Lifelong reductions of PKMζ in ventral hippocampus of nonhuman primates exposed to early-life adversity due to unpredictable maternal care

Sasha L. Fulton,1 Changchi Hsieh,2 Tobias Atkin,3 Ryan Norris,3 Eric Schoenfeld,3 Panayiotis Tsokas,2,4 André Antonio Fenton,2,5,6 Todd Charlton Sacktor,2,4,7,9 and Jeremy D. Coplan8,9

1Mount Sinai Medical Center, New York, New York 10029, USA; 2Department of Physiology and Pharmacology, 3College of Medicine, 4Department of Anesthesiology, SUNY Downstate Medical Center, Brooklyn, New York 11203, USA; 5Center for Neural Science, New York University, New York, New York 10003, USA; 6Neuroscience Institute at the NYU Langone Medical Center, New York, New York 10016, USA; 7Department of Neurology, 8Department of Psychiatry, SUNY Downstate Medical Center, Brooklyn, New York 11203, USA

Protein kinase Mζ (PKMζ) maintains long-term potentiation (LTP) and long-term memory through persistent increases in kinase expression. Early-life adversity is a precursor to adult mood and anxiety disorders, in part, through persistent disruption of emotional memory throughout life. Here we subjected 10- to 16-wk-old male bonnet macaques to adversity by a maternal variable-foraging demand paradigm. We then examined PKMζ expression in their ventral hippocampi as 7- to 12-yr-old adults. Quantitative immunohistochemistry reveals decreased PKMζ in dentate gyrus, CA1, and subiculum of subjects who had experienced early-life adversity due to the unpredictability of maternal care. Adult animals with persistent decrements of PKMζ in ventral hippocampus express timid rather than confrontational responses to a human intruder. Persistent down-regulation of PKMζ in the ventral hippocampus might reduce the capacity for emotional memory maintenance and contribute to the long-lasting emotional effects of early-life adversity.

Early-life adversity is associated with an increased vulnerability to stress-related disorders that is maintained into adulthood, suggesting a very long-lived effect on emotional memory by the early-life event (Coplan et al. 1996). Although several structural and neurochemical sequelae of early-life adversity have been reported (Teicher et al. 2003; Jackowski et al. 2011), the direct effects of early-life adversity on the molecular substrates maintaining long-term memory storage have not been explored.

Accumulating evidence supports a crucial role for the autonomously active, atypical protein kinase C (PKC) isoform protein kinase Mζ (PKMζ) in maintaining synaptic long-term potentiation (LTP), a putative physical substrate for memory, and long-term memory storage (Ling et al. 2002; Pastalkova et al. 2006; Glanzman 2013; Sacktor and Fenton 2018). The autonomous activity of PKMζ is due to its unusual structure that differs from other PKC isoforms (Sacktor et al. 1993). Most PKCs consist of two domains: a catalytic domain and an autoinhibitory regulatory domain that suppresses the catalytic domain. Therefore, most PKCs are inactive until second messengers bind to the regulatory domain and induce a conformational change that releases the autoinhibition. Because second messengers that activate PKCs are inactive until second messengers bind to the regulatory domain that suppresses the catalytic domain. Therefore, most PKCs are inactive until second messengers bind to the regulatory domain and induce a conformational change that releases the autoinhibition. Because second messengers that activate PKCs such as Ca²⁺ or diacylglycerol have short half-lives, most PKCs are only transiently activated.

PKMζ, in contrast, consists of an independent PKCζ catalytic domain, and the absence of an autoinhibitory regulatory domain results in autonomous and thus persistent activity once the kinase is synthesized. PKMζ mRNA is transcribed from an internal promoter within the PKCζ/PKMζ gene that is active only in neural tissue (Hernandez et al. 2003). The mRNA is translationally repressed and transported to dendrites of neurons (Muslimov et al. 2004). High-frequency afferent synaptic activity during LTP induction or learning derepresses PKMζ mRNA translation, triggering new synthesis of PKMζ protein (Osten et al. 1996; Hernandez et al. 2003; Tsokas et al. 2016; Hsieh et al. 2017).

Once increased, the steady-state amount of PKMζ remains elevated during LTP or long-term memory maintenance. Recent work with quantitative immunohistochemistry (IHC) shows that spatial conditioning induces persistent increases of PKMζ in somatic and selective dendritic compartments of dorsal hippocampal CA1 pyramidal cells that can last at least 1 mo (Hsieh et al. 2021). The persistent increases are preferentially expressed in CA1 pyramidal cells that were activated during the formation of the memory, specifically at the termination zone of the Schaffer collateral/commisural inputs from subfield CA3. In contrast, persistent PKMζ increases are not evident in stratum lacunosum-moleculare; the termination zone that originates in entorhinal cortex that nonetheless is capable of expressing PKMζ. Postsynaptic domain-specific PKMζ expression patterns hint at distinct circuit-specific modifications of cortical–hippocampal synaptic function by maturational and experiential factors.

Persistent changes in PKMζ expression are also associated with changes in the capacity for learning and memory across the
life span of animals. Decreased memory ability in aged rats is associated with decreased training-induced, persistent PKMζ expression in prelimbic cortex, and increases in PKMζ are crucial for the cognition-enhancing effects of environmental enrichment in the aged animals (Chen et al. 2016). Hara et al. extended the connection between PKMζ and cognitive function to nonhuman primates (NHPs), showing that levels of PKMζ expression in dentate gyrus (DG) axospinous synapses correlate with successful performance on cognitive tasks in young and aged monkeys (Hara et al. 2012). These studies suggest that persistent down-regulation of PKMζ may comprise an important pathophysiological mechanism for cognitive impairment.

Here we used a validated NHP model of early-life adversity, maternal variable-foraging demand (VFD), to explore the links between adversity in infancy and PKMζ expression in adulthood (Coplan et al. 1996; Jackowski et al. 2011). Previous studies of the VFD paradigm have revealed that both infants and their mothers exposed to VFD show significant cerebrospinal fluid (CSF) elevations of the stress neuropeptide, corticotropin-releasing factor (CRF). Moreover, the magnitude of CRF change in mothers and infants is positively correlated, suggesting synchronization of maternal–infant stress responses to the VFD stressor (Coplan et al. 2005). From a behavioral standpoint, maternal social rank plays a negligible role in determining an aggregate score of maternal–infant proximity, suggesting preferential attention of mothers to their infants. During the VFD condition, maternal social rank predicts >80% of the variance of maternal–infant proximity, suggesting preferential mothering patterns are interrupted by preferential orientations to social rank; the latter determines food accessibility (Coplan et al. 2015). Dominant females show relative increases in maternal–infant proximity, whereas subordinate females show relative reductions in maternal–infant proximity. Neither pattern of attachment ameliorates an abnormal association between CSF oxytocin concentrations and hypothalamic-pituitary-adrenal (HPA) axis activity (Coplan et al. 2015). Offspring exposed to VFD rearing assessed both as juveniles and as full adults demonstrate persistent increases in CSF CRF concentrations in comparison with controls reared under non-VFD conditions (Coplan et al. 1996, 2001).

Our prior neurohistological studies pointed to the DG as a region particularly vulnerable to VFD exposure, as shown by reduced trophic signaling and neurogenesis (Jackowski et al. 2011; Perera et al. 2011; Schoenfeld et al. 2021). We therefore hypothesized that early-life adversity due to unpredictable maternal care (for brevity, subsequently referred to as “early-life adversity”) reduces the persistent expression of PKMζ within the DG of ventral intra-hippocampal neurocircuitry that mediates affective memory processing (Fanselow and Dong 2010). We used PKMζ antisera validated by the lack of immunostaining in PKMζ-null mice (Hsieh et al. 2021) to examine PKMζ expression in ventral hippocampus (NHP) and hippocampus (in both DG granule cell layer and the stratum molecular of the suprapyramidal blade that receives direct input from entorhinal cortex, as well as other regions encompassing the hippocampal formation, including the hilus, CA3, CA1, and subiculum.

To assess behavioral correlates of hippocampal PKMζ expression, we used a stress-inducing paradigm designed specifically for singly housed bonnet macaque male NHPs, which we refer to as the “human exposure response” (Jackowski et al. 2011; Hamel et al. 2017), which is a variation of the paradigm used in human exposure studies by Kalin et al. in rhesus macaques (Kalin and Shulman 1989). On exposure to a direct human presence, singly housed adult male bonnet macaque responds with a dichotomy of responses—confrontational versus timid (see the Materials and Methods) (Jackowski et al. 2011). In our macaque colony, groups of fully adult males are necessarily housed individually to prevent injury sustained during male agonistic encounters, whereas adult females and/or juveniles are safely housed in social groups. Because group housing of nursing females and/or juveniles of both sexes elicits a range of behaviors intrinsic to the species’ social repertoire (Rosenblum et al. 2001; Coplan et al. 2015) that complicates behavioral analyses to human exposure, we restricted our current study to male macaques.

Results

Early-life adversity induces lifelong down-regulation of PKMζ in ventral hippocampus

On inspection, the expression of PKMζ is deficient in the ventral hippocampus of adult male animals who had experienced a 16-wk period of early-life adversity due to VFD initiated when they were 10- to 16-wk-old infants, as compared with control non-VFD subjects (Figs. 1 and 2). The reduction observed by PKMζ immunofluorescence (Fig. 1A) is corroborated by PKMζ immunohistochemistry using peroxidase staining, which reveals prominent decreases of PKMζ labeling in pyramidal cell bodies and dendrites (Fig. 2). Comparing animals who experienced VFD (n = 6) and controls (n = 4), the mean PKMζ immunofluorescence signal intensities in six ventral hippocampal formation subregions across two groups show a significant interaction of VFD and subregion (F(5,40) = 6.30, P = 0.0002, η² = 0.44; note effect size is at least threefold larger than stipulated by Cohen [η² ≥ 0.14 denotes a large effect size; Cohen 1973]), and a main effect of hippocampal subregion (F(5,40) = 17.69, P < 0.0001, η² = 0.69), but not a main effect of VFD (F(1,8) = 4.27, P = 0.07, η² = 0.35). The planned comparisons for region reveal a significant rearing group effect in three hippocampal subregions: stratum molecular of the DG, CA1, and subiculum, and no identifiable differences in the granule cell layer of DG, hilus, or CA3 (Fig. 1B).

Human exposure response

On exposure to a human stranger, macaques react with either species-specific confrontational behavior, or with behavior characterized by continual or intermittent timidity (Jackowski et al. 2011; Hamel et al. 2017). As vulnerability to environmental stress is a key sequela of early-life adversity leading to decreased hippocampal function (Coplan et al. 1996; Jackowski et al. 2011), we predict that timid responses to an intruder that had been measured antemortem would be associated with reductions in hippocampal PKMζ expression. The results reveal that subjects displaying timid responses show a main effect of decreased hippocampal PKMζ expression (n = 6), compared with animals exhibiting confrontationald responses (n = 3) (F(1,7) = 6.26, P = 0.04, η² = 0.47) (Table 1). In addition, there is a main effect of hippocampal subregion (F(5,35) = 10.44, P < 0.0001, η² = 0.60), but no interaction between human intruder response and hippocampal subregion (F(5,35) = 2.41, P = 0.06, η² = 0.26). The planned comparisons for region reveal subjects with timid responses express less PKMζ in CA3 and the stratum molecular and granule cell layer of DG.

Discussion

Here we present the first evidence that early-life adversity during infancy produces deficits of PKMζ expression in ventral hippocampus of adult NHPs. Limitations of the study include the relatively small number of subjects in line with other studies of NHPs. In addition, although VFD is carried out in social pens of maternal–infant dyads exposed to unpredictable foraging, grown VFD and non-VFD males are singly housed for their safety to avoid dangerous agonistic encounters. Single housing is an unavoidable limitation for measuring social behavior because socially housed adult
PKMζ loss after early-life adversity in primates

males will contest dominance with the harem male, with the like-
lihood of severe wounding. Another limitation is the lack of inclu-
sion of female subjects, which is a consequence of the complexity
of measuring behavioral responses to human exposure due to nor-
mative expression of the species-specific social repertoire intrinsic
to social housing. Unpublished data (JD Coplan) indicate that
group-housed females exhibit a role for ventral hippocampal
PKMζ in the maintenance of social affiliation.

Because persistent changes in the amount of PKMζ are thought
to maintain experience-dependent information in the cir-
cuity of the brain (Hsieh et al. 2017, 2021; Gao et al. 2018), the re-
duction of PKMζ associated with early-life adversity might be due
to ongoing, lifelong alterations in the induction of synaptic plastic-
ity in ventral hippocampal circuitry. Thus, early-life adversity
could cause long-lasting suppression of the induction of LTP that
persistently decreases PKMζ expression, and/or long-lasting aug-
mentation of the induction of long-term depression that persist-
tently decreases PKMζ (Harabtova and Sacktor 1996). This notion
is in line with other sequelae of early-life adversity in NHP, includ-
ing decreases in neurogenesis in DG in which the newborn neu-
rons may be more sensitive to LTP induction compared with mature
neurons (Snyder et al. 2001; Toda and Gage 2018). Future
studies will be required to determine the interactions between
VFD and stress-related changes in neuromodulatory signaling or
neural circuitry that may alter the induction of synaptic plasticity.
In addition to changes in induction, epigenetic modification, such as
hypermethylation of the PKMζ promoter that reduces PKMζ
gene expression (Chen et al. 2016), is a potential mechanism for the
long-lasting down-regulation in PKMζ expression after early-
life adversity.

The reductions of PKMζ in adulthood following early-life ad-
versity are selective to suprapyramidal DG stratum moleculare,
which receives input to the hippocampal formation from entorhi-
nal cortex, and the hippocampal formation output subregions,
CA1 and subiculum (Fig. 3). Although it is tempting to think of the entorhinal
cortex → DG → CA3 → CA1 → subiculum pathway as a feedforward sequence of re-
lays, these connections form a network of nested short and long loops because
each subfield receives multiple inputs. CA1, for example, receives both an input
from CA3 and a distinct direct input from the entorhinal cortex (Fig. 3; Kajiwara
et al. 2008). Moreover, electrophysiological studies demonstrate that each subfield
is strongly controlled by, and can main-
tain its own self-organizing activity due to local inhibitory networks (Colgin
et al. 2009; Dvorak et al. 2018). Thus, the reduction of the molecular substrate
of LTP maintenance preferentially at the postsynaptic sites of entorhinal inputs
to DG, CA1, and at the inputs to subic-
ulum might functionally weaken these projections. We speculate that this weak-
ening of inputs of sensory information might bias hippocampal processing in fa-
vor of local self-organizing activity at the expense of extrinsic entorhinal-originat-
ing activity. Such a VFD-induced bias might promote self-referential informa-
tion processing that resembles ruma-
ination, which is in line with the muted
behavioral responses we have observed in socially housed juvenile VFD subjects
in response to a human intruder donning a clown face (Rosenblum
et al. 2001).

Notably, the regions that show preferential lifelong reduc-
tions of PKMζ in ventral hippocampus after early-life adversity—
CA1 and suprapyramidal DG stratum moleculare—are also regions
that show preferential persistent increases in dorsal hippocampus
after spatial memory conditioning, as well as the layer 3 entorhinal
terminals in CA1 stratum lacunosum-moleculare, which do not change
with the conditioning (Hsieh et al. 2021). Thus, the hip-
 hippocampal regions that selectively store long-term information under
normative conditions by LTP maintenance through increases in
PKMζ expression in dorsal hippocampus are the ones that show reduc-
tions of PKMζ after early-life adversity in ventral hippocampus.
Whereas the dorsal/posterior hippocampus is critical to spatial
navigation (Ekstrom et al. 2003), the ventral/anterior hippocam-
pus may serve as a neural substrate facilitating “emotional naviga-
tion” of both the internal and external environment (Fanselow
and Dong 2010; Kheirbek et al. 2013). VFD deficits in behavioral
“navigatio” are evident (Rosenblum et al. 2001; Tavares et al.
2015), in line with the increase in timidity that we observed in an-
imals with decreased ventral hippocampal PKMζ.

VFD rearing is a contributing factor to timid responses to hu-
man exposure seen in adulthood, and the sites of down-regulation
in PKMζ associated with VFD rearing and with timid behavioral re-
sponses partially overlap. The VFD group shows selective PKMζ
down-regulation in input and output regions of the hippocampal
complex, whereas animals expressing timid responses show gene-
ral decreases in the hippocampus, particularly CA3 and DG. Of the
subregions examined, the stratum moleculare of DG shows numeri-
cally largest PKMζ decreases and large effect sizes both after
VFD (Fig. 1) and in association with timidity (Table 1), and there-
fore could be a key region by which early-life adversity contributes
to the timidity observed in response to human exposure. Survival of an organism enduring stressful environments may, under

Figure 1. Persistent decreases of protein kinase Mζ (PKMζ) expression in ventral hippocampal sub-
regions of adult nonhuman primate (NHP) subjects reared as infants under conditions of variable-foraging
demand (VFD), compared with adult, non-VFD-reared controls. (A) Representative PKMζ immunocyto-
chemistry shows that PKMζ decreases in the ventral hippocampus following VFD. (Top) Confocal images
of ventral hippocampus. White rectangles indicate regions shown in detail below. Color-coded scale bar
at right. (Bottom) Representative region of interest (ROI): CA1, (Mo) stratum moleculare of the suprapy-
amidal blade of the dentate gyrus (DG), (GC) granule cell layer of the suprapyramidal blade of the DG,
Hilus. Scale bar in top right panel, 500 µm in top panels and 80 µm in bottom panels. (B) Mean ± SEM of
PKMζ immunointensity in VFD-reared animals (n=6), compared with non-VFD controls (n=4, set at
100%). (Insert) Diagram outlining ROIs in the ventral hippocampus of bonnet macaque. Significant dif-
fferences, denoted by an asterisk, are for DG stratum moleculare, CA1, and subiculum. Effect sizes (partial
η²) is indicated on the X-axis for the subregions with significantly different P-values are shown in bold
below.
certain scenarios, be adaptively best served through diminution of “emotional navigation.” Impairment of emotional navigation may lead to uncertainty, timidity, fear, avoidance, and even immobilization during which time imminent threats to survival may dissipate or even abate.

It is of interest, in psychodynamic terms, that the VFD condition occurs at a time in macaque infancy that would correspond to a human age of childhood “amnesia” (<3 yr) prior to the recall of “personal memories,” which evidently requires the formation of explicit memories that can be encoded for long-term storage and retrieval (Bruce et al. 2000). We postulate that the early adversity induced by unpredictable maternal care implicit to VFD rearing persistently impacts the faculty of “affective memory,” a form of nondeclarative or implicit memory (Reber 2013). We speculate that affective memory may act as an implicit regulator of trait by subcicular outputs that include nucleus accumbens, amygdala, and prefrontal cortex (Barns et al. 1996). Affective memory potentially underlies components of personal identity (Karavanta 2013) and might modulate risk for the development of psychiatric disease, including posttraumatic stress disorder (PTSD) and depression (Bryant et al. 2007; van Vugt et al. 2012). An impairment of affective memory through reduction of PKMζ expression might also serve a protective or defensive role through reduction of the putative caloric demand required for the maintenance of memories of uncertainty and stress, including those formed early in infancy in the context of adversity (Coplan et al. 2018). Although we are unaware of published work on PKMζ in human depression or PTSD, studies on PKMζ in PTSD are ongoing (Coplan and Sacktor), and the results will be of interest in relation to these NHP data.

Last, our results also suggest that the conceptualization of the molecular treatment of PTSD and/or the consequences of early-life adversity is likely more complex than simply the erasure of traumatic memories. Here we see that the VFD paradigm, modeling at least a risk for PTSD (Heim et al. 1997), entails diminution of a molecular representation of memory traces in the ventral hippocampus.

In conclusion, the current report indicates that developmental adversity results in lifelong reductions of PKMζ expression in subregions of the ventral hippocampus in NHPs. We speculate that attenuation of a neural substrate critical to emotional navigation and affective memory maintenance may ensue. An adversity-induced reduction in PKMζ-maintained LTP in the afferent and efferent projections of the ventral hippocampus could, in turn, impair neocortical function in certain areas or circuitries, in line with a caloric “thrifty” hypothesis that we have proposed for depression (Coplan et al. 2018). The reduction in the molecular mechanism of LTP maintenance within the ventral hippocampus may therefore be relevant to the underlying mechanisms of mood disorders and posttraumatic disorders.

Materials and Methods

Approvals

All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council 2010). The SUNY Downstate Medical Center Institutional Animal Care and Use Committee (IACUC) approved the protocol.

Table 1. Subjects with timid responses to human exposure have lower amounts of protein kinase Mζ (PKMζ) in hippocampus, compared with subjects with confrontational responses

|                  | Timid | SEM | Confrontational | SEM | F(1,7) | P     | η²  |
|------------------|-------|-----|-----------------|-----|--------|-------|-----|
| Moleculare       | 15.4  | 1.4 | 22.0            | 1.4 | 9.11   | 0.02  | 0.57|
| GC               | 15.3  | 0.8 | 19.4            | 0.6 | 9.34   | 0.02  | 0.57|
| Hilus            | 15.7  | 1.0 | 18.6            | 0.8 | 3.59   | 0.10  | 0.34|
| CA3              | 14.5  | 1.1 | 19.1            | 0.7 | 7.89   | 0.03  | 0.53|
| CA1              | 16.4  | 1.4 | 20.7            | 0.5 | 4.05   | 0.08  | 0.37|
| Subiculum        | 19.4  | 1.5 | 22.1            | 0.5 | 1.52   | 0.26  | 0.18|

Mean ± SEM values of PKMζ immunocytochemical fluorescence intensity are expressed in arbitrary units (Timid, n = 6; Confrontational, n = 3). Values from subregions with significantly different P-values, together with F-values and effect sizes are shown in bold.
observer, used for inter-rater reliability, stood a meter behind the intruder. Each human exposure lasted 3 min. Emotional responsivity was rated by the two experimenters blind to rearing status using a 3-point scoring scale. To receive a score of “1” for intruder distress, subjects exhibited consistent “confrontational” behaviors including fang-baring, growling, direct eye contact, piloerection, ear-flexing, cage-shaking, and mouth-gaping. A “timid” response, which received a score of “3,” was characterized by an animal that was nonconfrontational, averting eye contact, submissive in posture, displaying lip-smacking behaviors, and receding to the back of the cage. Normative confrontational behavior in male macaques is consistent and without intervening timidity. Therefore, we partitioned behavioral responses between those with consistent confrontational behaviors and those with varying degrees of timidity, and subjects with intermediate or alternating levels of confrontational and timid behaviors (score of “2”) were classified under “timid.” Inter-rater reliability for the scoring system was κ > 0.90. In general, a timid response, or a posture of subordinance, seeks to avert threat and differs markedly from confrontational responses in which the macaque responds aggressively to threat, mounting a counter-response intended to intimidate the intruder.

Sacrifice
All subjects were sacrificed by transcardiac perfusion with normal saline (500 mL/kg) followed by 4% paraformaldehyde (500 mg/kg) under deep anesthesia with pentobarbital (15 mg/kg, IV). The macaques were born on different dates, and a necropsy team, which is assembled for any terminal study, requires extensive staffing and can only be convened for a limited number of sessions. Therefore, individual life spans will vary. The mean age of the animals at sacrifice was 8.9 yr ± 0.4 yr, and their weight, 10.1 kg ± 0.9 kg. —9 yr of age represents full adulthood, allowing us to address the question of persistence of PKMζ changes into adulthood.

PKMζ immunohistochemistry

PKMζ immunofluorescence
Left brain sections of ventral hippocampus cut to 40-μm thickness were rinsed three times for 5 min each in phosphate-buffered saline (PBS at pH 7.4), and transferred to PBS with 0.2% Triton (PBS-T) for permeabilization. Sections were incubated for 10 min with 0.1% glycine in PBS to quench free aldehydes, and rinsed again with PBS for 10 min. The sections were then incubated in blocking buffer (5% normal goat serum in PBS) for 1 h and then overnight in primary antibody (1:200, rabbit anti-PKMζ (Hernandez et al. 2003) in blocking buffer, or blocking buffer alone as control. Sections were rinsed in PBS, four times for 20 min each, and then incubated with goat anti-rabbit Alexa Fluor 488 (1:200) in blocking buffer overnight on a rotator at room temperature in the dark. The sections were rinsed three times in PBS, washed in distilled water, and mounted with DAPI Vectashield Mounting Medium on glass slides with ProLong Gold (Molecular Probes).

Slides were imaged using an Olympus B552 microscope fitted with the Olympus DP72 camera at 10x. Experimenters were blind to the rearing conditions of the animals. All parameters (pinhole, contrast, and brightness) were held constant for all sections from the same experiment, and the parameters were optimized for each group of control and experimental animals to account for differences in tissue quality. To compare the intensity profile of PKMζ immunostaining between images, we converted the images into grayscale and used the ‘Matlab’ functions ‘imread’ and ‘imager’ to scale the intensity distribution into the full range of a colormap. Background noise was subtracted using averaged region of interest (ROI) measurements from a no primary control slide. Hippocampal complex subregions were identified using a standard rhesus macaque brain atlas (Paxinos et al. 2000) adapted to bonnet macaque. ROIs from each subregion were selected in the grayscale images (Fig. 1B, insert), and mean PKMζ immunointensity calculated using ImageJ analysis software.
PKMζ immunohistochemistry using peroxidase staining
Sections taken from the same hippocampus as immunofluorescence were removed from cryoprotectant, and heat-induced epitope retrieval performed by boiling in citrate buffer (pH 6.0). Sections were washed in 0.2% PBS-T, and endogenous peroxidase activity quenched with 2% H2O2. The sections were then blocked in normal serum, incubated in primary PKMζ antiserum (1:500) for 72 h, washed in 0.2% PBS-T, and incubated in secondary antibody (1:200; Biotinylated goat anti-rabbit, Vector Laboratories), in avidin/biotin solution (ABC Elite, Vector Laboratories), and chromogen developed using 3,3'-diaminobenzidine (Vector DAB Substrate #SK-4100, Vector Laboratories). The sections were mounted, dehydrated, cleared, and cover-slipped with DPX Mountant (Sigma).

Statistics
The analysis used a general linear model (GLM) approach, using Statistica 13.0. In each statistical comparison, we used treatment group as the categorical variable, and hippocampal region was treated as a repeated measures-dependent variable. We also tested for an interactive effect between test condition X hippocampal region. If the overall GLM was significant, we then proceeded to perform post hoc univariate analysis within the GLM. For the human exposure test condition, responses were dichotomized as "confrontational" or "continually or intermittently timid" (see "Human Exposure Response," above), and used as the categorical variable in a separate GLM and region as a dependent variable. We also tested the interactive effect of the human exposure response by region. Weight and age were not used as covariates because they were not statistically different between test conditions. In the VFD experiment, the mean age was 8.4 yr ± 0.2 yr for VFD group, and 9.6 yr ± 0.8 yr for control group; t = -1.79, P = 0.11, Cohen’s d = 1.03. The mean weight was 10.2 kg ± 1.1 kg for VFD group, and 10.5 kg ± 1.8 kg for control group; t = -0.15, P = 0.88, Cohen’s d = 0.09. In the human exposure response analysis, the mean age was 9.7 yr ± 1.1 yr for confrontational group, and 8.5 yr ± 0.2 yr for timid group; t = 1.57, P = 0.16, Cohen’s d = 0.88. The mean weight was 8.0 kg ± 1.9 kg for confrontational group, and 10.9 kg ± 0.9 kg for timid group; t = -1.54, P = 0.17, Cohen’s d = 1.0. Probability of significance was set at P < 0.05 (two-tailed).

Competing interest statement
J.D.C. is a speaker for Sunovion, Abbvie, Teva, Otsuka, BMS, and Neurocrine. He has received grants from Pfizer Pharmaceuticals, GSK, Concept, and Neurocrine. He has served on the advisory board of Otsuka and Lundbeck. No other authors have interests to disclose.

Acknowledgments
This research was supported by grant numbers S001MH083990-05 (J.D.C.), 2R37MH057068 (T.C.S.), R01MH115304 (T.C.S. and A.A.F.), and R01NS108190 (Dr. Peter Bergold [State University of New York Downstate Medical Center] and T.C.S.).

References
Bruce D, Dolan A, Phillips-Grant K. 2000. On the transition from childhood amnesia to the recall of personal memories. Psychol Sci 11: 360–364. doi:10.1111/1467-9280.00271
Bryant RA, Sutherland K, Guthrie RM. 2007. Impaired specific autobiographical memory as a risk factor for posttraumatic stress after trauma. J Abnorm Psychol 116: 837–841. doi:10.1037/0021-843X.116.4 .837
Burns LH, Annett L, Kelley AE, Everitt BJ, Robbins TW. 1996. Effects of lesions to amygdala, ventral subiculum, medial prefrontal cortex, and nucleus accumbens on the reaction to novelty: implication for limbic- striatal interactions. Behav Neurosci 110: 60–73. doi:10.1037/0735-7044.110.1.60
Chen C, Meng SQ, Xue YX, Han Y, Sun CY, Deng JH, Chen N, Bao YP, Zhang H, Cao LL, et al. 2016. Epigenetic modification of PKMζ rescues aging-related cognitive impairment. Sci Rep 6: 22096. doi:10.1038/srep22096
Cohen J. 1973. Eta-squared and partial eta-squared in fixed factor ANOVA designs. Educ Psychol Meas 33: 107–112. doi:10.1177/001316447303000111
Colgin LL, Denninger T, Fynh M, Hafting T, Bonnevie T, Jensen O, Moser MB, Moser EL. 2009. Frequency of gamma oscillations routes flow of information in the hippocampus. Nature 462: 353–357. doi:10.1038/nature08573
Coplan JD, Andrews MW, Rosenblum LA, Owens MJ, Friedman S, Gorman JM, Nemeroﬀ CB. 1996. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: implications for the pathophysiology of mood and anxiety disorders. Proc Natl Acad Sci USA 93: 1619–1623. doi:10.1073/pnas.93.4.1619
Coplan JD, Smith EL, Altemus M, Scharf BA, Owens MJ, Nemeroﬀ CB, Gorman JM, Rosenblum LA. 2001. Variable foraging demand rearing: sustained elevations in cisternal cerebrospinal fluid corticotropin-releasing factor concentrations in adult primates. Biol Psychiatry 50: 200–204. doi:10.1016/S0006-3223(01)01175-1
Coplan JD, Altemus M, Mathijssen SJ, Smith EL, Shab B, Coplan PM, Kral JG, Gorman JM, Nemeroﬀ CB, et al. 2005. Synchronized maternal-infant elevations of primate CSF CRF concentrations in response to variable foraging demand. CNS Spectr 10: S30–S36. doi:10.1071/S109285290001018X
Coplan JD, Smith EL, Altemus M, Mathijssen SJ, Peerera T, Kral JG, Gorman JM, Owens MJ, Nemeroﬀ CB, Rosenblum LA. 2006. Maternal-infant response to variable foraging demand in nonhuman primates: effects of timing of stressor on cerebrospinal fluid corticotropin-releasing factor and circulating glucocorticoid concentrations. Ann NY Acad Sci 1071: 525–533. doi:10.1196/annals.1364.057
Coplan JD, Karim A, Chandra P, St Germain A, Abdallah CG, Altemus M. 2015. Neurobiology of maternal stress: role of social rank and central oxytocin in hypothalamic-pituitary adrenal axis modulation. Front Psychiatry 6: 100. doi:10.3389/fpsyt.2015.00110
Coplan JD, Rozenboom AV, Fulton SL, Panthangi V, Tang J, Thiramangalkal I, Peerera TD, Liu Y, Kamran H, Owens MJ, et al. 2018. Reversal left ventricular dimension and function following early-life stress: a thriﬁe phenotype hypothesis engendering risk for mood and anxiety disorders. Neurobiol Stress 8: 202–210. doi:10.1016/j.ynstr.2017.01.001
Dweck DS, Radwan B, Sparks FT, Talbot ZN, Fenton AA. 2018. Control of recollection by slow gamma dominating mid-frequency gamma in hippocampus C1A. PLoS Biol 16: e2003154. doi:10.1371/journal.pbio.2003154
Ektrom AD, Kallana MJ, Caplan JB, Fields TA, Isham EA, Newman EL, Fried I. 2003. Cellular networks underlying human spatial navigation. Nature 425: 184–188. doi:10.1038/nature01964
Fanselow MS, Dong HW. 2010. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 65: 7–19. doi:10.1016/j.neuron.2009.11.031
Gao PP, Goodman JH, Sacktor TC, Francis JT. 2017. Persistent increased PKMζ in sensorimotor cortex maintain procedural long-term memory storage. Science 359: 90–98. doi:10.1126/science.aap8022
Glanzman DL. 2015. PKMζ and the maintenance of memory. F1000 Biol Rep 5: 4. doi:10.1246/fbr.14085
Hamel AF, Lutz CK, Coleman K, Worlein JM, Peterson EJ, Rosenberg KL, Novak MA, Meyer JS. 2017. Responses to the Human Intruder Test are related to hair cortisol phenotype and sex in zebus macaques (Macaca mulatta). Ann J Prinatol 79: 1–10. doi:10.1002/apj.22526
Hara Y, Punsoni M, Yus F, Park CS, Janssen WG, Rapp PR, Morrison JH. 2012. Synaptic distributions of GluA2 and PKMζ in the monkey dentate gyrus and their relationships with aging and memory. J Neurosci 32: 7336–7344. doi:10.1523/JNEUROSCI.0605-12.2012
Heim C, Owens MJ, Plotisky PM, Nemeroﬀ CB. 1997. Persistent changes in corticotropin-releasing factor systems due to early life stress: relationship to the pathophysiology of major depression and post-traumatic stress disorder. Psychopharmacol Bull 33: 185–192.
Hernandez AI, Blace N, Crary JF, Serrano PA, Leitges M, Libien JM, Glanzman DL, Novak MA, Meyer JS, Gorman JM, Coplan JD, Smith EL, Altemus M, Scharf BA, Owens MJ, Nemeroﬀ CB, Gorman JM, Rosenblum LA. 2001. Variable foraging demand rearing: sustained elevations in cisternal cerebrospinal fluid corticotropin-releasing factor concentrations in adult primates. Biol Psychiatry 50: 200–204. doi:10.1016/S0006-3223(01)01175-1
Hernandez AI, Blace N, Crary JF, Serrano PA, Leitges M, Libien JM, Glanzman DL. 2013. PKMζ and the maintenance of memory. Nat Neurosci 16: 10. doi:10.1002/ajp.22526
Hsieh C, Tsokas P, Grau-Perales A, Lesburguees E, Bukai J, Khanna K, Chorniy J, Chung A, Jou C, Burghardt NS, et al. 2021. Persistent increases of PKMζ in memory-activated neurons trace LTP maintenance during spatial long-term memory storage. *Eur J Neurosci*; doi:10.1111/ejn.15137

Jackowski A, Perera TD, Abdallah CC, Garrido G, Tang CY, Martinez J, Mathew SJ, Gorman JM, Rosenblum LA, Smith EL, et al. 2011. Early-life stress, corpus callosum development, hippocampal volumetrics, and anxious behavior in male nonhuman primates. *Psychiatry Res* 192: 37–44. doi:10.1016/j.psychres.2010.11.006

Kajiwara R, Wouterlood FG, Sah A, Boekel AJ, Baks-te Bulte LT, Witter MP. 2008. Convergence of entorhinal and CA3 inputs onto pyramidal neurons and interneurons in hippocampal area CA1—an anatomical study in the rat. *Hippocampus* 18: 266–280. doi:10.1002/hipo.20385

Kalin NH, Shelton SE. 1989. Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. *Science* 243: 1718–1721. doi:10.1126/science.2564702

Karavanta M. 2013. The injunctions of the spectre of slavery: affective memory and the counterwriting of community. *Fem Rev* 104: 42–60. doi:10.1057/fr.2013.4

Kheirkhah MA, Drew LJ, Burghardt NS, Costantini DO, Tannenholz L, Almari B, Zeng H, Fenton AA, Hen R. 2013. Differential control of learning and anxiety along the dorsoventral axis of the dentate gyrus. *Neuron* 77: 955–968. doi:10.1016/j.neuron.2012.12.038

Ling DS, Bernardo IS, Serrano FA, Blace N, Kelly MT, Cray JP, Sacktor TC. 2002. Protein kinase Mζ is necessary and sufficient for LTP maintenance. *Nat Neurosci* 5: 295–296. doi:10.1038/nn829

Musilová I, Nimmrich V, Hernandez AI, Tcherepanov A, Sacktor TC, Tiedge H. 2004. Dendritic transport and localization of protein kinase Mζ mRNA: implications for molecular memory consolidation. *J Biol Chem* 279: 52613–52622. doi:10.1074/jbc.M409240200

National Research Council. 2010. *Guide for the care and use of laboratory animals*. National Academies Press, Washington, D.C.

Osten P, Valsamis H, Jiang X, Naik MU, Sublette E. 1993. Persistent activation of the ζ isofrom of protein kinase C in the maintenance of long-term potentiation. *Proc Natl Acad Sci* 90: 8342–8346. doi:10.1073/pnas.90.18.8342

Reber PJ. 2013. The neural basis of implicit learning and memory: a review of neuropsychological and neuroimaging research. *Neuropsychologia* 51: 2026–2042. doi:10.1016/j.neuropsychologia.2013.06.019

Rosenblum LA, Forger C, Noland S, Trost RC, Coplan JD. 2001. Response of adolescent bonnet macaques to an acute fear stimulus as a function of early rearing conditions. *Dev Psychobiol* 39: 40–45. doi:10.1002/dev.1026

Sacktor TC, Fenton AA. 2018. What does LTP tell us about the roles of CaMKII and PKMζ in memory? *Mid Brain* 11: 77. doi:10.1186/s13041-018-0420-5

Sacktor TC, Osten P, Valsamis H, Jiang X, Naik MU, Sublette E. 1993. Persistent activation of the ζ isofrom of protein kinase C in the maintenance of long-term potentiation. *Proc Natl Acad Sci* 90: 8342–8346. doi:10.1073/pnas.90.18.8342

Tavares RM, Mendelsohn A, Grossman Y, Williams CH, Shapiro M, Trope Y, Schiller D. 2015. A map for social navigation in the human brain. *Neuron* 87: 231–243. doi:10.1016/j.neuron.2015.06.011

Toda T, Gage FH. 2018. Review: adult neurogenesis contributes to hippocampal plasticity. *Cell Tissue Res* 373: 693–709. doi:10.1007/s00441-017-2735-4

Received July 5, 2021; accepted in revised form July 20, 2021.