the unfamiliar adult male reduced plasma cortisol levels of the pups as effectively as did the mother. Additionally, the presence of the unfamiliar adult male resulted in an increase of Fos induction in the prelimbic cortex as well as high levels of social interactions. The presence of the mother was not associated with prelimbic activity. These results confirm the ability of the mother to reduce active behavioral and HPA responses, and suggest a specific maternal buffering effect on proinflammatory mediated depressive-like behavioral responses. The findings also demonstrate an unexpected ability of adult males to reduce HPA responses and raise the possibility that different social partners can buffer HPA activity through different underlying processes.

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HIEARCHICAL RULE LEARNING AND GENERALIZATION IN INFANCY

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The ability to learn and generalize abstract rules is a hallmark of flexible human cognition. Adults spontaneously learn and generalize hierarchical 'rule-set' structures without instruction through interactions between prefrontal cortex and striatum. However, there have been no investigations of this learning mechanism in infancy. We presented twenty 8 month-old infants with cue-target location pairs during a learning and a generalization task. The cues varied by shape and color and the target location consisted of an animated toy presented in one of four quadrants of the computer screen. These pairings could be learned as four separate cue-target location rules, using simple associative learning mechanisms. Alternatively, if infants used one dimension (color or shape) as a higher-order context, the lower-order pairings could be learned as abstract rule-sets, allowing them to be generalized to novel instances. Our data indicated that infants spontaneously created hierarchical rule sets during incidental learning—despite no cues or incentive to do so. Infants also generalized rule sets to support learning in a novel context. Eye blink rate, a physiological indicator of striatal dopamine activity, mirrored behavioral learning patterns. These results are the first to demonstrate hierarchical rule learning in infancy, providing evidence that the human brain is predisposed to create efficient hierarchical rule representations from environmental input.
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NEONATAL ETHANOL EXPOSURE IMPAIRS INCIDENTAL SPATIAL LEARNING IN THE JUVENILE RAT: EFFECTS OF EXPOSURE SCENARIO

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Fetal alcohol spectrum disorders (FASDs) are neurocognitive disorders that include behavioral impairments resulting from exposure to alcohol during gestation. Early exposure to alcohol leads to deficits in spatial memory (Dokovna, Jablonski, & Stanton, 2013; Jablonski & Stanton, 2014; Goodlett & Johnson, 1997; Goodlett & Pearson, 1995; Murawski & Stanton, 2010, 2011). Using the object location recognition task, incidental (non-reinforced) spatial learning was examined in a rat model of FASDs in which rats received binge-like (5.25 g/kg/day) alcohol (EtOH) exposure during the third trimester-equivalent or sham-intubations (SI). In Experiment 1, rats exposed to EtOH or SI from postnatal day (PD) 7-9 and tested on PD26 performed the object location (OL) task by preferentially exploring the object in the novel location compared to the object in the familiar location after both 5-min and 24-hr retention intervals. In Experiment 2, rats exposed to EtOH from PD4-9 and tested on PD26 did not preferentially explore the displaced object after either delay, while the SI rats did show a preference for the displaced object. However, PD26 rats exposed to EtOH or SI from PD4-9 display novel object recognition, suggesting that the PD4-9 EtOH deficit in the OL task is not a performance effect. The presence of an alcohol deficit in the OL task in juvenile rats following PD4-9 exposure but not PD7-9 exposure suggests that the different exposure windows may target different brain areas and/or processes underlying incidental spatial learning tasks.
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DOES CONGENITAL HEART DISEASE IMPACT NEWBORN HEART RATE AND HEART RATE VARIABILITY?

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Newborn heart rate (HR) and HR variability are known predictors of developmental outcomes in later childhood. We explored differences in HR and HR variability between newborns with and without congenital heart disease (CHD) in active (AS) and quiet sleep (QS). HR was recorded during 10 minutes of supine sleep from control and unrepaired CHD neonates. Differences between HR and HR variability at low (0.008-0.05 Hz), mid (0.05-0.1 Hz), and high (0.1-0.5 Hz) frequency were assessed using Student's t test and ANOVA. We obtained 113 recordings from 72 control and 41 CHD newborns with hypoplastic left heart syndrome, HLHS ($N = 15$), transposition of the great arteries ($N = 11$), and tetralogy of Fallot ($N = 15$). The CHD group demonstrated higher HR (QS: 145 ± 12 vs 119 ± 9 bpm, $p < 0.001$; AS: 143 ± 13 vs 127 ± 10 bpm, $p < 0.001$) and diminished variability ($p < 0.001$) compared with controls. While controlling for gestational age, hours of life at recording, prostaglandin administration, respiratory support, and sex in a multivariable model, CHD status remained an independent predictor of mean HR, median HR and low frequency HR variability in quiet sleep and mean HR, median HR, and low, mid, and high frequency HR variability in active sleep. HLHS demonstrated the largest effects of all diagnostic groups. Newborn HR is higher and HR variability is lower in CHD newborns compared with controls. Thus, these infants begin their postnatal lives with atypical autonomic profiles and altered capacities to deal with environmental challenges. Whether these differences predict later neurodevelopment remains to be investigated.
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PUBERTY-INDUCED CHANGES IN NEURON AND GLIA NUMBER THE PREFRONTAL CORTEX OF MALE AND FEMALE RATS

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Adolescence is a critical period for brain maturation, characterized by the reorganization of many complex neural networks. The medial prefrontal cortex (mPFC), a region highly involved in executive function, is particularly known to undergo synaptic pruning during this time. We have previously shown that rats lose neurons in the mPFC between early adolescence and adulthood. However, neuronal loss was substantially greater in females during this time. Previous data from our lab also show that an increase in ovarian hormones during puberty play a significant role in neuronal loss in females, as OVX prevented this cellular loss. In the present study, we describe neuronal and glial changes in the male and female mPFC at multiple time points from preadolescence to adulthood (P25, P35, P45, P60 and P90). In addition, we correlate the cellular changes during this time with the observance of puberty markers. Our preliminary analysis confirmed a greater neuronal loss in the female mPFC between adolescence and adulthood, and that the timing of neuronal loss in females coincides with the onset of puberty (between P35 and P45). Elucidation of the precise timing of cellular pruning of the mPFC will have clinical implications in respect to mental illnesses characterized by mPFC dysfunction, such as depression, ADHD and schizophrenia, and could potentially explain the observed sex differences in prevalence of these disorders.
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ADOLESCENT EXPOSURE TO BISPHENOL A AFFECTS NEURON AND GLIA NUMBER IN THE ADULT RAT PREFRONTAL CORTEX DIFFERENTLY BETWEEN THE SEXES

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Bisphenol A (BPA), an endocrine disruptor used in a variety of consumer products, has been found to alter neuroanatomical structure in multiple brain areas. However, few studies have examined long-term effects on the prefrontal cortex, an area where both the number of neurons and glia change during adolescence. In the current study, Long-Evans male and female rats were administered 0, 4, 40, or 400 μg/kg/day BPA during

adolescent development (postnatal days 27-46). In adulthood (postnatal day 150), the number of neurons and glia in the prefrontal cortex were stereologically assessed. There were no changes in the number of neurons, but there was a significant dose x sex interaction in number of ($p = .05$). Pairwise comparisons between controls and each dose show a significant increase in the number of glia between 0 and 40 μg/kg/day in females ($p = .04$), and a trend towards a significant decrease in the number of glia between 0 and 4 μg/kg/day in males ($p = .06$). In order to determine the type of glia, immunohistochemistry was conducted to assess astrocyte number in the prefrontal cortex. There is a sex difference between male and female control animals with females having fewer astrocytes in layer 5/6 than the males ($p = .04$). There is also a trend towards an increase in astrocytes between 0 and 40 μg/kg/day in females ($p = .08$). There was no change in astrocyte number between 0 and 4 μg/kg/day in males. Ongoing research includes immunohistochemistry to assess the number of microglia in the prefrontal cortex.
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EEG ALPHA POWER INCREASES IN RESPONSE TO A NOVEL ODOR IN THE SLEEPING NEWBORN

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In human newborns, the capacity to learn and discriminate between familiar and novel odors during sleep may be critical to adaptive functioning in the extra-uterine environment, and aid mother-infant attachment. In this study, we investigated if sleeping newborn infants were able to discriminate between a novel odor (orange) and a familiar odor (infant formula). High-density electroencephalogram (EEG) was recorded from healthy full-term infants ($n = 6$; 12-24h postnatal age; 37-41w gestational age). The stimuli were presented in 10 blocks of 4 minutes duration, each block consisting of contiguous 1 minute presentations of clean air, formula, air, and orange. A constant airflow of 1L/min was delivered to the vicinity of the nostrils. During the first 20 minutes of the paradigm, there was a 47% increase ($p < .001$) in frontal alpha power from baseline during presentations of the novel odor (orange), and a non-significant decrease (1%) in frontal alpha power from baseline during presentation of the familiar odor. During the second 20 minutes, there was a 51% increase ($p < .001$) in frontal alpha power from baseline during presentation of the novel odor. Again, there was no significant response to the odor of infant formula. We hypothesize that the increased EEG alpha power during presentation of a novel odor may be related to arousal during sleep. This paradigm has potential utility as a non-invasive means to assess the salience of various odors and, in turn, their potential role in learning and memory formation in sleeping newborns.
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ANTI-INFLAMMATORY INFLUENCES ON FEBRILE AND BEHAVIORAL RESPONSES DURING REPEATED MATERNAL SEPARATION

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Attachment disruption and other childhood stressors can increase vulnerability for developing depression in later life. This process may involve sensitization of stress-induced proinflammatory reactions due to these early insults. In guinea pig pups, which exhibit evidence of a strong filial attachment, a several-hour separation from the mother produces a passive, depressive-like response that appears mediated by proinflammatory activity. The behavioral reaction is accompanied by an increase in core temperature, both of which sensitize with repeated separation. Recently, we found 14 mg/kg of the anti-inflammatory compound, naproxen, given before an initial separation reduced the depressive-like behavioral, but not the temperature, response to separation up to 10 days later. If the increased core temperature is, indeed, mediated by proinflammatory activity (i.e., is stress-induced fever), one would expect naproxen to reduce the response. Therefore in the present study, pups were injected with a larger dose of naproxen (28 mg/kg) or vehicle for three consecutive days and then separated on three occasions: 1 hour after the last injection, 24 hours after the first separation, and 10 days after the first separation. We again observed sensitization of the behavioral and core temperature response. The higher dose of naproxen had little effect on behavior, but reduced core temperature during separation. These results indicate that the elevation of core temperature is, indeed, a stress-induced febrile response. Considering the two studies together, the differential effects of the two doses of naproxen on behavior and fever suggest that different specific proinflammatory mediators underlie the two classes of response.
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