

Early experience in humans is associated with changes in neuropeptides critical for regulating social behavior

Alison B. Wismer Fries*[†], Toni E. Ziegler*[‡], Joseph R. Kurian[§], Steve Jacoris[‡], and Seth D. Pollak*^{†¶}

*Department of Psychology, University of Wisconsin, 1202 West Johnson Street, Madison, WI 53706-1696; [†]Waisman Center for Human Development, University of Wisconsin, 1500 Highland Avenue, Madison, WI 53705; [‡]Wisconsin National Primate Research Center, 1220 Capitol Court, Madison, WI 53715; and [§]Molecular and Environmental Toxicology Center, University of Wisconsin, 777 Highland Avenue, STE 2233, Madison, WI 53705-2222

Edited by William T. Greenough, University of Illinois at Urbana–Champaign, Urbana, IL, and approved September 20, 2005 (received for review June 7, 2005)

The formation of social attachments is a critical component of human relationships. Infants begin to bond to their caregivers from the moment of birth, and these social bonds continue to provide regulatory emotional functions throughout adulthood. It is difficult to examine the interactions between social experience and the biological origins of these complex behaviors because children undergo both brain development and accumulate social experience at the same time. We had a rare opportunity to examine children who were reared in extremely aberrant social environments where they were deprived of the kind of care-giving typical for our species. The present experiment in nature provides insight into the role of early experience on the brain systems underlying the development of emotional behavior. These data indicate that the vasopressin and oxytocin neuropeptide systems, which are critical in the establishment of social bonds and the regulation of emotional behaviors, are affected by early social experience. The results of this experiment suggest a potential mechanism whose atypical function may explain the pervasive social and emotional difficulties observed in many children who have experienced aberrant care-giving. The present findings are consistent with the view that there is a critical role for early experience in the development of the brain systems underlying basic aspects of human social behavior.

attachment | emotion | oxytocin | vasopressin | child abuse

The social attachments formed between human infants and their caregivers begin very early in postnatal life and play a critical role in children's survival and healthy adaptation. Typically, adults provide infants with a social environment that is fairly consistent. Caregivers learn how to recognize and respond to the infants' needs, thereby creating predictable contingencies in the environment; these regularities, in turn, make the infant's environment secure and conducive to further social learning (1, 2). Multiple perceptual, sensory, cognitive, and affective systems must become synchronized so that a social bond can develop between an infant and caregiver; this bond is then reflected in the child's adaptive behavioral responses to the environment. The goal of this experiment was to address a fundamental evolutionary and developmental question: To what extent are the neurobiological systems that regulate affiliative behaviors dependent on the social experiences afforded to most infants by their caregivers?

It is difficult to evaluate the effects of early social experience on the organization of the developing brain because, within seconds of postnatal life, the human infant experiences a vast amount of emotional input. In this regard, the maturation of the brain is confounded with the accumulation of social experience. One way to address this problem is to experimentally manipulate the social environment. Although this approach is successful with nonhuman animals, such studies are neither possible nor

desirable to undertake with human children. Therefore, to examine the effects of early experience on emotional development, we studied a sample of children who did not receive the kind of emotionally responsive care-giving typically received by human infants. These children were reared in institutionalized (orphanage) settings, where a prominent lack of emotional and physical contact from caregivers is a consistent adverse feature of the environment (3). Previously institutionalized children frequently experience problems in establishing social bonds and regulating social behavior, including a lack of developmentally appropriate wariness of strangers, atypical and disinhibited patterns of attachment, and difficulties developing close friendships (4–6). Fortunately, when these children are adopted, they move into family environments that provide normative contexts for child development. In this regard, children's adoption marks a dramatic (and measurable) termination of social deprivation that allows us to assess the impact of a circumscribed period of early neglect on the neurobiological mechanisms implicated in the regulation of emotional behavior.

To explore the neurobiological mechanisms underlying the formation of social attachments, we examined the oxytocin (OT) and arginine vasopressin (AVP) neurohypophyseal peptide systems in previously institutionalized children. We chose to study OT (7–12) and AVP (13–16) based upon research with nonhuman animals and humans suggesting that these systems are an integral part of mammalian emotional circuitry. These neuropeptides are associated with the emergence of social bonding, parental care, stress regulation, social communication, and emotional reactivity (17–22). OT and AVP levels are increased by socially pleasant sensory experiences, such as comforting touches and smells. Studies with nonhuman animals have demonstrated that as levels of these hormones rise, animals increase their positive social interactions: they form social bonds (23–25), display selective infant–parent attachments (26, 27), and form memories of these social interactions (28). OT receptors are part of the neural system of reward circuitry that includes the nucleus accumbens (29); a critical feature of this system for infant development is that it likely confers a sense of security and protection that makes social interactions rewarding. Indeed, higher levels of OT are associated with decreases in stress (24).

Our goal was to illuminate the role of early social experience in subsequent brain–behavioral development. Little is currently known about how developmental history can influence the

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Freely available online through the PNAS open access option.

Abbreviations: OT, oxytocin; AVP, arginine vasopressin.

[¶]To whom correspondence should be addressed. E-mail: spollak@wisc.edu.

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organization and functioning of this emotion-regulatory system in humans. However, a growing body of research suggests that early social experience, through changes in corticotrophin-releasing hormone, may alter OT and AVP receptor binding (30–34). Therefore, we predicted that early social experience would influence the feedback loops involving social reward circuitry, thereby affecting the regulatory capacities of the OT and AVP systems. This mechanism would have developmental implications for stress reactivity and behavioral regulation as the infant matures. Toward this end, we compared the children who began their lives in institutionalized settings to a group of children who were raised in typical family environments.

Children were tested in their homes on two occasions. During one visit, children interacted with their mothers, and on the second visit, children engaged in the same physical interactions with an unfamiliar adult female. The order of these visits was counterbalanced. To ensure that the interactions were similar across children and familiar–unfamiliar adult conditions, an animated computer program guided the physical contacts between child and adult in the form of a game. To avoid the stress involved in the physical discomfort of measuring cerebrospinal fluid or blood, we developed a noninvasive procedure to obtain peripheral measures of OT and AVP (see supporting information, which is published on the PNAS web site).

Little is known about the relationship between central and peripheral OT and AVP levels in humans, and available data suggest that the relationship is complex. However, research with nonhuman animals indicates that under certain conditions, central and peripheral release is coordinated (35–37). For example, suckling leads to increases in OT levels both within the CNS and peripherally in rats (38). Similarly, increased central (39) and peripheral (40) OT levels have been demonstrated after exposure to an emotional stressor. Although little is actually known about the peripheral–central relationship of these peptide systems in humans, nonhuman primates reared in socially deprived environments exhibit alterations of the central OT system (41), consistent with the findings reported here with humans.

Method

Participants. The sample of children who experienced early neglect consisted of 18 children (12 females) who had resided in orphanages for an average of 16.6 months (range, 7–42 months) immediately after birth. To ensure that children had time to acclimate to their new home environments, we studied children who had been residing in their adoptive homes for an average of 34.6 months (range, 10–48 months). Twenty-one children (12 females) who were being reared by their biological parents in a typical home environment served as a comparison group. The two groups of children were equivalent in age (previous neglect, 53.7 months; control, 54.2 months), and the families were drawn from similar high socioeconomic backgrounds (parent's education, 15.8 years for previous neglect, 16.2 years for control). The previously neglected children in this study were drawn from a larger study of postinstitutionalized children adopted into Wisconsin homes from institutions abroad. These children were screened for fetal alcohol exposure, birth defects, and developmental disabilities.

Procedure. In counterbalanced order, children engaged in an interactive computer game while sitting on either their mother's or an unfamiliar female experimenter's lap. Throughout the 30-min interaction, the mother (or unfamiliar adult) and child engaged in regularly timed physical contact (e.g., tickling, patting on the head, counting each others fingers, whispering in each others ears, etc.). After the task, a urine sample was collected as soon as the child was able to void (range, 15–20 min after task).

Each assessment was separated by at least 7 days, but not more than 14 days, and occurred in the children's homes. Research with nonhuman animals has reported peak plasma oxytocin levels ≈ 15 min after the onset of sensory stimulation (42). In humans, one study detected an increase in plasma oxytocin after 5 min of massage (43). Thus, the length of the task was deemed sufficient to produce an increase in OT and AVP.

To avoid the stress involved in the physical discomfort of measuring cerebrospinal fluid or blood, OT and AVP were assayed from urinary samples. Basal levels of OT and AVP were assessed by averaging 12-h overnight urine collections from four separate mornings. Urine from the first void of the morning was collected and immediately frozen.

After release into the bloodstream, OT circulates as a free peptide and has a short half-life of ≈ 10 min. The release of these peptide hormones into the bloodstream is pulsatile, whereas measurement in urine reflects the accumulation of the hormones over time, and the excretion of OT by the kidneys is related to the concentration in blood and to the total volume of urine passed (44). Therefore, concentration levels should be higher than those found in plasma; additionally, the acidic environment of urine should preserve these small peptides, whereas the enzymes in blood are likely to break down these peptides (45). Thus, hormone detection in urine may be more desirable than plasma detection for these peptide hormones. In addition, the relationship between plasma and urine hormone concentrations have been investigated in i.v. infusion studies, which show a linear association between OT in urine and blood during a 90-min infusion period (45).

Within 1 month, urine samples were thawed and a portion of the urine acidified by the addition of 0.05 M HCl (1:1) for peptide measurement and a portion used directly for creatinine measurement. Sample preparation for HPLC was modified from the techniques described (46). One milliliter of acidified urine was purified by using solid-phase extraction (Oasis, HOB 1 cc, Waters or Strata, 30 mg/ml, Phenomenex). The method consisted of preparing the column with 2 ml of methanol and 2 ml of distilled water, applying the 1-ml sample, washing with 2 ml of 1.5% acetic acid, and eluting with 2 ml of methanol. Samples were dried and resuspended in 20 μ l of acetonitrile/water/0.13% trifluoroacetic acid (TFA) (1:1). Samples were injected onto the column by an automated sample injector (catalog no. 508, Beckman). Human child samples and standards for small peptides were separated by HPLC using Beckman dual HPLC pumps (model no. 126, Beckman) connected to a diode array analyzer for UV detection at all wavelengths (model no. 168, Beckman). All instruments were connected to a computer, and the samples were run and data analyzed by detection software (Beckman, Nouveau, System Gold). The technique used a 3 μ , C18-A 50 \times 4.6 mm column for separating the peptides (MetaChem), with mobile phase A, 0.15% TFA in water, and phase B, 0.13% TFA in 95% acetonitrile/5% water. The flow rate was 3.0 ml/min at UV of 216 nm, and the gradient was 15% B at 0, at 3.5–7.0 min, 15–25% B. Retention times for AVP and OT were 2.7 and 5.5 min, respectively. Serial dilutions of the standards were linear between 220 ng and 14 μ g, $R^2 = 0.982$ for AVP and $R^2 = 0.988$ for OT. Mean recovery for added OT is 107.69 ± 3.96 SEM and for AVP is 96.29 ± 0.07 SEM. Interassay coefficient of variation for OT is 8.24% and for AVP is 6.9%. AVP and OT were expressed per mg of creatinine to control for fluid variability. The creatinine assay has been described (47). Intra and interassay coefficient of variation was 2.1% and 9.9%, respectively, for the low pool and 0.9% and 7.4%, respectively, for the high pool.

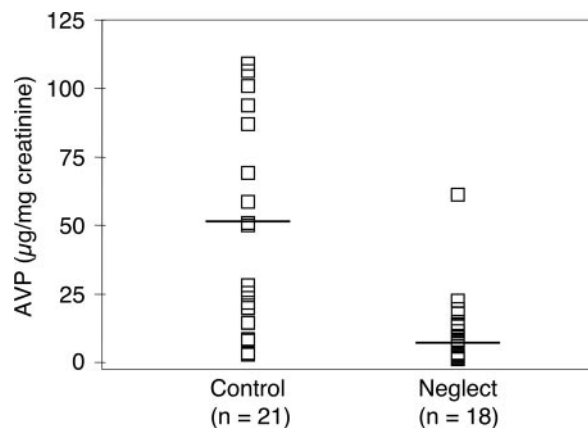


Fig. 1. Children's baseline levels of AVP were sampled from urine collections pooled across 4 days before the experimental sessions. Children who had experienced early neglect had lower overall levels of AVP than family-reared children [$F(1,37) = 7.61, P < 0.01$].

Results

We first examined basal levels of OT and AVP to determine whether early experience affected the functioning of these systems in the absence a discrete social manipulation. OT and AVP levels (given in $\mu\text{m}/\text{mg}$ creatinine) were equivalent for boys and girls in both groups (OT: girls = 17.03, boys = 13.88; AVP: girls = 31.66, boys = 32.07). Basal levels of OT did not differ between the previously neglected and control children (previous neglect: $M = 12.12, SD = 10.61$; control: $M = 18.99, SD = 20.96$). However, as shown in Fig. 1, children who had experienced early neglect had lower overall levels of AVP than family-reared children. These results suggest that social deprivation may inhibit the development of the AVP system. Functionally, central AVP appears to be critical for recognizing familiar individuals, a key component of forming social bonds (48).

Because emotions are inherently regulatory processes, we evaluated how these neuropeptide systems responded to dynamic social interactions. To do so, we examined hormone levels ≈ 20 min after children completed their interactions with their own mothers and an unfamiliar adult. Based on what is known about these systems in nonhuman animals, children were expected to show hormonal responsivity after physical interactions with their caregivers. As predicted, OT levels for family-reared children increased after physical contact with their mothers. Children who experienced early neglect did not show this response after physical contact with their mothers (Fig. 2). These altered peripheral levels of OT are consistent with central defects in peptide synthesis or mutations in the peptide genes (22). There were no group differences in OT levels after interaction with the unfamiliar adult (previous neglect: $M = 22.09, SD = 28.23$; control: $M = 16.42, SD = 12.49$). AVP levels after interactions with both mother (previous neglect: $M = 9.84, SD = 9.61$; control: $M = 20.92, SD = 30.56$) and stranger (previous neglect: $M = 12.42, SD = 9.68$, control: $M = 15.42, SD = 13.87$) were similar across groups.

The goal of this experiment was to address a fundamental evolutionary question that would be otherwise difficult to study. This unusual population, children who moved from severely deprived and aberrant environments to species-appropriate social environments, provides a window into the relationship between early postnatal experience and the emergence of a complex biobehavioral system. To what extent are the neurobiological mechanisms underlying human emotional behavior dependent on the social experiences afforded to most infants by

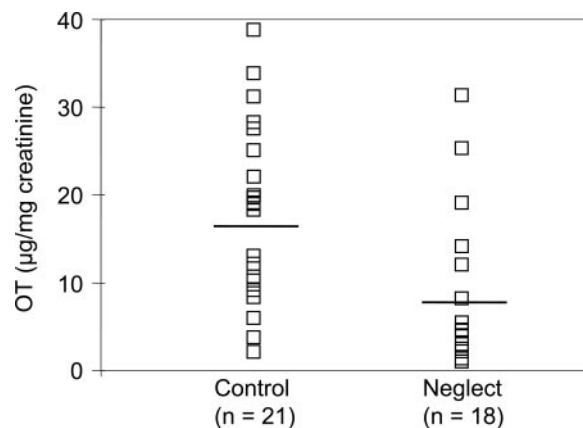


Fig. 2. Control children had higher OT levels after the interaction with their mothers than early neglected children [$F(1,37) = 3.91, P = 0.056$].

their caregivers? These results suggest that a failure to receive species-typical care disrupts the normal development of the OT and AVP systems in young children. Perturbations in this system may interfere with the calming and comforting effects that typically emerge between young children and familiar adults who provide care and protection.

Importantly, at the time of testing, children had experienced an average of 3 years of rearing in relatively stable, enriched, and nurturing family environments. However, this environmental change does not seem to have completely overridden all of the effects of early neglect. Indeed, consistent with these data, children reared in institutionalized settings, and other psychologically neglectful environments, often experience problems in social development that persist even after children settle into family environments (49). However, it is critical to note that not all children who experience early neglect develop the same kinds of problems, and children with lower hormonal reactivity may, over time, develop satisfactory interpersonal relationships. In fact, examination of Fig. 2 makes clear that there are potentially important individual differences operating across both the previously neglected and control groups of children. Therefore, future studies should seek to address this issue by relating detailed aspects of the early environment to longitudinal outcomes and determine the degree of plasticity in these systems. The current experiment cannot address questions concerning the direction of influence between social difficulties and alterations in this biobehavioral affiliative system. Nonetheless, the present data provide a potential explanation for how the nature and quality of children's environments shape the brain-behavioral systems underlying complex human emotions. These findings not only inform understanding of normal human development, but will hopefully foster the development of targeted interventions for children exposed to environmental risks.

We thank Christopher Coe, Megan Gunnar, Catherine Marler, Jenny Saffran, and Charles Snowdon for early discussion about this project and helpful comments on a previous draft of this manuscript. This research was funded by National Institute of Mental Health Grant R01 MH 068858 and grants from the Jane Bradley Pettit Foundation and the University of Wisconsin Graduate School (to S.D.P.). Additional support was provided by Waisman Center, University of Wisconsin Core Grant P30 HD03352, National Institute of Mental Health Grant R01 035215 (to Charles T. Snowdon and T.E.Z.), and the National Center for Research Resources–Wisconsin National Primate Research Center Grant 5P51 RR 000167. A.B.W.F. was supported by National Institutes of Health Training Program in Emotion Research Grant T32 MH 18931. The institutional review board of the University of Wisconsin approved this study.

