Early Life Stress: Long-Term Physiological Impact in Rodents and Primates

Christopher R. Pryce, Daniela Rüedi-Bettschen, Andrea C. Dettling, and Joram Feldon

Behavioral Neurobiology Laboratory, Swiss Federal Institute of Technology Zurich, CH-8603 Zurich, Switzerland

Rat, monkey, and human infants have evolved to expect certain patterns of care. Spontaneous or experimental deviations of care from the norm result in infant stress responses. Hyperactivity of immature stress systems such as the limbic-hypothalamic-pituitary-adrenal轴 and the limbic-sympatho-adrenomedullary axis can alter their subsequent reactivity across the life span.

The sequencing of the human genome is a monumental advance and will lead to marked facilitation of the identification of candidate genes involved in the regulation of complex physiological and behavioral traits. A logical consequence of this is that great interest will now be stimulated in identifying the external and internal environmental factors that regulate the individual- and organ-specific expression of these genes (7). In psychobiology, it has long been recognized that postnatal parental care is one of, if not the, most important environmental regulatory factors in terms of individual, i.e., offspring, development (10). Hofer (10) has been pioneering in describing how observable behavioral interactions of parent and offspring mediate nonobservable sensorimotor, thermal, and nutrient-based events that have important and widespread regulatory effects. Most importantly, the impact of the quality and quantity of maternal care received by the offspring is not restricted to acute effects; rather, via these acute effects the trajectory of development is also altered, leading to chronic effects. Accordingly, there is now a growing body of human epidemiological evidence and animal experimental evidence that early social experiences influence the functioning of some vital physiological processes in adulthood (8, 10, 14). This situation is presented in Fig. 1. Infancy/childhood constitutes a sensitive period for responsiveness to external environmental factors, parental care constitutes the major such factor at this stage of development, and the impact of this responsiveness is both short and long term.

Human stress and animal models

Ultimately, this life span chain of events will need to be understood at the many epigenetic, molecular, and proteomic levels at which it is occurring. At present, however, considerable research effort is being made to identify 1) the physiological and behavioral traits that are distinctive in human adults who experienced extreme environments, notably abuse or neglect, in early life and 2) in vivo animal models in which specific manipulations of the mother-infant relationship yield robust acute and/or chronic effects on these same traits.

Recent epidemiological studies indicate that early exposure to adverse experience (physical or emotional neglect and/or physical or sexual abuse) increases the risk for the development of, among others, posttraumatic stress, depression, and anxiety disorders (8). This association may be mediated by chronically altered activity in and sensitization of the neuroendocrine stress axes, namely the hypothalamic-pituitary-adrenocortical (HPA) system and the autonomic sympatho-adrenomedullary (SAM) system. Therefore, the chemical messengers corticotropin-releasing factor (CRF), adrenocorticotropic hormone (ACTH), and glucocorticoids (GC) in the case of the HPA axis and adrenaline and noradrenaline in the case of the SAM axis, as well as their respective receptors, are key targets for study in both the periphery and the brain. Women who suffered abuse as children, including both those with and those without current depression, have been shown to exhibit basal plasma cortisol titers that are lower than in their respective control women. When challenged with a standardized and validated psychosocial stress protocol, abused women exhibited greater plasma ACTH responsiveness than did the controls; the increase was more pronounced in abused women with current depression, and these women also exhibited greater cortisol responsiveness and greater heart rate responsiveness than controls. In a second study, complementary to the first, subjects were examined in terms of their stress responses to CRF- and ACTH-provocative neuroendocrine challenge tests, with blood sampling being conducted before and after challenge via indwelling catheters. In terms of challenge responsiveness, CRF challenge stimulated a greater ACTH response in abused women without current depression than in their control group, whereas abused depressives and control depressives both exhibited blunted ACTH responsiveness. In the ACTH stimulation test, abused women who were not depressed exhibited blunted cortisol responsiveness relative to each of the other three groups, who responded similarly. The authors interpret their findings in terms of sensitization of the pituitary (CRF-ACTH) and counterregulative adaptation of the adrenal gland (ACTH-cortisol) in abused women without current depression. On exposure to stress, they propose, such women may hypersecrete CRF, resulting in depression but pituitary CRF receptor downregulation (see Ref. 8 and additional references by the same authors).

With regard to animal models, in this brief review we summarize the current status of rodent and primate research in this very important area, including some of the recent, published findings from our own laboratory. We are in the unusual position of being able to perform animal model work with both...
rodents and primates, namely rats and marmoset monkeys, respectively. This comparative approach allows for direct investigation of the relative responsiveness of specific stress-system factors to manipulation of maternal care in species with different phylogenetic relationships to humans. We will therefore also be able to directly assess the relative validity of these different species for modelling the life span impact of postnatal experience on physiological status and disease susceptibility in humans.

### Rats and marmosets

Before focusing on the effects of abnormal postnatal social environments, it is important to consider some relevant, species-typical characteristics of rats and marmosets (Fig. 2). Rat dams typically give birth to ~12 pups per litter, and these pups are poorly developed at birth and grow rapidly, with weaning at 3–4 wk. Maternal care occurs in bouts of retrieving, licking, and nursing, interspersed with periods when the dam is absent from the nest and litter (18). Very importantly, rat pups aged postnatal days (PNDs) 2–14 are said to be in a “stress-hyporesponsive period” (SHRP), because they have low basal levels of ACTH and GC (Fig. 3) and are also hyporesponsive to environmental events that elicit a marked hormone response in older conspecifics, e.g., saline injection (20). The identification of the rat SHRP has added considerable weight to the theory that avoidance of early life stress is of marked physiological and biomedical importance (5, 10).

Marmosets are about the same body size as rats, i.e., 300–400 g. Females typically give birth to twin infants that are well developed at birth and grow moderately postnatally, with weaning at ~10–12 wk (Fig. 2). The infant is able to cling on to the parent from the moment of birth, and the first 3–4 wk are characterized, in contrast to the rat, by continuous parent-infant body contact. In addition to maternal nursing, both mothers and fathers provide parental care in the form of carrying, licking, and grooming (16). Also, in stark contrast to the rat (and indeed to other primates, including humans), basal ACTH and GC levels are actually elevated in infant marmosets compared with older conspecifics (Fig. 3; Ref. 19). Despite such high basal activity, infant marmosets, like infants in other primate species, are able to exhibit ACTH and GC stress responses that are of adult-like magnitude (19).

### Rat postnatal manipulations

The regulation of maternal care is itself highly complex in mammals, with many determining factors (15, 16). Therefore, although maternal care must be of a minimum quantity if it is to ensure infant survival, above this minimum there is marked interfemale variation in the maternal care that infants receive. Based on this natural variation, longitudinal correlational studies in rodents and primates have provided quite persuasive indirect evidence that physiological and behavioral traits/states in weaned and adult offspring are related to the history of positive mother-infant behaviors (e.g., anogenital licking) as well as negative mother-infant behaviors (e.g., aggression) received during infancy (6, 12).

Of course, it is controlled manipulative studies in animals that will provide direct evidence for the presence or absence
of acute or chronic effects of specific experiences in infancy on physiological and neurobehavioral development. For laboratory rats, there is now quite substantial data on this subject from a number of laboratories (12, 14, 20), although the field is complex because the effects of a number of different manipulation paradigms have been investigated, and these in different strains (11). The major paradigms are as follows: maternal separation (MS), involving either a single 24-h separation of the intact litter from the dam (11, 20) or repeated such separations for a shorter (but nonetheless prolonged) period of 3/6 h/day (11, 14); early handling (EH), involving daily human handling of pups to separate them from the mother, and usually also from the littermates, for a short time period, typically 15 min/day (11, 12, 14, 17); and early deprivation or isolation (ED), comprising repeated (typically daily) human handling of pups and separation from the mother and littermates for a prolonged period of 1–6 h/day (11, 13, 17). The nature of the control group is also complex; in many studies, the manipulated rats have been compared against rats that do not experience any direct human environmental disturbance during infancy, so-called early nonhandling (NH) (11–14, 17); in other studies, the control dams and litters experience the lab’s routine husbandry regimen, including occasional brief handling for cage cleaning (CON) (11, 17).

**Acute effects of postnatal stress in rats**

Beginning with acute effects of these environmental manipulations on rat pups and in particular on the SHRP, the maintenance of the SHRP is dependent on the pup receiving at least a certain minimum level of maternal care. MS for 8–24 h (but not less) induces increased ACTH and GC levels directly, as well as “allowing” an additional stress response of these hormones to discrete stressors such as saline injection. These peripheral endocrine changes are accompanied by downregulation of brain expression of the genes coding for the GC receptors (GRs) that mediate HPA negative feedback, including GRs in the paraventricular nucleus of the hypothalamus and mineralocorticoid receptors (MRs) in the Ammon horn of the hippocampus (20). How much, or indeed how little, maternal care is actually required to maintain the SHRP is revealed by an elegant study in which occasional anogenital stroking (to stimulate urination and defecation) and cannulated feeding of pups was found to be sufficient to maintain the SHRP in 24-h MS pups (20). Although a single MS must therefore last longer than 8 h before an increase in pup stress hormones is apparent, daily ED manipulation appears to sensitize the rat pup’s HPA system to shorter stress experiences, because ED for 1 h on PND 8 leads to increased ACTH and GC following ED on PND 9 (13). Equally interesting is the finding that EH does not induce such HPA sensitization in pups; in fact the opposite is the case, with EH leading to downregulation of basal hypothalamic CRF mRNA levels and a reduced ACTH stress response, both during the SHRP and at weaning (1). In the next section, we summarize the evidence that these acute physiological effects of manipulations of the pup-dam relationship are associated with, i.e., lead to, altered functioning of the same stress systems in adulthood.

**Chronic effects of postnatal stress in rats**

As adults, rat offspring that experienced 24-h MS during and therefore disruption of, the SHRP also exhibit at least some features of HPA hyperactivity in adulthood. Compared with NH adults, basal ACTH and GC are elevated and hypothalamic and hippocampal MR and GR binding capacity are reduced, suggesting GC feedback resistance (5). However, hippocampal MR and GR mRNA levels are unaffected in these 24-h MS adults, whereas hypothalamic CRF mRNA levels are (somewhat paradoxically, given ACTH levels) reduced. Therefore, the actual chronic pathways via which 24-h MS leads to an adult rat phenotype of basal pituitary-adrenal hyperactivity are presently little understood. Relative to NH, repeated 3-h MS leads to increased hypothalamic CRF content but is without effect on basal and stress-related plasma GC titers (14). Compared with NH, EH adults demonstrate reduced stress
responsiveness in terms of ACTH and GC. This has been reported (12, 14) to be associated with reduced hypothalamic CRF gene expression and CRF immunoreactivity, increased hippocampal GR gene expression, and increased GC negative feedback sensitivity. This impressive complex of chronic EH effects, it has been proposed, results in increased feedback inhibition of CRF synthesis and therefore reduced pituitary ACTH release during stress (12, 14).

In our laboratory, we have investigated the chronic effects of 15-min EH vs. 4-h ED on stress-related physiology and behavior; in addition to direct comparison of these two manipulations, we also used two control groups, namely NH and CON. In terms of the HPA system, we observed the classic effect of EH adult offspring demonstrating a reduced GC stress response relative to NH adults (17). However, as given in Fig. 4, the GC stress response of ED and CON adult male rats was very similar to that of EH rats; that is, NH is the distinctive “manipulation” and EH, ED, and CON all lead to a similar GC stress response in male adulthood. This increased physiological stress responsiveness of NH adults cooccurred with increased stress-related behavioral responsiveness; for example, the reflex startle response to acoustic stimulation was increased in NH male and female adults relative to EH, ED, and CON, whereas the latter three groups were again quite similar in their responses (Fig. 4). Relative to NH, one common characteristic of these three postnatal conditions is that they all expose dams and pups to higher levels of stimulation, including handling and changes in the physical environment (see below).

Mediating mechanisms

Therefore, a complex picture is emerging in terms of the chronic effects of different postnatal manipulations in the rat. As stated at the beginning of this review, the cellular and molecular mechanisms mediating these chronic effects are potentially of profound biomedical importance. We would attach equal importance to the mechanisms via which the external environment impinges on the whole organism to trigger the cellular and molecular events. As noted above, a characteristic common to all postnatal manipulations is pup stimulation. Depending on the manipulation, this stimulation constitutes: 1) increased maternal care following reunion, as stimulated by increased emission of care-eliciting pup cues (e.g., following EH, ED); 2) loss of homeostasis related to prolonged absence of the mother (e.g., during MS) or of the mother and littermates (e.g., during ED); or 3) a combination of these factors.

EH pups receive more maternal care, in the form of dam-pup licking and nursing in the upright crouching posture (kyphosis), than do NH pups, and Liu et al. (12) propose that this increased maternal care is the primary mediator of the long-term stress system effects of EH. As correlational evidence for this, they report that, within NH cohorts, adult offspring that received relatively high levels of maternal licking and kyphotic nursing exhibit relatively low stress-related physiological responses. This proposed causal relationship between EH stimulation of maternal care received in infancy and reduced HPA activity in adulthood may, however, be difficult to reconcile with the evidence, described above, that brief human stroking and feeding at intervals of several hours is quite sufficient to reverse at least the acute effects of 24-h MS on the HPA system (20). We measured maternal care in our EH/ED studies (18). We found that EH litters received significantly more kyphotic nursing than CON litters. In ED litters, which were deprived of potential maternal care for 4 h/day, post-ED reunion invariably
stimulated an intense bout of licking and kyphotic nursing, but thereafter CON-like levels of maternal care returned (18). Variation in maternal care was marked between dams within treatments. Furthermore, the observed differences between EH, ED, and CON offspring in terms of maternal care received did not lead to adulthood differences in either the GC stress response or stress-related behavior (Fig. 4) (17).

Regarding the second proposed mediating mechanism for long-term physiological effects of postnatal manipulations, namely loss of homeostasis in infancy, there has been very little experimental research in this direction to date. However, Catalani and colleagues (2) have provided the very exciting evidence that NH pups exposed to high GC levels via maternal milk develop into adults with reduced stress-induced GC secretion and increased hippocampal MR binding. As noted above, ED (but not EH) stimulates pup GC levels via sensitization (13). This leads to the interesting possibility that, although we observed similar long-term effects of EH and ED in our studies (Fig. 4), these might be mediated by very different mechanisms: increased maternal care in the case of EH and acute activation of stress systems due to the absence of maternal care in the case of ED.

Stress effects of postnatal manipulations in primates

Relative to the laboratory rat, effects of postnatal manipulations on neurobiological development of stress systems have received little attention in laboratory primate species such as the rhesus macaque, squirrel monkey, and common marmoset. The classic rhesus macaque studies (see Refs. 3 and 9) have focused on complete separation of the infant from the biological mother, followed by human rearing in a nursery situation. Although sufficient to ensure infant survival, the sensory stimulation and two-way interactions provided by the biological mother are absent, and severe, chronic behavioral abnormalities are induced by such maternal privation compared with biological mother rearing. In terms of the long-term impact of maternal privation on macaque stress physiology, different laboratories report either a reduction or an increase in basal ACTH and GC levels, and for HPA stress responsiveness there is evidence that this is reduced (3, 9). A study in bonnet macaques has taken the novel approach of reducing maternal care via manipulation of the predictability of food availability: mothers that have to forage for their food in an unpredictable environment are more aggressive toward and less nurturing of their infants. As adults, offspring raised by such mothers exhibit elevated basal cerebrospinal fluid CRF levels and reduced basal CSF GC levels (4). This evidence for increased CRF levels in adult primate offspring of aggressive, less-nurturing mothers is broadly consistent with the evidence for increased CRF levels in adult rats exposed to MS as infants (14). The experimental approach of attempting to manipulate infant neurobiological development via manipulation of the maternal environment is elegant and could also be informative in rat studies.

Integrated rodent-primate approach

On the basis of the evidence currently available, it is not possible to make robust statements about the effects of daily life stress on the long-term development of physiological stress systems, not to mention underlying mediating mechanisms, in nonhuman primates. Furthermore, there exists very little overlap between the approaches used in rodents and primates. Our common marmoset studies are attempting to address this issue. The manipulation we are using is very much an ED manipulation as described above for the rat: On PNDs 2–28, the marmoset infant is removed from the parent to which it is clinging and placed in an isolation chamber for 0.5–2.0 h, with the control condition involving brief restraining of the parent and infant in the home cage followed by release. The monkey ED is less severe than the rat ED in terms of daily duration, but its severity is probably increased by our varying the time of day at which ED is performed. Regardless of duration, intuitively the severity of ED will be greater in primates than in rodents because the loss of parental contact is a completely abnormal experience for monkey infants, whereas experiencing bouts of
maternal absence is the norm for rat pups. We are studying acute physiological effects of ED in the marmoset infant by measuring GC and catecholamine metabolites in urine samples collected immediately before and after ED and medium-term behavioral effects in terms of parental care and infant responses to this care. Chronic physiological effects are being studied in terms of basal and stress-related HPA axis and SAM axis endocrine activity, basal and stress-related blood pressure and heart rate as measured by telemetry, and postmortem brain MR and GR expression. Chronic behavioral effects are being studied in terms of social behavior and performance on neuropsychological tasks that allow us to measure emotionality and cognition.

Relative to other primates, the small body size and therefore short maturation time of the marmoset renders it a suitable species for such longitudinal study. The high postnatal pituitary-adrenal activity that we have recently identified in this species (Fig. 3) is not typical for primates, including humans. Given that high GC is one of the major hypothetical mechanisms via which early life stress can have long-term impact, then the infant marmoset's basal hypercortisolism (specifically, its apparently massive GC tolerance; Fig. 3) might reduce its overall suitability as a model in this respect. We have at least now established, for the first time in any primate, that a paradigm of repeated early deprivation of parental care in very young infants, as applied in rat studies, is possible in at least this monkey species. Our future findings should provide a very valuable complementary approach, both to the equivalent rodent-based research and to the human evidence for the marked impact of early life stress on individual physiology and well-being.

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References


