1   **Urocortin-3 neurons in the perifornical area mediate the impact of chronic stress on**
2   **female infant-directed behavior**

3   Brenda Abdelmesih[1], Robyn Anderson[1], Ilaria Carta[1], Anita E. Autry[1,2*]

4   1.   Dominick P. Purpura Department of Neuroscience, Albert Einstein College of Medicine,
5   Bronx, NY
6   2.   Department of Psychiatry and Behavioral Sciences, Albert Einstein College of Medicine,
7   Bronx, NY

8   *Correspondence should be addressed to Anita E. Autry, anita.autry@einsteinmed.edu

9   **Abbreviated title:** Ucn3 neurons mediate impact of stress on female parenting

10

Abstract

Infant avoidance and aggression are promoted by activation of the Urocortin-3 expressing neurons of the perifornical area of hypothalamus (PeFA^{Ucn3}) in male and female mice. PeFA^{Ucn3} neurons have been implicated in stress, and stress is known to reduce maternal behavior. We asked how chronic restraint stress (CRS) affects infant-directed behavior in virgin and lactating females and what role PeFA^{Ucn3} neurons play in this process. Here we show that infant-directed behavior increases activity in the PeFA^{Ucn3} neurons in virgin and lactating females. Chemogenetic inhibition of PeFA^{Ucn3} neurons facilitates pup retrieval in virgin females. CRS reduces pup retrieval in virgin females and increases activity of PeFA^{Ucn3} neurons but does not affect maternal behavior in mothers. Inhibition of PeFA^{Ucn3} neurons blocks stress-induced deficits in pup-directed behavior in virgin females. Together, these data illustrate the critical role for PeFA^{Ucn3} neuronal activity in mediating the impact of chronic stress on female infant-directed behavior.

Significance statement

While a large body of research has studied the impact of maternal stress on offspring, few studies have focused on the neural circuitry underlying reduced maternal behavior in stressed mothers. In this study, we examine the neural substrates involved in reduced infant-directed behavior caused by chronic stress. We find that perifornical area neurons expressing the neuropeptide urocortin-3 are critical mediators of the impact of stress on infant-directed behavior in females.

Introduction

Many decades of research have focused on the neurobiology of maternal behavior, revealing common mechanisms and pathways involved in infant caregiving behavior across a variety of species (Numan & Insel, 2003; Numan, 2020). Studies have converged on the critical role of the medial preoptic area of hypothalamus in orchestrating the behavioral responses of mothers to their young in frogs, fish, birds, and rodents (Numan, 1974; Slawski & Buntin, 1995; Fischer *et al.*, 2019; Maruska *et al.*, 2020). Recently, it has been appreciated that these mechanisms may also be involved in paternal behaviors as well, suggesting a core circuitry that exists in both sexes to promote caregiving (O'Connell *et al.*, 2012; Wu *et al.*, 2014; Kohl *et al.*, 2018). Moreover, it has been well-documented that neural plasticity mechanisms underlie the facilitation of infant care behavior, including alloparental care towards unrelated young, particularly in females (Numan & Insel, 2003).

In the absence of caregiving behavior, it is possible to observe neglect or even aggression toward infants by adults. Studies have identified circuit nodes in the brain, including the medial and posterior amygdala, the bed nucleus of stria terminalis, and the perifornical area of hypothalamus, that modulate expression of infant-directed neglect and aggression (Tsuneoka *et al.*, 2015; Chen *et al.*, 2019; Sato *et al.*, 2020; Autry *et al.*, 2021). We wondered if this anti-parental circuitry may be active in neglectful animals including virgin females or stressed virgin and lactating females.

Clinical research clearly shows that stress is a critical risk factor for postpartum mental illnesses including postpartum depression or anxiety which affect up to 25% of women and 10% of men annually in the United States (Paulson & Bazemore, 2010; Wisner *et al.*, 2013). However, there are few preclinical studies that examine the neurobiology underlying reduced parent-infant bonding or associated symptoms in animal models (Nephew & Bridges, 2011; Zoubovsky *et al.*, 2020; Rosinger *et al.*, 2021). Extant research has focused on the impact of maternal stress as a model of early life stress either pre- or postnatally on behavior outcomes in

59　　offspring, often with profound behavioral, physiological, and neurobiological impacts on young

60　　raised by stressed mothers (Cameron *et al.*, 2005; Wang *et al.*, 2011; Singh-Taylor *et al.*, 2015;

61　　Delpech *et al.*, 2016; Feifel *et al.*, 2017; Kronman *et al.*, 2021; Rincon-Cortes & Grace, 2021).

62　　During lactation, females are hyporesponsive to acute stress due to hormonal changes that

63　　impact the Hypothalamic-Pituitary-Adrenal (HPA) axis regulation (Walker *et al.*, 2001; Brunton *et*

64　　*al.*, 2008). HPA axis hypo-responsivity is thought to be protective of anxiety-related behavior

65　　and adult-pup interactions in lactating females (Miller *et al.*, 2011; Medina *et al.*, 2021).

66　　However, chronic stress has been documented to have a long-lasting impact on the regulation

67　　of the HPA axis, leading to reduced parenting, and how the underlying neurobiology is affected

68　　remains poorly understood (Carini *et al.*, 2013; Murgatroyd & Nephew, 2013; Murgatroyd *et al.*,

69　　2015).

70　　　　Ucn3 is a member of the corticotropin releasing factor (CRF) family of stress hormones

71　　and has the highest endogenous binding affinity for CRF receptor 2 (CRFR2). Previous studies

72　　of this group of neurons suggest that they are sensitive to stress and adrenalectomy (loss of

73　　stress hormones) (Jamieson *et al.*, 2006). Overexpression of Ucn3 in the brain leads to

74　　increased anxiety- and depression-related behaviors and results in a blunted HPA response to

75　　stress (Neufeld-Cohen *et al.*, 2012). Furthermore, overexpression of Ucn3 specifically in the

76　　PeFA is associated with enhanced anxiety-like behaviors in mice (Kuperman *et al.*, 2010).

77　　Social discrimination abilities are altered in a sex-specific manner in total Ucn3 knockout mice

78　　(Deussing *et al.*, 2010). Taken together, these studies suggest that PeFA Ucn3 cells mediate

79　　stress-induced behavioral changes.

80　　　　Thus, we hypothesized that chronic stress would negatively affect infant-directed

81　　behavior in females and that this disruption is dependent on activation of perifornical area

82　　urocortin-3 expressing neurons. We set out to determine if anti-parental circuit components,

83　　specifically the urocortin-3 positive neurons in the perifornical area of hypothalamus were more

84　　active in naïve or stressed females, and if we could recover parental behavior by blocking

4

85 activation of this anti-parental circuit node. We find that increased parental behavior is

86 accompanied by decreased activity in perifornical area urocortin-3 expressing neurons and

87 blocking activity in these cells enhances parental behavior in naïve females. Chronic stress

88 reduces alloparental behavior in naïve females and this stress-induced behavioral effect is

89 occluded by inhibition of perifornical area urocortin-3 cells. In stressed lactating females,

90 parental behavior is preserved and perifornical area urocortin-3 cells are less activated in stress.

91 Together, these data reveal a critical role for perifornical urocortin-3 neurons in the expression

92 of alloparental behavior in female mice under both normal and pathological conditions.

93 **Materials and Methods**

94 Animals

95 Mice were maintained on a 12h:12h dark light cycle (10:30am-10:30 pm dark phase)

96 with access to food and water ad libitum. All experiments were performed in accordance with

97 NIH guidelines and approved by the Albert Einstein College of Medicine Institutional Animal

98 Care and Use Committee (IACUC; protocol 20180110; 20180111; 00001386).

99 C57BL/6J sexually naïve female and pregnant female (E14) mice were ordered from

100 Jackson Laboratories (Bar Harbor, ME) aged at 6-8 weeks. Ucn3::Cre BAC transgenic line

101 (STOCK Tg(Ucn3-cre) KF43Gsat/Mmcd 032078-UCD; obtained from laboratory of Catherine

102 Dulac, Harvard University) were genotyped at weaning (3 weeks of age) and used in

103 experiments at age 2-5 months. Animals received from Jackson Laboratories habituated to our

104 facility for 7 days prior to behavioral testing.

105 Corticosterone Measure

106 Trunk blood samples were taken at the time of sacrifice and blood serum was isolated

107 from blood samples by centrifugation. A high-sensitivity corticosterone (CORT) enzyme

108 immunoassay (EIA) was used and analyzed according to manufacturer's instructions

109 (Immunodiagnostic Systems Ltd, Fountain Hills, AZ, USA) as previously described (Autry *et al.*,

110 2009). Briefly, percent binding (B/Bo%) of each calibrator, control and sample was calculated by

111   dividing the mean absorbance over the mean absorbance for '0' calibrator and multiplied by

112   100. A calibration curve was used to plot B/Bo% on the ordinate against concentration of

113   corticosterone. A 4pl curve fit was applied.

114   <u>Chronic restraint stress model</u>

115   Virgin and lactating female mice were used. Lactating females were restrained starting

116   from approximately postpartum (PP) day 2. Both stressed and unstressed mice were brought to

117   a test room under dim red light during their dark cycle. All animals were weighed, and females

118   were either placed back into their home cage or placed into a 50 mL conical tube for one hour.

119   Humidity and temperature in the test room was recorded each day. On the last day of stress,

120   females remained in the test room for 1-2 hours before being exposed to a foreign-born pup

121   (see Parental Behavior).

122   Animals injected with AAV1/DIO-hM4Di (see chemogenetics) recovered from surgery at

123   least 1 week before the start of restraint. For the stressed virgin female group, mice were

124   excluded from the stressed group (n=4 females) based on open field test behavior that was

125   indistinguishable from control. For the groups that received AAV1/DIO-hM4Di injections,

126   females were excluded if they did not show adequate recombination of the DREADD construct

127   (n=2, virgin females Figure 2; n=0 stressed virgin females Figure 6).

128   <u>Intruder stress model</u>

129   Lactating females were stressed starting from approximately postpartum (PP) day 2.

130   Both stressed and unstressed mice were brought to a test room under dim red light during their

131   dark cycle. All animals were weighed, and females were placed back into their home cage and

132   either placed back on the housing rack or had an intact adult male intruder (C57, ~2 months old)

133   introduced into their home cage for 10 minutes as described in previous studies (Carini *et al.*,

134   2013; Murgatroyd *et al.*, 2016). In the event the male was too aggressive toward pups, the

135   intruder stress period was curtailed. Humidity and temperature in the test room was recorded

136   each day. On the last day of stress, females remained in the test room for 1-2 hours before

137   being exposed to a foreign-born pup (see Parental Behavior).

138

139   <u>Behavior assays</u>

140   Mice were individually housed for at least 1 week prior to testing. Experiments were

141   conducted during the dark phase under dim red light. Tests were recorded by Fly Capture

142   cameras (Point Grey, Richmond, BC, Canada) and behaviors were scored by an observer blind

143   to experimental condition using Observer XT13 Software or Ethovision XT 13 (Noldus

144   Information Technology, Leesburg, VA, USA). Animals were tested for a single behavior per

145   session with at least 24 hours between sessions.

146   *Parental behavior*

147   Parental behavior tests were conducted in the mouse's home cage as previously

148   described (Wu *et al.*, 2014). Mice were habituated to the testing environment for 10 minutes.

149   One to two C57BL6/J pups 1-4 days old were presented in the cage in the opposite corner to

150   the nest. Test sessions started either at pup introduction or pup approach (female first touches

151   the pup with its snout) and lasted for 10- 15 minutes. If the mouse became aggressive by biting

152   and wounding the pup, the session was immediately halted, and the pup was euthanized. The

153   following behaviors were quantified: latency to retrieve, pup investigation (sniffing, close contact

154   with snout), grooming (handling with forepaws and licking), nest building, time spent in the nest,

155   crouching, latency to attack (latency to bite and wound), aggression (roughly handling,

156   aggressively grooming, aggressive carrying with no retrieval), and tail rattling. A 'parenting

157   behavior' index was calculated as the sum of duration of grooming, nest building, time spent in

158   the nest, and crouching.

159   *Open field*

160   Mice were assessed for activity in a 45cm x 45 cm open field at 40 lux for 5 min as

161   previously described (Autry *et al.*, 2009). Center was considered 15 cm x 15 cm and borders

162 were 5 cm around the perimeter of the box. Time and frequency in center and borders as well

163 as distance and velocity were calculated using Ethovision XT13.

164 Behavioral ethograms were made in Matlab using custom code.

165 Fluorescence in situ hybridization

166 Fluorescence in situ hybridization (FISH) was performed as recommended by ACD Bio

167 (Newark, CA, USA) using V1 RNAscope reagents. Briefly, fresh brain tissue was collected from

168 animals housed in their home cages or 35 min after the start of the behavior tests for immediate

169 early gene (*Fos)* studies. Brains were embedded in OCT (Tissue-Tek) and frozen with dry ice.

170 25µm cryosections were used for mRNA in situ. Adjacent sections from each brain

171 were collected over replicate slides to stain with multiple probes. Protease 3 was used to digest

172 tissue. Fos (Cat No. 316921), Ucn3 (Cat No. 464861), and Crh (Cat No. 316091) probes were

173 used as per manufacturer's instructions. Slides were mounted using Prolong Gold with DAPI.

174 Zeiss Axioscan was used to image DAPI, Alexa 488, Atto-550, and Alexa 647 at 20X

175 magnification.

176 Immunostaining and histology

177 To visualize c-Fos protein in combination with AAV-hM4Di, perfused tissue was sliced

178 on a freezing microtome at 30 µm, and every third section throughout the PeFA was stained.

179 Sections were rinsed with 0.1% PBS with Triton (PBST), blocked with 5% donkey serum diluted

180 in PBST (blocking solution) for 1 hour at room temperature. Primary antibody chicken anti-

181 mCherry (Millipore AB3566481) and rabbit anti c-Fos (Cell Signaling 2250S) were diluted at

182 1:1000 in blocking solution and sections were incubated overnight at 4°C. After rinsing with

183 PBST, secondary anti-chicken-A594 (Sigma CF594) and anti-rabbit-A647 (Life Technologies

184 A31573) were applied at 1:200 and 1:1000 dilutions, respectively, in blocking solution and

185 incubated overnight at 4°C. Sections were rinsed in PBS, mounted to Superfrost Plus slides,

186 coverslipped with Prolong Gold containing DAPI, and imaged on the Zeiss Axioscan as

187 described previously.

188   Chemogenetics

189   Ucn3::Cre virgin female mice (or Cre negative littermates as controls) 8-20 weeks old were

190   used for these experiments. We stereotaxically injected ~225 nL of conditional inhibitory

191   designer receptor exclusively activated by designer drug (DREADD) virus bilaterally into the

192   PeFA (AP -0.6mm, ±ML 0.3mm, DV -4.2mm). For the naïve virgin female DREADD

193   experiment, we used a custom prep from UNC Vector Core (AAV1-hSyn-DIO-HM4D(Gi)-

194   mCherry; Chapel Hill, North Carolina, USA) and for the stressed virgin female DREADD

195   experiment we used a custom prep from Vector Builder (AAV1-hSyn-FLEX-HM4D(Gi)-mCherry;

196   Chicago, Illinois, USA). Animals used for stress study recovered from surgery for 1 week before

197   the start of restraint and around two-three weeks before behavioral testing.

198   Cre-positive and Cre-negative females were administered intraperitoneally (i.p) with

199   either 1x PBS (vehicle) or 0.3mg/kg clozapine-n-oxide (CNO) dissolved in 1x PBS and

200   habituated to the testing environment for two-three hours prior to pup assay. Females were

201   presented one to two C57BL6/J pups in the corner of their home-cage opposite the nest and

202   parental behaviors were recorded for 10-15 minutes.

203   Four control animals used in the stress study were administered either CNO or saline i.p

204   and 2 hours later were exposed to a pup. Animals were then perfused 90 minutes later to stain

205   for c-Fos protein expression. (See Immunostaining and histology)

206   Data analysis and Statistics

207   Data was analyzed by Graphpad Prism 9.0 or Matlab scripts. For colocalization

208   experiments, we used Fisher's exact test to compare the total number of fos+/marker+ positive

209   cells to the total number of fos-/marker+ positive cell populations across all mice and expressed

210   the data as percentages from each individual mouse. Pup retrieval percentages are analyzed

211   by Kolmogorov-Smirnoff (2 groups) or Friedman test (3 groups). For experiments comparing

212   one manipulation to control (i.e., stress or neuronal inactivation), we used t-test for parameters

213   with normally distributed data and Mann-Whitney test for non-normally distributed data. To

214   compare one manipulation and control across several sessions, we used one-way repeated

215   measures ANOVA tests followed by post-hoc correction. In experiments with comparison of two

216   manipulations in several sessions (stress and neuronal inactivation), we used two-way repeated

217   measures ANOVA followed by post-hoc correction. P values reported as follows: <0.05 *, **

218   P<0.01, *** P<0.001, **** P<0.0001. All data are expressed as mean ± SEM.

219   <u>Image analysis</u>

220   Images were exported from Zen Blue software and cells were manually counted for

221   colocalization using FIJI Cell Counter. Graphpad Prism 9 was used to plot graphs and perform

222   statistics.

223   <u>Fiber Photometry</u>

224   Ucn3-cre animals were injected with a cocktail of 150 nL of AAV-syn-jGCaMP7f-WPRE

225   (Addgene 104488-AAV9) and 225 nL of AAV-hSyn-DIO-hM4D(Gi)-mCherry into PeFA (ML:

226   0.3mm; AP -0.6mm; DV 4.2mm). In the same surgery, a 200μM fiber optic cannula was

227   implanted (ML: 0.3mm; AP -0.6mm; DV 4.2mm). Animals recovered for at least 3 weeks before

228   behavioral experiments. Animals were brought up to the test room in dim red light and injected

229   with either vehicle (session 1) or 0.3mg/kg CNO (session 2) i.p. Two hours later, a fiber optic

230   patch cable (Doric) was attached to the cannula and adjusted to attachment for 10 minutes

231   before recording. Using a multi-channel fiber photometry system (Neurophotometrics LTD), a

232   470 nm LED and 415 nm LED (isosbestic control) alternatively illuminated at 60μW via a 20X

233   objective and fluorescence emission was collected using a CMOS camera sensor. After 1-2

234   minutes of recording, animals underwent 6 tail suspensions for approximately 5 seconds per

235   suspension. Data were acquired using the open-source software Bonsai.

236   Photometry data was analyzed using custom MATLAB code. To correct for

237   photobleaching and motion artifact, we used normalization similarly described by Hvratin et al

238   2020 (Hrvatin *et al.*, 2020). In short, the isosbestic signal was fit with a biexponential and then

239   linearly scaled to fit signal emitted by GCaMP. GCaMP signal was then divided by the scaled fit

240   for ΔF/F. Tail suspension events were aligned to normalized photometric signal and peri-events

241   were taken from 5 sec before tail suspension ("pre") to 5 sec after ("post"). The pre-event

242   baseline was used to calculate the z score $\frac{\chi_i - \chi_{baseline}}{s_{baseline}}$. The mean ΔF/F of each pre- and post- tail

243   suspension event was taken and averaged across animals per group (vehicle vs CNO) and

244   compared using a paired t test. Area under curve was calculated with the mean ΔF/F of each

245   pre- and post- tail suspension event using the MATLAB built-in function "trapz". Standard error

246   mean is plotted with the average z-score.

247   <u>Code availability</u>

248   Custom Matlab code for ethogram generation and analysis of photometry data is

249   available upon request.

250

251   **Results**

252   To identify the activation levels of Urocortin-3 in the rostral perifornical area of the

253   hypothalamus, we exposed C57 virgin females as well as lactating females (postpartum day 2)

254   to either a foreign pup (P0-P4) or ~25 mg of fresh bedding (control) in their home cage. Animals

255   were subsequently sacrificed 30 minutes after exposure (Figure 1A). To control for number of

256   pups as well and foreign pup discrimination (Ostermeyer & Elwood, 1983; Mogi *et al.*, 2017), we

257   utilized two groups of lactating females that either had their litter removed 10 minutes prior to

258   foreign pup introduction or kept their litter (Figure 1A). Visualization of immediate early gene Fos

259   and Ucn3 in PeFA revealed increases in PeFA[Ucn3] cell activation in virgin females exposed to a

260   pup compared to controls (Figure 1B, C; Supplemental Figure 1-1). However, in lactating

261   females, Fos levels in PeFA[Ucn3] decrease with pup exposure if litter has been removed but

262   increase if litter is present. These results suggest that PeFA[Ucn3] cells respond to pup exposure

263   similarly in virgin females and lactating females that keep their litter, while we observe opposite

264   impact on PeFA[Ucn3] cell activity in mothers when her litter is removed.

11

265   Because virgin females are not as parental as lactating females (Lonstein & De Vries,

266   2000; Kuroda *et al.*, 2011; Marlin *et al.*, 2015; Carcea *et al.*, 2021) and PeFA^{Ucn3} neurons are

267   activated by infanticide in females (Autry *et al.*, 2021), we wanted to test if suppression of

268   PeFA^{Ucn3} neuronal activity could enhance alloparental behavior in virgin females. To

269   accomplish this, we used a conditional viral strategy to express the inhibitory designer receptor

270   exclusively activated by designer drug (DREADD hM4Di) in Ucn3::Cre positive and Cre

271   negative animals in the PeFA of virgin females naïve to pups. Two-three weeks after viral

272   injection, both groups of animals were administered CNO (0.3 mg/kg intraperitoneally) and 2-3

273   hours later, exposed to two pups for fifteen minutes in their home cage (Figure 2A;

274   Supplemental Figure 2-1). We confirmed viral recombination to include females in subsequent

275   behavioral analyses (Figure 2B). Cre+ females retrieved more pups in a shorter amount of time

276   relative to Cre- females (Figure 2C & D). However, there was no difference in latency to retrieve

277   the 2nd pup (Figure 2F). Furthermore, Cre+ animals spent more time in the nest with pups and

278   started nest-building earlier compared to Cre- females (Figure 2K &N). While other behaviors

279   were not improved (Figure 2 G-J, L, M,O-P), suppression of PeFA^{Ucn3} neurons improved certain

280   aspects of alloparenting behavior (Figure 2Q), particularly pup retrieval and time spent in the

281   nest with the pups.

282   To understand the impact of chronic stress on alloparental behavior in virgin females, we

283   employed a chronic restraint stress paradigm in which females were placed into a 50 mL Falcon

284   conical tube for 1 hour a day for 20 days (Figure 3A). Stressed females weighed significantly

285   less than control females (Figure 3B). On day 19, females were tested for exploratory behavior

286   in a 5-minute open field task (Figure 3C-F). Stressed females spent less time in the center of

287   the field compared to control females (Figure 3C). On the last day of stress, day 20, females

288   were exposed to a newborn pup (P0-4) for 15 minutes and their alloparental behavior was

289   recorded and analyzed (Figure 3G-R). Only 2 out of 9, or 22%, of stressed females retrieved

290   pups compared to control females (5 of 9, or 55% retrieved) (Figure 3G). Other than retrieval

291 latency, virgin stressed females did not show any significant changes in other measures of

292 alloparental behavior. Altogether, we find that chronic restraint stress significantly reduces pup

293 retrieval in virgin females.

294   Next, to understand the impact of chronic stress on maternal behavior in lactating

295 females, we utilized the same chronic restraint paradigm in females from postpartum day 2-18

296 (Figure 4A), before weaning age for pups. Like stressed virgin females, stressed lactating

297 females weighed significantly less than control females (Figure 4B), indicating that chronic

298 restraint induced physiological changes. On day 16 of chronic restraint, females were tested for

299 anxiety-related behavior in a 5-minute open field task (Figure 4C-F). Surprisingly, stressed

300 females spent more time in the center of the field and less time in the borders (Figure 4C, D).

301 Stressed females also showed an increase in velocity and distance traveled relative to control

302 females (Figure 4E, F). On the last day of stress, day 17, females had their litters removed and

303 10 minutes later we introduced a foreign-born pup to their home cage (Figure 4G-R). All females

304 retrieved pups before the end of the 10 minutes session (Figure 4G, H) and there was no

305 difference in latency to retrieve between groups. Stressed mothers showed similar levels of

306 parenting toward pups as control mothers. We also attempted to use an intruder stress

307 paradigm that has previously been reported to impact parental behavior in lactating females

308 (Carini *et al.*, 2013; Murgatroyd *et al.*, 2016). We did not see any weight changes or parenting

309 measures (Supplemental Figure 4-1).

310   Next, we investigated molecular and physiological impacts of chronic restraint stress in

311 virgin or lactating females.   In situ hybridization revealed increases in PeFA^{Ucn3}/Fos

312 colocalization in stressed virgin females (Figure 5A, B; Supplemental Figure 5-1). In control

313 virgin females, percentage of PeFA^{Ucn3}/Fos colocalization was negatively correlated with time

314 spent parenting, indicating that activation of PeFA^{Ucn3} may reduce alloparental behaviors (Figure

315 5E). Because activation of corticotropin releasing factor (CRF) cells in the paraventricular

316 hypothalamus (PVH) is postulated to disrupt maternal behavior and is critical for physiological

13

317   stress responses (Herman & Tasker, 2016; Klampfl & Bosch, 2019), we also quantified

318   PVH^{CRF}/Fos colocalization (Figure 5C,F). We found that PVH^{CRH}/Fos levels were significantly

319   reduced in both stressed virgin females and lactating females, and like $PeFA^{Ucn3}$, PVH^{CRF}

320   neuronal activation is negatively correlated with parental behaviors (Figure 5F). Chronic

321   restraint stress did not affect circulating CORT levels in virgin females (Figure 5D). In mothers,

322   chronic stress led to a decrease in $PeFA^{Ucn3}$/Fos levels compared to control lactating females,

323   opposite to the effect we observed in virgin females (Figure 5G, H). Like virgin females,

324   however, PVH^{CRF} cell activation was significantly decreased in chronically stressed lactating

325   females (Figure 5G, I; Supplemental Figure 5-1), consistent with previous literature (Girotti *et*

326   *al.*, 2006; Radley & Sawchenko, 2015; Matovic *et al.*, 2020). We observed a similar negative

327   trend for correlation of $PeFA^{Ucn3}$ neuronal activation and parental behaviors in control lactating

328   females that we observed in virgin females, but the trend for PVH^{CRF} cell activation is positively

329   correlated in lactating females (Figure 5 K, L). We observed that CORT levels were significantly

330   decreased in stressed lactating females (Figure 5J), suggesting adaptive habituation to the

331   repeated stress. In our intruder stress experiment, we did not observe molecular changes in

332     $PeFA^{Ucn3}$ activation or in circulating corticosterone levels, consistent with no changes in weight

333   or parental behavior (Supplemental Figure 5-2). Altogether, chronic restraint stress induces

334   differential $PeFA^{Ucn3}$ activation patterns in virgin and lactating females in response to pups,

335   while chronic stress reduces PVH^{CRF} neuronal activation in both virgins and mothers.

336   Because our chronic restraint stress paradigm dampened alloparental behavior in virgin

337   females and increased $PeFA^{Ucn3}$ neuronal activation, we sought to ameliorate deficits in

338   parenting by inhibiting $PeFA^{Ucn3}$ neurons during pup exposure. To accomplish this, we injected

339   virgin Ucn3::Cre positive and negative females with AAV1-eF1a-DIO-hM4Di-mCherry and then

340   started restraint stress 1 week after recovery from surgery. After 16 days of chronic restraint

341   stress, we injected either vehicle or CNO on 2 consecutive days after the last day of stress.

342   After several days we then employed randomized CNO/Vehicle open field trials. On day 32, we

14

343   performed an additional pup exposure with vehicle treatment (Figure 6A). Stressed females had

344   a significant difference in weight compared to control females (Figure 6B). During open field,

345   CNO administration did not induce changes in exploratory behaviors in either group (Figure 6C-

346   F). In the pup exposure assay, both stressed and control virgin females had improved

347   cumulative retrieval with CNO treatment, which we did not observe in the Cre negative group

348   (Figure 6 G, H; Supplemental Figure 6-1). Strikingly, stressed females treated with CNO

349   displayed improved latency to retrieve, time spent crouching, time spent in nest and overall time

350   spent parenting which did not occur in unstressed controls or Cre negative controls, or in the

351   final vehicle session (Figure 6I, L, M, N; Supplemental Figure 6-1). No significant changes were

352   observed in pup grooming (Figure 6K). Interestingly, pup investigation was significantly

353   decreased in both groups which may be due to increased familiarity (Figure 6J) (Bielsky *et al.*,

354   2005; Richter *et al.*, 2005; Moy *et al.*, 2008). CNO administration did not improve any of the

355   parenting measures in stressed Cre negative females (Supplemental Figure 6-1).   We

356   confirmed that CNO administration reduced activity in the PeFA^{Ucn3} neurons using both

357   histology and fiber photometry recording (Supplemental Figure 6-2). Altogether, inhibition of

358   PeFA^{Ucn3} neuronal activity leads to enhancement in parenting behaviors in stressed virgin

359   females (Figure 6O, P).

360

361   **Discussion**

362   Previously, we have observed that virgin females showing alloparental behavior toward

363   pups have a low-level of activation of PeFA^{Ucn3} neurons while activating these cells highly with

364   chemogenetic or optogenetic methods leads to infant-directed neglect and aggression toward

365   pups (Autry *et al.*, 2021). In the present study, we further explored the role of urocortin-3

366   neurons of the perifornical area during female alloparental and maternal behavior. We aimed to

367   examine the intersection between the role of these neurons in infant-directed behavior and their

368   putative role in the hypothalamic-pituitary-adrenal axis. We hypothesized that PeFA^{Ucn3} neurons

369   become more active with stress and increased PeFA^{Ucn3} cell activity would lead to deficits in

370   pup-directed behavior. Therefore, we studied activation levels of PeFA^{Ucn3} neurons in virgin

371   females and mothers exposed to pups with and without stress and studied the effect of

372   PeFA^{Ucn3} neuron inhibition on alloparental behavior under non-stressed and stressed

373   conditions.

374   We find that around 20% of PeFA^{Ucn3} neurons are active during infant-directed behavior

375   in virgin females, replicating our previous findings (Autry *et al.*, 2021). In addition, we replicated

376   our finding that mothers have a lower level of PeFA^{Ucn3} neuronal activation during pup exposure

377   compared to virgin females. However, we noticed that the controls for our mother group, in

378   which we typically remove the litter, had a high baseline of PeFA^{Ucn3} neuron activity compared

379   to virgin females exposed to bedding. We therefore added a group of mothers who did not have

380   their litters removed. This experiment revealed that our control bedding exposure and

381   experimental pup exposure conditions impact PeFA^{Ucn3} neural activity differentially in mothers

382   depending on whether the mother's litter is present. These results indicate that PeFA^{Ucn3}

383   neurons may be sensitive to social contexts; these neurons appear to have high baseline

384   activity in mothers with their litters removed during a control bedding exposure and this activity

385   level plummets with introduction of foreign pups. In the future, it will be important to tease apart

386   whether this heightened PeFA^{Ucn3} neural activity after litter removal may be related to an

387   aversive or stressed state, or possibly a social motivation set point that is altered after a female

388   gives birth. Indeed, previous studies have shown that maternal separation can impact both a

389   mother and their offspring's behavior in measures related to anxiety, social behavior, and

390   cognition (Lemaire *et al.*, 2000; Weinstock, 2001; Chapillon *et al.*, 2002).

391   We next assessed the impact of inhibiting this low-level of activity during infant-directed

392   behavior. We found that there was a subtle but significant effect of inhibition PeFA^{Ucn3} neurons

393   on pup-retrieval latency in virgin females. We previously observed that activation of the

394   excitatory PeFA^{Ucn3} neuron projections to the ventromedial hypothalamus or lateral septum

16

395   mediate infant avoidance and neglect. Our current results suggest that inhibition of PeFA^{Ucn3}

396   neurons in virgin females may lead to decreased activity in these target areas responsible for

397   negative pup directed behavior, allowing for faster infant retrieval. While the behavioral impact

398   of this manipulation is relatively minor, it is in line with the low-level of activation we observe at

399   the cellular level.

400   In a parallel set of experiments, we tested the impact of chronic stress on infant-directed

401   behavior and PeFA^{Ucn3} neuronal activity in virgin females and mothers. We started by testing a

402   chronic restraint stress paradigm in virgin females. With this paradigm, we observed significant

403   weight loss in females exposed to daily restraint compared to unstressed females. Prior to

404   testing infant-directed behavior, we tested open-field behavior to ensure that the stress

405   paradigm had a behavioral impact after two weeks of chronic restraint, and indeed we observed

406   a reduction in exploration time of the center of the arena in stressed females. Thus, we

407   continued with the infant-directed behavior assay and observed that females with chronic

408   restraint showed significantly less pup retrieval compared to unstressed females, with 2 out of 9

409   stressed females displaying infant-directed aggression behavior. When we examined the

410   physiological effects of stress, we found that circulating CORT levels were not impacted though

411   PVH^{CRF} neuron activation was significantly reduced in females subjected to stress, suggestive

412   of HPA axis habituation to the repeated stress. However, we did find enhanced PeFA^{Ucn3}

413   neuron activity in chronically stressed females. Intriguingly, we found a strong negative

414   correlation between PeFA^{Ucn3} neuronal activity levels and overall alloparenting behavior in

415   unstressed females, and this correlation was lost in stressed females. Together, these data

416   support our hypothesis that stress increases activity of PeFA^{Ucn3} neurons, and heightened

417   activity in PeFA^{Ucn3} neurons negatively impacts infant-directed behavior.

418   We therefore proceeded with the chronic restraint paradigm in lactating females. We

419   used a similar timeline for testing, with daily weighing and an open field test prior to pup

420   exposure. We observed significant weight loss in lactating females exposed to stress, however

17

421   our open field test revealed that females with chronic restraint stress displayed increased

422   exploration of the center of the open field relative to control females. This behavior may be a

423   sign of hypervigilance and may be explained by an increase in distance traveled as well as

424   velocity (Cabib *et al.*, 1988; Sequeira-Cordero *et al.*, 2019; Rudolph *et al.*, 2020). In our

425   subsequent infant-directed behavior assessment, we found no differences in maternal behavior

426   between unstressed and chronically restrained mothers. In our physiological measures, we

427   observed that CORT levels were decreased in mothers with chronic restraint stress with a

428   decrease in both CRF and Ucn3 neuronal activation. The decrease in CORT and CRF neuronal

429   activation are indicative of HPA axis habituation to the repeated stress. Indeed, previous

430   studies have illustrated a reduction in CRF/Fos colabeling or electrophysiological properties of

431   CRF neurons in the PVH with repeated restraint stress in rodents (Bonaz & Rivest, 1998;

432   Matovic *et al.*, 2020). We interpret the decrease in Ucn3 neuronal activity as a protective

433   mechanism, preserving maternal behavior under stressful conditions. Indeed, previous studies

434   show that lactating mothers display changes in HPA axis responsivity to stress (Johnstone *et*

435   *al.*, 2000; Douglas *et al.*, 2003; Klampfl & Bosch, 2019), and reduction in PeFA[Ucn3] neuronal

436   activity may contribute to behavioral adaptations to stress.

437   To overcome the habituation to repeated restraint we observed in mothers, we

438   attempted to perform chronic social stress in lactating females (Supplemental Figure 4-1).

439   However, the stress did not result in weight changes or impact maternal behavior. In the future,

440   we hope to identify a stress paradigm for lactating females that impacts maternal behavior.

441   Furthermore, we aim to study the role of PeFA[Ucn3] neurons in HPA axis hypo-responsivity in

442   mothers to uncover their potential role in maternal behavior preservation.

443   We plotted female infant-directed behaviors as ethograms to gain broader insight into

444   how stress affects this complex interaction. Surprisingly, we found that virgin females display

445   longer bouts of infant-directed behaviors during the pup exposure assay relative to mothers,

446   whose behavioral motifs appear to be more sporadic from one behavior to the next (Zoubovsky

18

447   *et al.*, 2020). We suspect that this difference is at least in part due to the removal of the litter

448   during habituation for mothers that is not required for testing virgin females. However, this

449   result does imply that, at least in terms of studying the neural architecture of infant-directed

450   behavior, (1) we can collect a rich dataset from virgin females and (2) that we must be diligent in

451   considering the conditions under which we test behavior in mothers in the laboratory given that

452   litter removal may have a significant impact on some experimental parameters (Lonstein, 2005;

453   Smith & Lonstein, 2008; Miller *et al.*, 2011).

454   Finally, we tested if we could rescue stress-induced deficits in alloparental behavior by

455   inhibiting PeFA[Ucn3] neurons. We confirmed that our chronic restraint stress paradigm led to

456   weight reductions and proceeded with our pup exposure assay. We found that chemogenetic

457   inhibition of PeFA[Ucn3] neurons did lead to improved alloparental behavior on several measures

458   including latency to retrieve, time in nest, and crouching. We designed the experiment to

459   observe the effect of stress alone on the first day with vehicle administration followed by

460   inhibition of PeFA[Ucn3] neurons by CNO treatment on the second day, with a final vehicle test

461   after drug washout. We settled on this design based on our previous observation that blocking

462   PeFA[Ucn3] neuronal activity optogenetically led to prolonged improvement of infant-directed

463   behavior. We included two control groups, the nonstressed Cre positive group, and the

464   stressed Cre negative group to control for the effects of repeated testing. Our data illustrate that

465   inhibiting PeFA[Ucn3] neurons in stressed females leads to more substantial effects on alloparental

466   behavior compared to either control group.

467   Overall, we find that PeFA[Ucn3] neuronal activity is higher in females showing lower levels

468   of positive infant-directed behavior, a trend that can be observed regardless of physiological

469   status. Chronic stress leads to reduced alloparental behavior accompanied by higher numbers

470   of active PeFA[Ucn3] neurons in virgin females. Blocking activity in PeFA[Ucn3] neurons rescues

471   infant-directed behavioral deficits in virgin females. Together with previous studies, our results

472   suggest the important role for the level of PeFA[Ucn3] neuronal activity in the expression of pro-

19

473   and anti-parental behavior (Supplemental Figure 6-3). These results support the critical role for

474   PeFA^{Ucn3} neurons in the neural circuitry controlling female parental behaviors and the sensitivity

475   of these behaviors to stress.

476

477   **Author Contributions**

478   B.A. and A.E.A. designed and performed experiments, analyzed, and plotted data, and

479   interpreted data and wrote the paper. R.A. supported animal experiments and analyzed data.

480   I.C. analyzed and plotted data.

481

493

References

Autry, A.E., Adachi, M., Cheng, P. & Monteggia, L.M. (2009) Gender-specific impact of brain-derived neurotrophic factor signaling on stress-induced depression-like behavior. *Biol Psychiatry*, **66**, 84-90.

Autry, A.E., Wu, Z., Kapoor, V., Kohl, J., Bambah-Mukku, D., Rubinstein, N.D., Marin-Rodriguez, B., Carta, I., Sedwick, V., Tang, M. & Dulac, C. (2021) Urocortin-3 neurons in the mouse perifornical area promote infant-directed neglect and aggression. *Elife*, **10**.

Bielsky, I.F., Hu, S.B., Ren, X., Terwilliger, E.F. & Young, L.J. (2005) The V1a vasopressin receptor is necessary and sufficient for normal social recognition: a gene replacement study. *Neuron*, **47**, 503-513.

Bonaz, B. & Rivest, S. (1998) Effect of a chronic stress on CRF neuronal activity and expression of its type 1 receptor in the rat brain. *Am J Physiol*, **275**, R1438-1449.

Brunton, P.J., Russell, J.A. & Douglas, A.J. (2008) Adaptive responses of the maternal hypothalamic-pituitary-adrenal axis during pregnancy and lactation. *J Neuroendocrinol*, **20**, 764-776.

Cabib, S., Kempf, E., Schleef, C., Mele, A. & Puglisi-Allegra, S. (1988) Different effects of acute and chronic stress on two dopamine-mediated behaviors in the mouse. *Physiol Behav*, **43**, 223-227.

Cameron, N.M., Champagne, F.A., Parent, C., Fish, E.W., Ozaki-Kuroda, K. & Meaney, M.J. (2005) The programming of individual differences in defensive responses and reproductive strategies in the rat through variations in maternal care. *Neurosci Biobehav Rev*, **29**, 843-865.

Carcea, I., Caraballo, N.L., Marlin, B.J., Ooyama, R., Riceberg, J.S., Mendoza Navarro, J.M., Opendak, M., Diaz, V.E., Schuster, L., Alvarado Torres, M.I., Lethin, H., Ramos, D., Minder, J., Mendoza, S.L., Bair-Marshall, C.J., Samadjopoulos, G.H., Hidema, S., Falkner, A., Lin, D., Mar, A., Wadghiri, Y.Z., Nishimori, K., Kikusui, T., Mogi, K., Sullivan, R.M. & Froemke, R.C. (2021) Oxytocin neurons enable social transmission of maternal behaviour. *Nature*, **596**, 553-557.

Carini, L.M., Murgatroyd, C.A. & Nephew, B.C. (2013) Using chronic social stress to model postpartum depression in lactating rodents. *J Vis Exp*, e50324.

Chapillon, P., Patin, V., Roy, V., Vincent, A. & Caston, J. (2002) Effects of pre- and postnatal stimulation on developmental, emotional, and cognitive aspects in rodents: a review. *Dev Psychobiol*, **41**, 373-387.

536

537 Chen, P.B., Hu, R.K., Wu, Y.E., Pan, L., Huang, S., Micevych, P.E. & Hong, W. (2019) Sexually
538   Dimorphic Control of Parenting Behavior by the Medial Amygdala. *Cell*, **176**, 1206-1221
539   e1218.

540

541 Delpech, J.C., Wei, L., Hao, J., Yu, X., Madore, C., Butovsky, O. & Kaffman, A. (2016) Early life
542   stress perturbs the maturation of microglia in the developing hippocampus. *Brain Behav*
543   *Immun*, **57**, 79-93.

544

545 Deussing, J.M., Breu, J., Kuhne, C., Kallnik, M., Bunck, M., Glasl, L., Yen, Y.C., Schmidt, M.V.,
546   Zurmuhlen, R., Vogl, A.M., Gailus-Durner, V., Fuchs, H., Holter, S.M., Wotjak, C.T.,
547   Landgraf, R., de Angelis, M.H., Holsboer, F. & Wurst, W. (2010) Urocortin 3 modulates
548   social discrimination abilities via corticotropin-releasing hormone receptor type 2. *The*
549   *Journal of neuroscience : the official journal of the Society for Neuroscience*, **30**, 9103-
550   9116.

551

552 Douglas, A.J., Brunton, P.J., Bosch, O.J., Russell, J.A. & Neumann, I.D. (2003) Neuroendocrine
553   responses to stress in mice: hyporesponsiveness in pregnancy and parturition.
554   *Endocrinology*, **144**, 5268-5276.

555

556 Feifel, A.J., Shair, H.N. & Schmauss, C. (2017) Lasting effects of early life stress in mice:
557   interaction of maternal environment and infant genes. *Genes Brain Behav*, **16**, 768-780.

558

559 Fischer, E.K., Roland, A.B., Moskowitz, N.A., Tapia, E.E., Summers, K., Coloma, L.A. &
560   O'Connell, L.A. (2019) The neural basis of tadpole transport in poison frogs. *Proc Biol*
561   *Sci*, **286**, 20191084.

562

563 Girotti, M., Pace, T.W., Gaylord, R.I., Rubin, B.A., Herman, J.P. & Spencer, R.L. (2006)
564   Habituation to repeated restraint stress is associated with lack of stress-induced c-fos
565   expression in primary sensory processing areas of the rat brain. *Neuroscience*, **138**,
566   1067-1081.

567

568 Herman, J.P. & Tasker, J.G. (2016) Paraventricular Hypothalamic Mechanisms of Chronic
569   Stress Adaptation. *Front Endocrinol (Lausanne)*, **7**, 137.

570

571 Hrvatin, S., Sun, S., Wilcox, O.F., Yao, H., Lavin-Peter, A.J., Cicconet, M., Assad, E.G., Palmer,
572   M.E., Aronson, S., Banks, A.S., Griffith, E.C. & Greenberg, M.E. (2020) Neurons that
573   regulate mouse torpor. *Nature*, **583**, 115-121.

574

575 Jamieson, P.M., Li, C., Kukura, C., Vaughan, J. & Vale, W. (2006) Urocortin 3 modulates the
576   neuroendocrine stress response and is regulated in rat amygdala and hypothalamus by
577   stress and glucocorticoids. *Endocrinology*, **147**, 4578-4588.

578

22

579 Johnstone, H.A., Wigger, A., Douglas, A.J., Neumann, I.D., Landgraf, R., Seckl, J.R. & Russell,
580   J.A. (2000) Attenuation of hypothalamic-pituitary-adrenal axis stress responses in late
581   pregnancy: changes in feedforward and feedback mechanisms. *J Neuroendocrinol*, **12**,
582   811-822.

583
584 Klampfl, S.M. & Bosch, O.J. (2019) Mom doesn't care: When increased brain CRF system
585   activity leads to maternal neglect in rodents. *Front Neuroendocrinol*, **53**, 100735.

586
587 Kohl, J., Babayan, B.M., Rubinstein, N.D., Autry, A.E., Marin-Rodriguez, B., Kapoor, V.,
588   Miyamishi, K., Zweifel, L.S., Luo, L., Uchida, N. & Dulac, C. (2018) Functional circuit
589   architecture underlying parental behaviour. *Nature*, **556**, 326-331.

590
591 Kronman, H., Torres-Berrio, A., Sidoli, S., Issler, O., Godino, A., Ramakrishnan, A., Mews, P.,
592   Lardner, C.K., Parise, E.M., Walker, D.M., van der Zee, Y.Y., Browne, C.J., Boyce, B.F.,
593   Neve, R., Garcia, B.A., Shen, L., Pena, C.J. & Nestler, E.J. (2021) Long-term behavioral
594   and cell-type-specific molecular effects of early life stress are mediated by H3K79me2
595   dynamics in medium spiny neurons. *Nat Neurosci*, **24**, 667-676.

596
597 Kuperman, Y., Issler, O., Regev, L., Musseri, I., Navon, I., Neufeld-Cohen, A., Gil, S. & Chen, A.
598   (2010) Perifornical Urocortin-3 mediates the link between stress-induced anxiety and
599   energy homeostasis. *Proceedings of the National Academy of Sciences of the United
600   States of America*, **107**, 8393-8398.

601
602 Kuroda, K.O., Tachikawa, K., Yoshida, S., Tsuneoka, Y. & Numan, M. (2011) Neuromolecular
603   basis of parental behavior in laboratory mice and rats: with special emphasis on
604   technical issues of using mouse genetics. *Prog Neuropsychopharmacol Biol Psychiatry*,
605   **35**, 1205-1231.

606
607 Lemaire, V., Koehl, M., Le Moal, M. & Abrous, D.N. (2000) Prenatal stress produces learning
608   deficits associated with an inhibition of neurogenesis in the hippocampus. *Proc Natl
609   Acad Sci U S A*, **97**, 11032-11037.

610
611 Lonstein, J.S. (2005) Reduced anxiety in postpartum rats requires recent physical interactions
612   with pups, but is independent of suckling and peripheral sources of hormones. *Horm
613   Behav*, **47**, 241-255.

614
615 Lonstein, J.S. & De Vries, G.J. (2000) Sex differences in the parental behavior of rodents.
616   *Neurosci Biobehav Rev*, **24**, 669-686.

617
618 Marlin, B.J., Mitre, M., D'Amour J, A., Chao, M.V. & Froemke, R.C. (2015) Oxytocin enables
619   maternal behaviour by balancing cortical inhibition. *Nature*, **520**, 499-504.

620

Maruska, K.P., Butler, J.M., Field, K.E., Forester, C. & Augustus, A. (2020) Neural Activation Patterns Associated with Maternal Mouthbrooding and Energetic State in an African Cichlid Fish. *Neuroscience*, **446**, 199-212.

Matovic, S., Ichiyama, A., Igarashi, H., Salter, E.W., Sunstrum, J.K., Wang, X.F., Henry, M., Kuebler, E.S., Vernoux, N., Martinez-Trujillo, J., Tremblay, M.E. & Inoue, W. (2020) Neuronal hypertrophy dampens neuronal intrinsic excitability and stress responsiveness during chronic stress. *J Physiol*, **598**, 2757-2773.

Medina, J., De Guzman, R.M. & Workman, J.L. (2021) Lactation is not required for maintaining maternal care and active coping responses in chronically stressed postpartum rats: Interactions between nursing demand and chronic variable stress. *Horm Behav*, **136**, 105035.

Miller, S.M., Piasecki, C.C. & Lonstein, J.S. (2011) Use of the light-dark box to compare the anxiety-related behavior of virgin and postpartum female rats. *Pharmacol Biochem Behav*, **100**, 130-137.

Mogi, K., Takakuda, A., Tsukamoto, C., Ooyama, R., Okabe, S., Koshida, N., Nagasawa, M. & Kikusui, T. (2017) Mutual mother-infant recognition in mice: The role of pup ultrasonic vocalizations. *Behav Brain Res*, **325**, 138-146.

Moy, S.S., Nadler, J.J., Young, N.B., Nonneman, R.J., Segall, S.K., Andrade, G.M., Crawley, J.N. & Magnuson, T.R. (2008) Social approach and repetitive behavior in eleven inbred mouse strains. *Behav Brain Res*, **191**, 118-129.

Murgatroyd, C.A., Hicks-Nelson, A., Fink, A., Beamer, G., Gurel, K., Elnady, F., Pittet, F. & Nephew, B.C. (2016) Effects of Chronic Social Stress and Maternal Intranasal Oxytocin and Vasopressin on Offspring Interferon-gamma and Behavior. *Front Endocrinol (Lausanne)*, **7**, 155.

Murgatroyd, C.A. & Nephew, B.C. (2013) Effects of early life social stress on maternal behavior and neuroendocrinology. *Psychoneuroendocrinology*, **38**, 219-228.

Murgatroyd, C.A., Pena, C.J., Podda, G., Nestler, E.J. & Nephew, B.C. (2015) Early life social stress induced changes in depression and anxiety associated neural pathways which are correlated with impaired maternal care. *Neuropeptides*, **52**, 103-111.

Nephew, B.C. & Bridges, R.S. (2011) Effects of chronic social stress during lactation on maternal behavior and growth in rats. *Stress*, **14**, 677-684.

Neufeld-Cohen, A., Kelly, P.A., Paul, E.D., Carter, R.N., Skinner, E., Olverman, H.J., Vaughan, J.M., Issler, O., Kuperman, Y., Lowry, C.A., Vale, W.W., Seckl, J.R., Chen, A. &

24

664   Jamieson, P.M. (2012) Chronic activation of corticotropin-releasing factor type 2
665   receptors reveals a key role for 5-HT1A receptor responsiveness in mediating behavioral
666   and serotonergic responses to stressful challenge. *Biological psychiatry*, **72**, 437-447.

668 Numan, M. (1974) Medial preoptic area and maternal behavior in the female rat. *J Comp
669   Physiol Psychol*, **87**, 746-759.

671 Numan, M. (2020) *The parental brain : mechanisms, development, and evolution*. Oxford
672   University Press, New York.

674 Numan, M. & Insel, T.R. (2003) *The neurobiology of parental behavior*. Springer-Verlag, New
675   York.

677 O'Connell, L.A., Matthews, B.J. & Hofmann, H.A. (2012) Isotocin regulates paternal care in a
678   monogamous cichlid fish. *Horm Behav*, **61**, 725-733.

680 Ostermeyer, M.C. & Elwood, R.W. (1983) Pup recognition in Mus musculus: parental
681   discrimination between their own and alien young. *Dev Psychobiol*, **16**, 75-82.

683 Paulson, J.F. & Bazemore, S.D. (2010) Prenatal and postpartum depression in fathers and its
684   association with maternal depression: a meta-analysis. *JAMA*, **303**, 1961-1969.

686 Radley, J.J. & Sawchenko, P.E. (2015) Evidence for involvement of a limbic paraventricular
687   hypothalamic inhibitory network in hypothalamic-pituitary-adrenal axis adaptations to
688   repeated stress. *J Comp Neurol*, **523**, 2769-2787.

690 Richter, K., Wolf, G. & Engelmann, M. (2005) Social recognition memory requires two stages of
691   protein synthesis in mice. *Learn Mem*, **12**, 407-413.

693 Rincon-Cortes, M. & Grace, A.A. (2021) Postpartum scarcity-adversity disrupts maternal
694   behavior and induces a hypodopaminergic state in the rat dam and adult female
695   offspring. *Neuropsychopharmacology*.

697 Rosinger, Z.J., Mayer, H.S., Geyfen, J.I., Orser, M.K. & Stolzenberg, D.S. (2021) Ethologically
698   relevant repeated acute social stress induces maternal neglect in the lactating female
699   mouse. *Dev Psychobiol*, **63**, e22173.

701 Rudolph, S., Guo, C., Pashkovski, S.L., Osorno, T., Gillis, W.F., Krauss, J.M., Nyitrai, H.,
702   Flaquer, I., El-Rifai, M., Datta, S.R. & Regehr, W.G. (2020) Cerebellum-Specific Deletion
703   of the GABAA Receptor delta Subunit Leads to Sex-Specific Disruption of Behavior. *Cell
704   Rep*, **33**, 108338.

705

706 Sato, K., Hamasaki, Y., Fukui, K., Ito, K., Miyamichi, K., Minami, M. & Amano, T. (2020)
707   Amygdalohippocampal Area Neurons That Project to the Preoptic Area Mediate Infant-
708   Directed Attack in Male Mice. *J Neurosci*, **40**, 3981-3994.

709

710 Sequeira-Cordero, A., Salas-Bastos, A., Fornaguera, J. & Brenes, J.C. (2019) Behavioural
711   characterisation of chronic unpredictable stress based on ethologically relevant
712   paradigms in rats. *Sci Rep*, **9**, 17403.

713

714 Singh-Taylor, A., Korosi, A., Molet, J., Gunn, B.G. & Baram, T.Z. (2015) Synaptic rewiring of
715   stress-sensitive neurons by early-life experience: a mechanism for resilience? *Neurobiol*
716   *Stress*, **1**, 109-115.

717

718 Slawski, B.A. & Buntin, J.D. (1995) Preoptic area lesions disrupt prolactin-induced parental
719   feeding behavior in ring doves. *Horm Behav*, **29**, 248-266.

720

721 Smith, C.D. & Lonstein, J.S. (2008) Contact with infants modulates anxiety-generated c-fos
722   activity in the brains of postpartum rats. *Behavioural brain research*, **190**, 193-200.

723

724 Tsuneoka, Y., Tokita, K., Yoshihara, C., Amano, T., Esposito, G., Huang, A.J., Yu, L.M., Odaka,
725   Y., Shinozuka, K., McHugh, T.J. & Kuroda, K.O. (2015) Distinct preoptic-BST nuclei
726   dissociate paternal and infanticidal behavior in mice. *EMBO J*, **34**, 2652-2670.

727

728 Walker, C.D., Tilders, F.J. & Burlet, A. (2001) Increased colocalization of corticotropin-releasing
729   factor and arginine vasopressin in paraventricular neurones of the hypothalamus in
730   lactating rats: evidence from immunotargeted lesions and immunohistochemistry. *J*
731   *Neuroendocrinol*, **13**, 74-85.

732

733 Wang, X.D., Rammes, G., Kraev, I., Wolf, M., Liebl, C., Scharf, S.H., Rice, C.J., Wurst, W.,
734   Holsboer, F., Deussing, J.M., Baram, T.Z., Stewart, M.G., Muller, M.B. & Schmidt, M.V.
735   (2011) Forebrain CRF(1) modulates early-life stress-programmed cognitive deficits. *J*
736   *Neurosci*, **31**, 13625-13634.

737

738 Weinstock, M. (2001) Alterations induced by gestational stress in brain morphology and
739   behaviour of the offspring. *Prog Neurobiol*, **65**, 427-451.

740

741 Wisner, K.L., Sit, D.K., McShea, M.C., Rizzo, D.M., Zoretich, R.A., Hughes, C.L., Eng, H.F.,
742   Luther, J.F., Wisniewski, S.R., Costantino, M.L., Confer, A.L., Moses-Kolko, E.L., Famy,
743   C.S. & Hanusa, B.H. (2013) Onset timing, thoughts of self-harm, and diagnoses in
744   postpartum women with screen-positive depression findings. *JAMA Psychiatry*, **70**, 490-
745   498.

746

747   Wu, Z., Autry, A.E., Bergan, J.F., Watabe-Uchida, M. & Dulac, C.G. (2014) Galanin neurons in
748   the medial preoptic area govern parental behaviour. *Nature*, **509**, 325-330.

749

750   Zoubovsky, S.P., Hoseus, S., Tumukuntala, S., Schulkin, J.O., Williams, M.T., Vorhees, C.V. &
751   Muglia, L.J. (2020) Chronic psychosocial stress during pregnancy affects maternal
752   behavior and neuroendocrine function and modulates hypothalamic CRH and nuclear
753   steroid receptor expression. *Transl Psychiatry*, **10**, 6.

754

755   **Figures and legends**

756

757

758
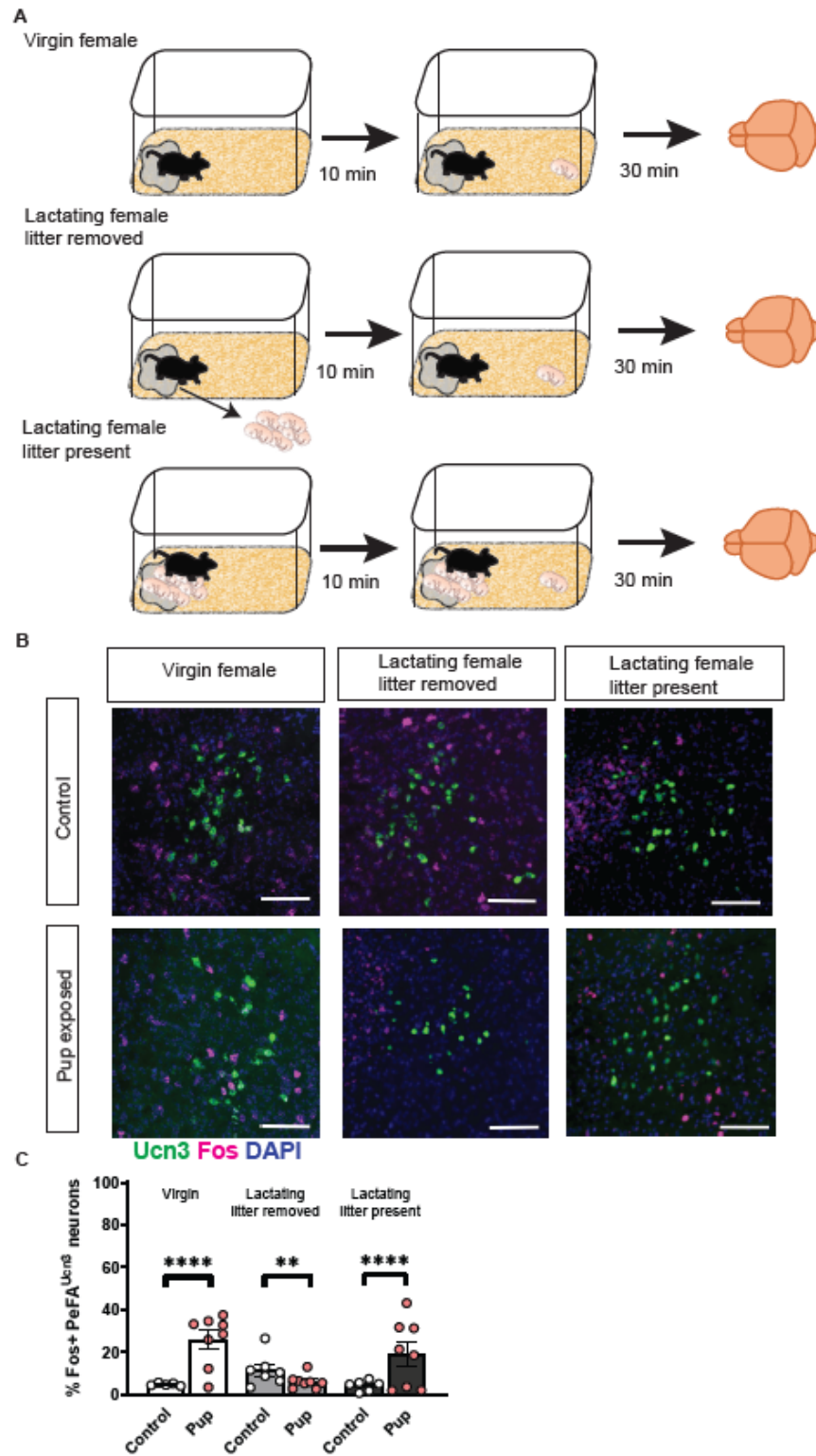
Figure 1. PeFA Urocortin-3 neuronal activation levels in response to foreign pups

depends on physiological context. (A) Schematic of behavioral paradigm. C57 virgin females

761   were exposed to a newborn pup and sacrificed 30 minutes after pup exposure or addition of

762   fresh bedding into home cage (control). Lactating females either had a litter removed or litter

763   intact and exposed to a foreign-born pup or fresh bedding. **(B)** Rostral perifornical area cells

764   containing *Urocortin-3* and *Fos* RNA were counted for colocalization in each group. (scale bar

765   100 µm**) (C)** Quantification of percentage of Ucn3+ cells colocalized with Fos across groups.

766   Fisher exact test reveals that pup exposure increases PeFA^{Ucn3} activation in virgin females

767   (Control n=605 N=5; Pup exposed n=655 N=8; ****p=<0.0001), reduced PeFA^{Ucn3} activation in

768   lactating females with litter removed (Control n=622 N=7; pup exposed n=828 N=8;

769   **p=0.0044), and increased activation in lactating females that did not have litter removal

770   compared to control bedding exposure (Control n=816 N=6; pup exposed n=988 N=8

771   ****p<0.0001).

772

773
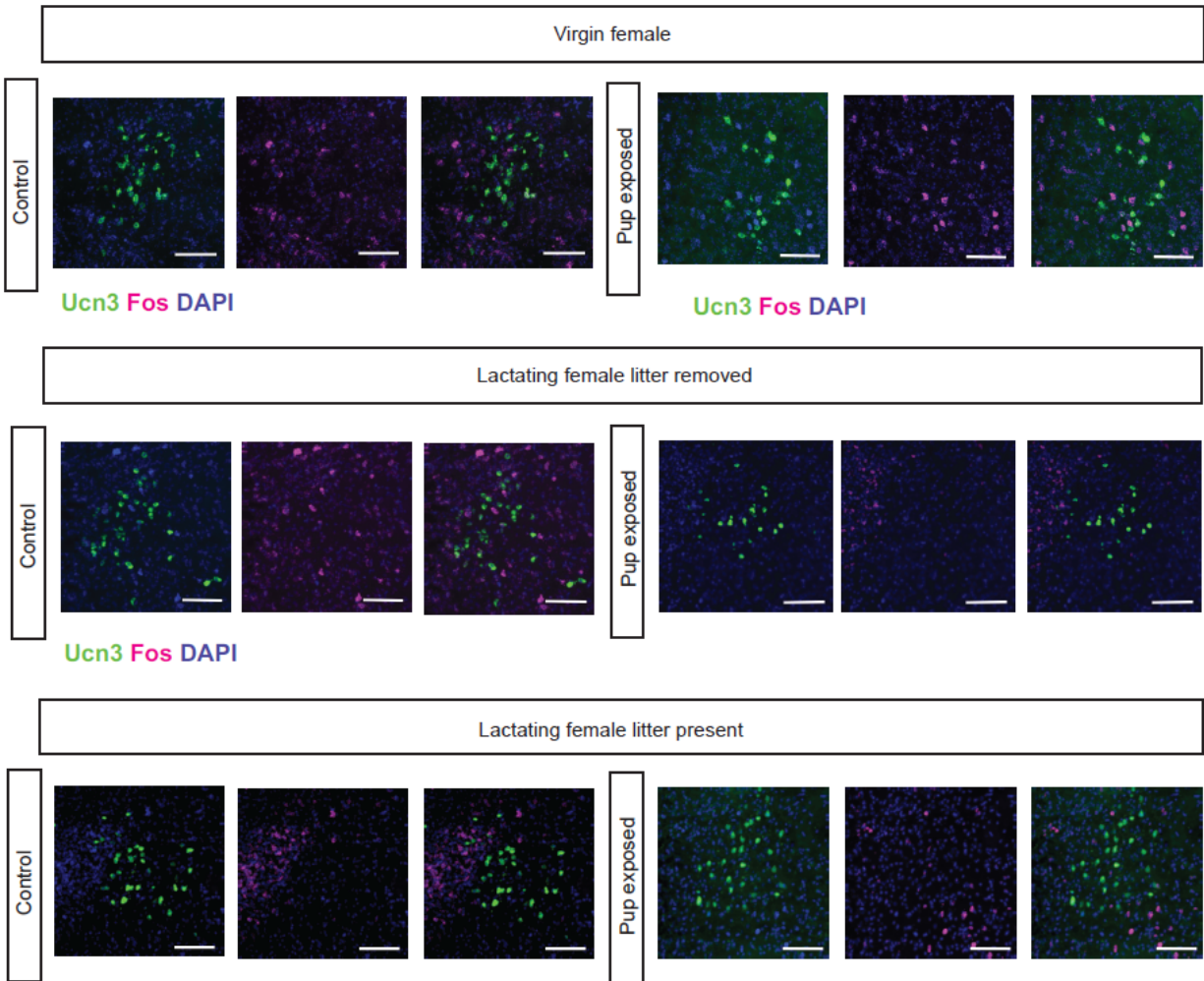
Supplemental Figure 1-1. Colocalization of PeFA Urocortin-3 neurons with Fos in response to foreign pups. Representative images from Figure 1 with Ucn3 and Fos channels separated (scale bar 100 μm).
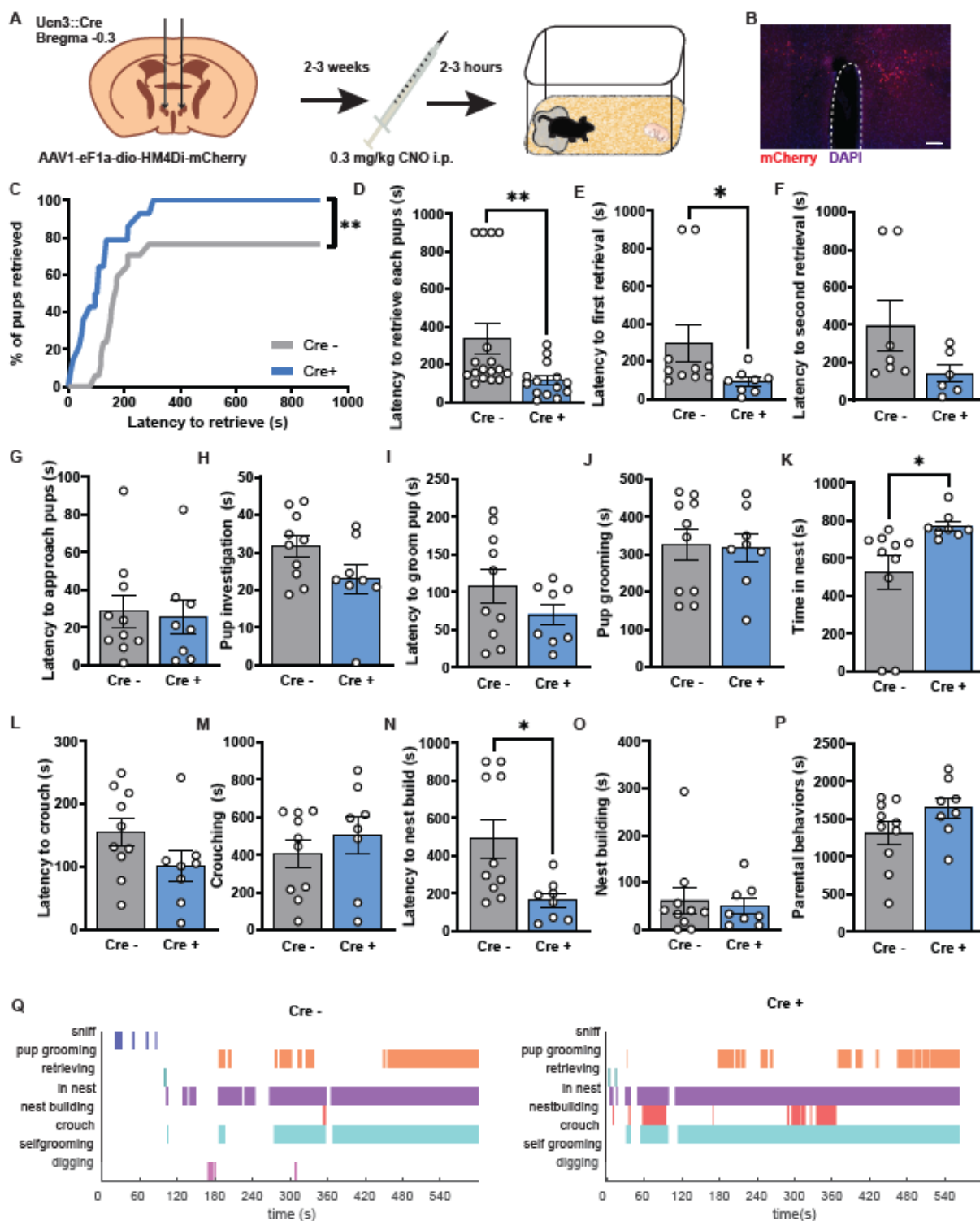
Figure 2. Inhibition of PeFA^Ucn3 neurons enhances alloparental behavior in virgin female mice. (A) Schematic of viral injection strategy and behavior timeline (n=10 Cre- females; n=8

780 Cre+ females). **(B)** Representative image of mCherry reporter expression in Ucn3::Cre+

781 female injected with inhibitory DREADD virus (magenta: mCherry; blue: DAPI; scale bar 100

782 µm). **(C)** Percentage of pups retrieved by Cre+ females is significantly increased compared to

783 Cre- females (Kolmogorov-Smirnov test p=0.0048). **(D)** Latency to retrieve pups is significantly

784 faster in Cre+ females compared to Cre- females (Two-tailed Mann-Whitney test; p=0.0033), as

785 well as **(E)** latency to retrieve the first pup (Two-tailed Mann-Whiney test; p=0.0259), but there

786 was no difference between groups in **(F)** latency to retrieve the second pup. **(G-H)** Latency to

787 approach pups was not significantly different, but time spent investigating pups trended lower in

788 Cre+ animals (Unpaired t test; p=0.0854). **(I-J)** We observed no significant difference in latency

789 to pup groom or in time spent pup grooming in Cre+ animals compared to Cre- females. **(K)**

790 Time spent in nest with pups significantly increased in Cre+ animals (Unpaired t test; p=0.0289).

791 **(L-M)** Latency to crouch trended lower in Cre+ animals (Two-tailed Mann-Whiney test;

792 p=0.1011), but we did not observe a significant difference in time spent crouching. **(N-O)**

793 Latency to nest build was significantly faster in Cre+ animals (Two-tailed Mann-Whiney test;

794 p=0.0152) but time spent nest building was not significantly different between groups. **(P)**

795 Cumulative time spent parenting was unchanged between groups **(Q)** Representative behavior

796 trace of a Cre- animal (left) and a Cre+ animal (right) during pup assay (time 0 is when pup was

797 added to home cage).

798

799
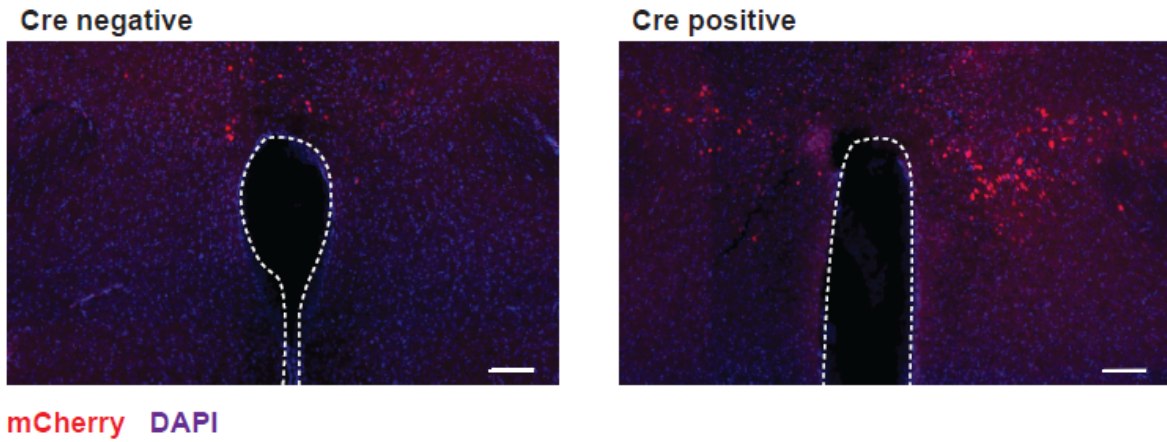
Supplemental Figure 2-1. Inhibitory DREADD expression in virgin female mice.

Representative image of AAV-mediated hM4di DREADD expression in the perifornical area

(PeFA) of Ucn::Cre- (left) or Ucn3::Cre+ (right) females. Third ventricle indicated by outline

(scale bar 100 μm).
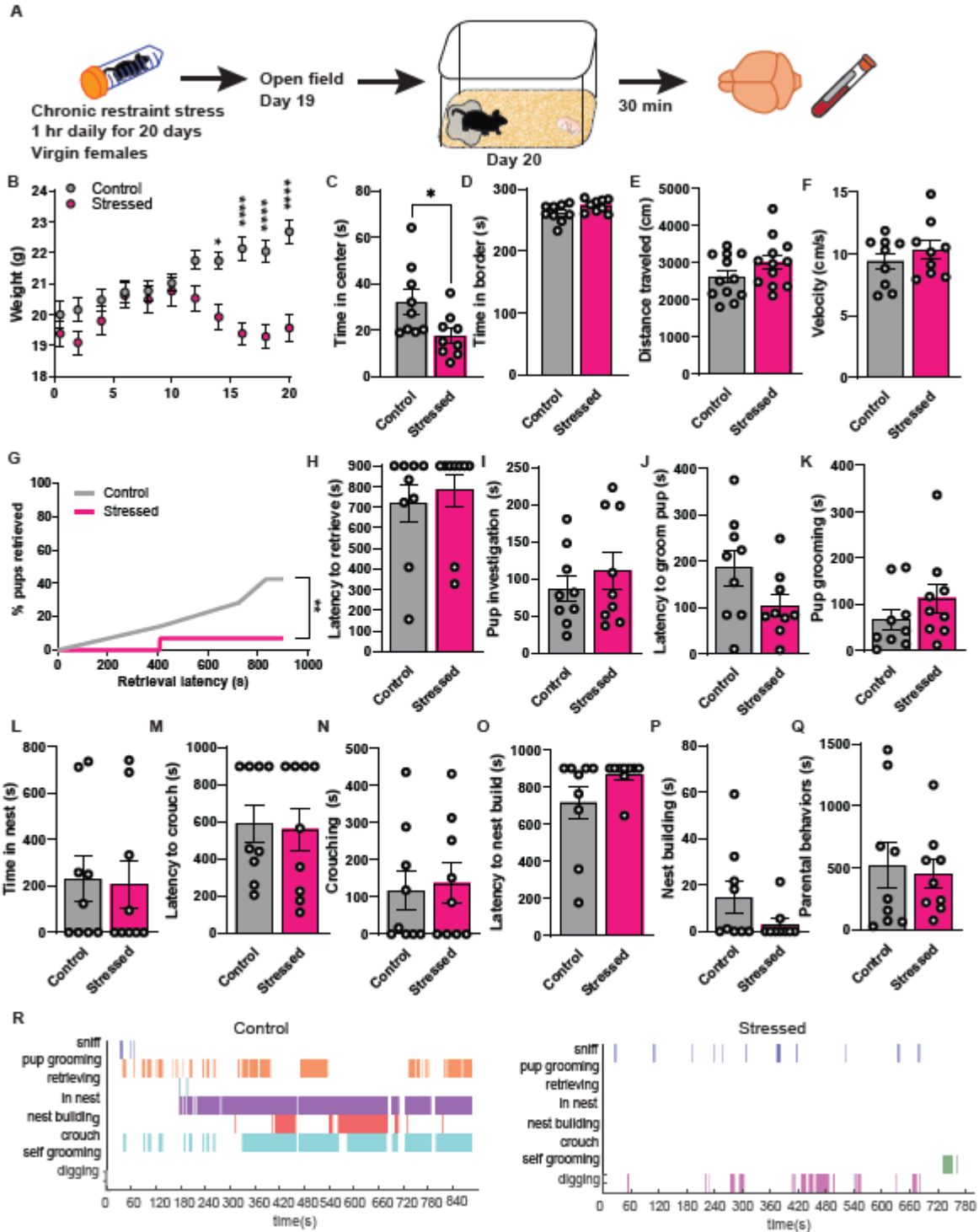
804

805

806   **Figure 3. Chronic restraint stress dampens alloparental behavior in virgin female mice.**

807   **(A)** Schematic of timeline using restraint stress paradigm and behavioral testing in virgin

808   females (n=9 control; n=9 stress). **(B)** Weights taken from each group during the period of

809   chronic restraint stress. Stressed females have significant difference in weight compared to

810   controls (Two-way repeated measures ANOVA; main effect of interaction of stress x time

811    $F_{(10,220)}$=22.92 p<0.0001; main effect of time $F_{(10, 220)}$ = 20.35; Bonferroni's multiple comparisons

812   test) **(C-F)** Time spent in center of open field was significantly reduced for stressed females

813   compared to controls (C) (unpaired t test; p=0.0289) but no other parameter in open field was

814   changed. **(G)** Chronic restraint stress significantly decreased cumulative pup retrieval in females

815   (Kolmogorov-Smirnov test p=0.0059).**(H-Q)** Chronic restraint stress did not significantly change

816   other parenting measures such as retrieval latency, or time spent pup grooming, time in nest,

817   crouching, and nest building. **(R)** Representative behavior trace for a control female (left) and

818   stressed female (right) during pup assay. (*p=0.05; **p=0.01; ***p=0.001; ****p=0.0001).

819
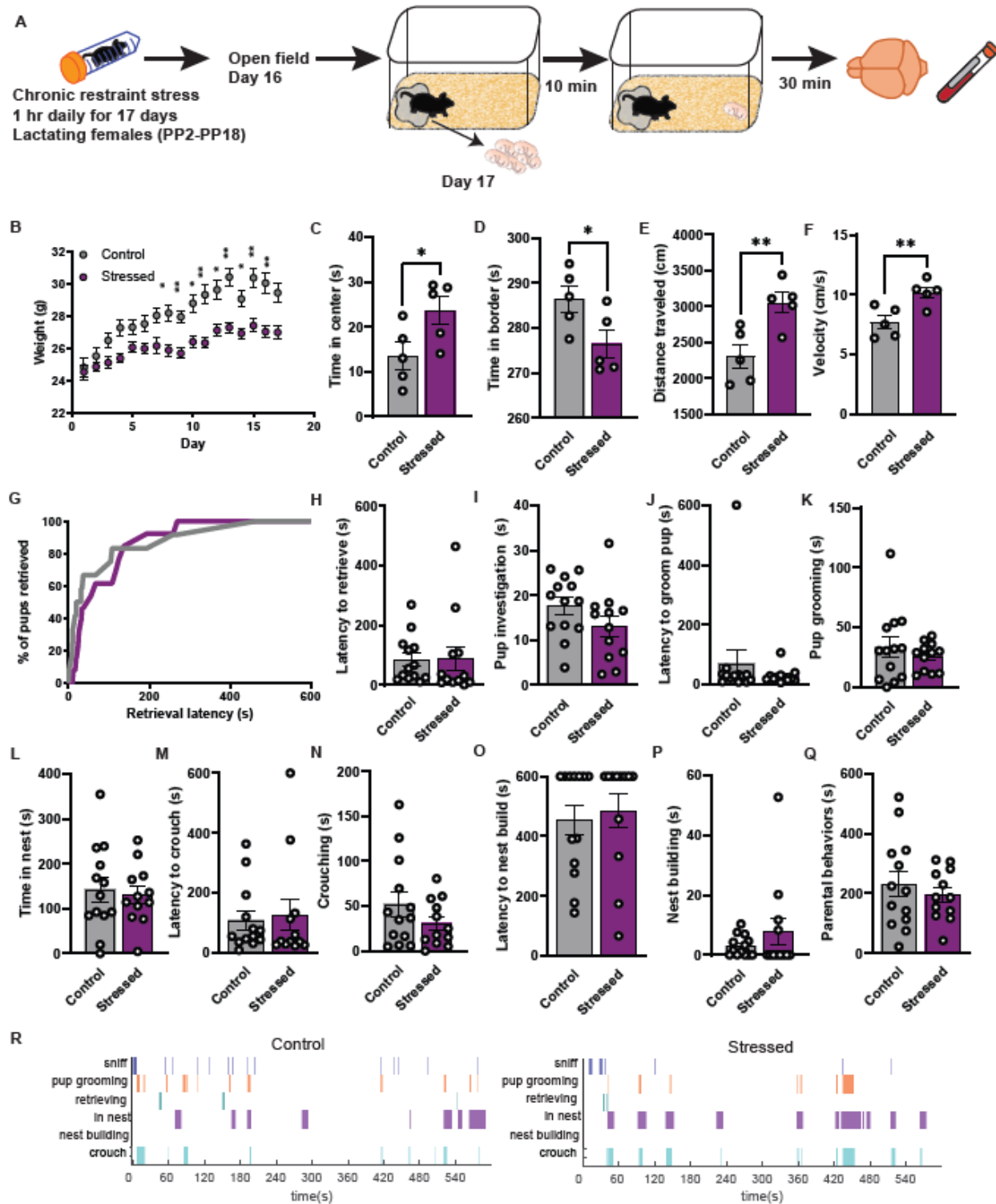
Figure 4. Chronic restraint stress does not induce changes in parental behavior in

lactating females. (A) Schematic of timeline using restraint paradigm and behavioral testing in

820

821

822   lactating females (Control n=12; Stressed n=13). **(B)** Weights taken from each group during the

823   period of chronic restraint stress from postpartum day 1 to 18. Stressed females have a

824   significant difference in weight compared to controls (Two-way repeated measures ANOVA;

825   main effect of interaction of stress x time $F_{(17,368)}$=8.286 p<0.0001; main effect of time

826    $F_{(7.579,174.3)}$=75.28 p<0.0001; Sidak's multiple comparisons). **(C-F)** Time spent in center of open

827   field was significantly increased for stressed females compared to controls (unpaired t test;

828   p=0.0459) and time spent in borders decreased (unpaired t test; p=0.0459) accompanied by

829   increased distance traveled (unpaired t test; p=0.0089) and velocity (unpaired t test; p=0.0089)

830   **(G-Q)** Chronic restraint stress did not significantly change any parenting measures as retrieval,

831   pup grooming, time in nest, crouching, and nest building compared to females that did not

832   receive stress. **(R)** Representative behavior trace for a control female (left) and stressed female

833   (right) during pup assay.

834
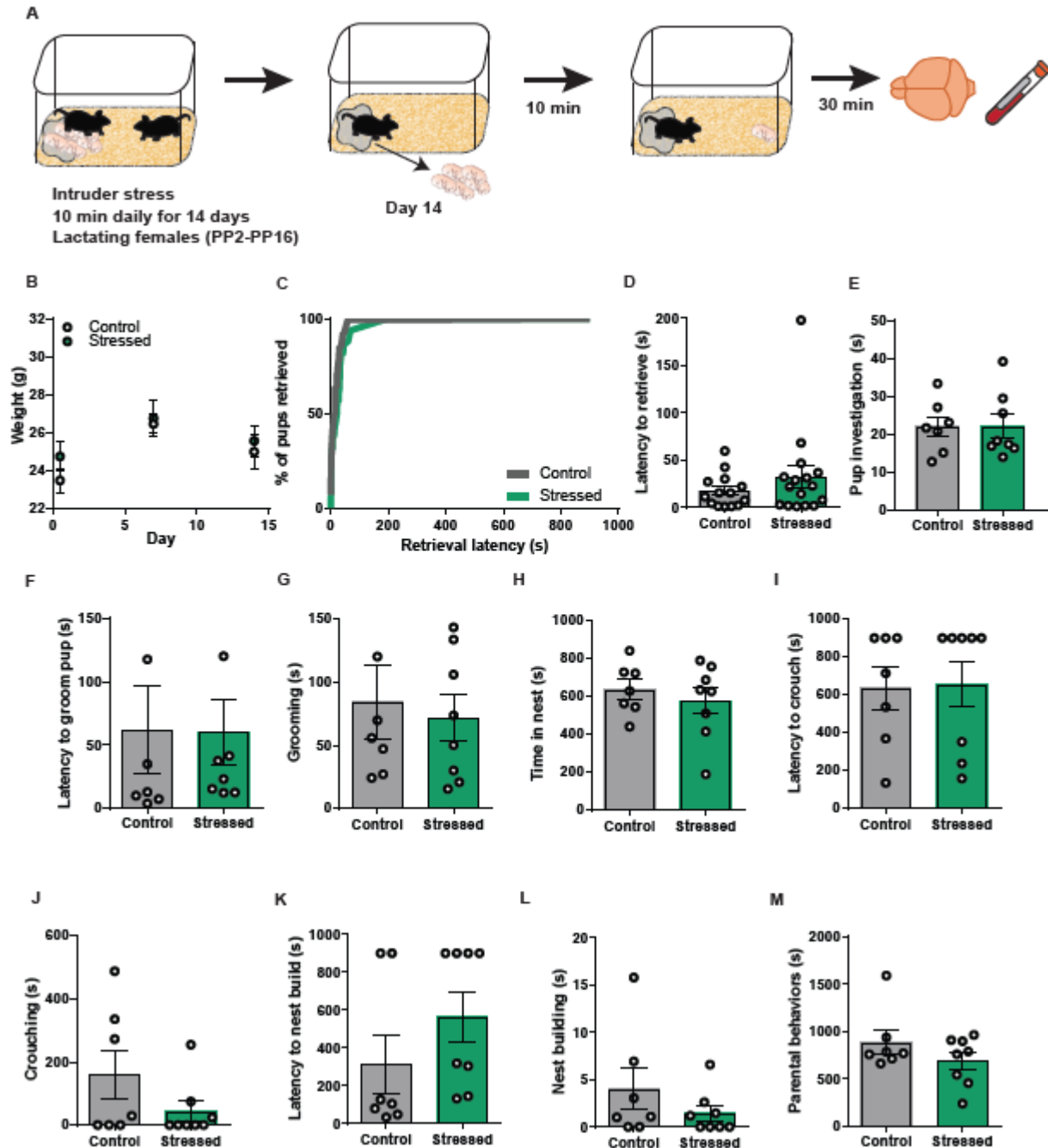
Supplemental Figure 4-1. Intruder stress in lactating females does not impact weight or parental behavior. (A) Experimental paradigm for intruder stress in lactating females. **(B)** Weight was not affected by stress condition. **(C)** Percentage of pups retrieved was not significantly different between control a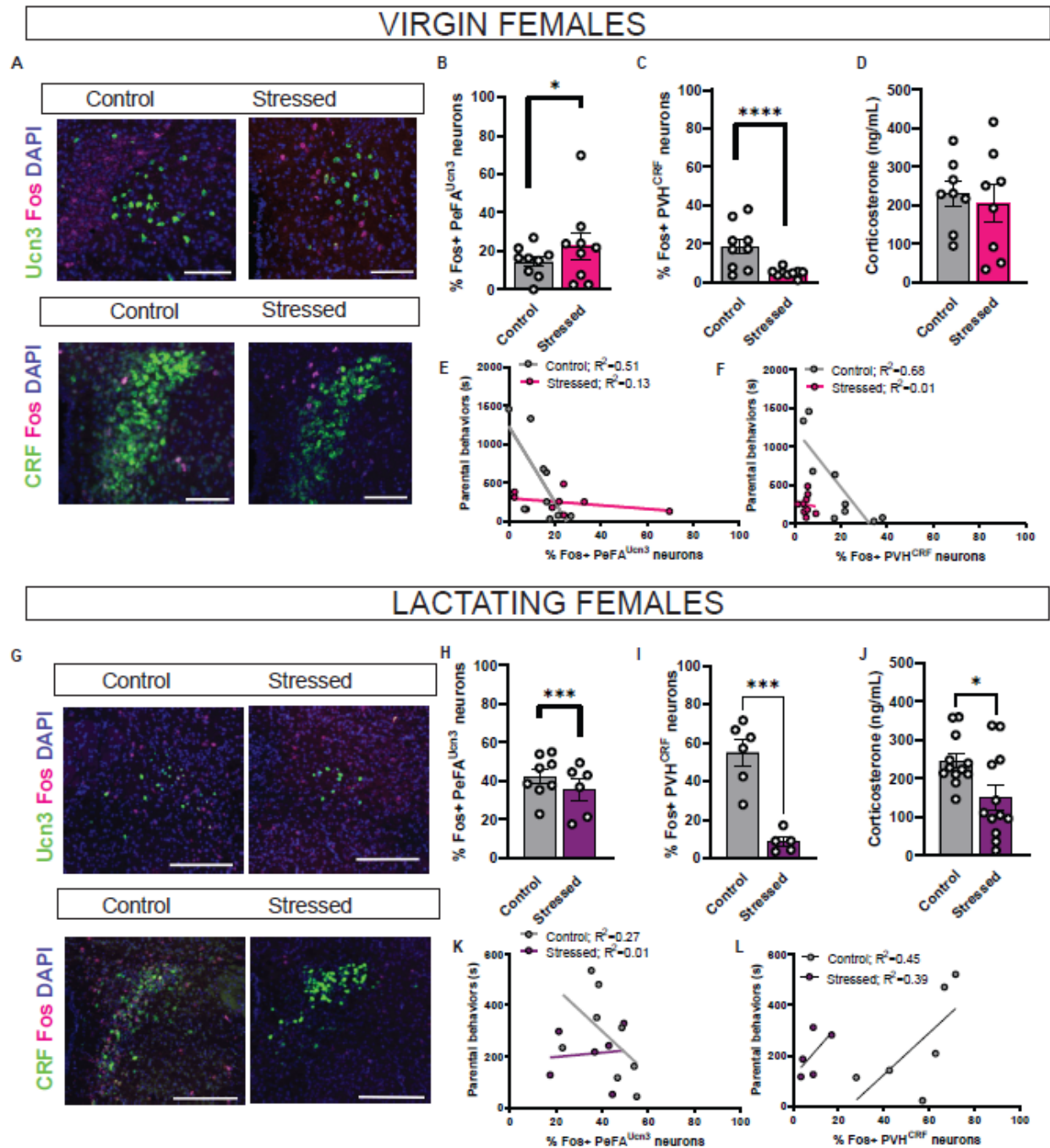nd stressed females. **(D)** Latency to retrieve **(E)** Pup investigation **(F)** Latency to groom **(G)** Grooming duration **(H)** Time in the nest **(I)** Latency to

39

841   crouch **(J)** Crouching duration **(K)** Latency to nest build **(L)** Nest building duration and **(M)**

842   Parental behaviors were unchanged between stress and control mothers.

843

844

845

846 **Figure 5. Chronic restraint leads to contrasting molecular changes between virgin and**

847 **lactating females in parenting. (A)** Rostral perifornical area cells containing *Ucn3, CRF,* and

848 *Fos* RNA were counted for colocalization in each virgin female group. (scale bar 100 μm). **(B)**

849   Colocalization of *Ucn3* and *Fos* in perifornical area reveals increased of activation of PeFA[Ucn3]

850   neurons in in stressed virgin females compared to control virgin females (Fisher exact test:

851   Control n=490 N=9; Stressed n=472 N=9; p=0.038). **(C)** Colocalization of *CRF* and *Fos* in

852   paraventricular nucleus of the hypothalamus reveals decreased activation of PVH[CRF] neurons in

853   stressed virgin females compared to control virgin females (Fisher exact test: Control n=1305

854   N=9; Stressed n=1272 N=9; p<0.0001). **(D)** Serum corticosterone levels were measured using

855   ELISA in virgin females. Chronic restraint stress did not contribute to altered corticosterone in

856   virgin females. **(E)** Plotting time spent parenting against PeFA[Ucn3] activation levels shows

857   marked negative correlation between parenting and PeFA[Ucn3] neuron activation in controls but

858   this relationship is abolished in stressed virgin females (Control R^2=0.51 Stressed R^2=0.13;

859   difference in slope F= 10.73 DFn=1, DFd=14; p=0.0055). **(F)** PVH[CRF] activation levels in

860   negatively correlated with time spent parenting in control virgin females but this relationship is

861   indiscernible in stressed virgin females (Control R^2=0.68 Stressed R^2=0.01). **(G)** Rostral

862   perifornical area cells containing *Ucn3, CRF,* and *Fos* RNA were counted for colocalization in

863   each lactating female group (scale bar 100 μm). **(H)** PeFA[Ucn3] neuron activation levels are

864   reduced in stressed lactating females (Fisher exact test: Control n=338 N=8; Stressed n=360

865   N=6; p=0.0005). **(I)** Activation levels of CRF cells in the paraventricular nucleus of the

866   hypothalamus are significantly decreased in stressed lactating females (Control n=919 N=6;

867   Stressed n=2268 N=5; p<0.0001). **(J)** Chronic restraint stress significantly reduced

868   corticosterone in lactating females (unpaired t test; p=0.0198). **(K)** Linear regression analysis

869   reveals a trend towards negative correlation between time spent parenting and PeFA[Ucn3]

870   activation levels in control lactating females but not in the stressed group (Control R^2=0.27

871   Stressed R^2=0.01). **(L)** PVH[CRF] activation is positively correlated with time spent parenting

872   (Control R^2=0.45 Stressed R^2=0.39).

873

874
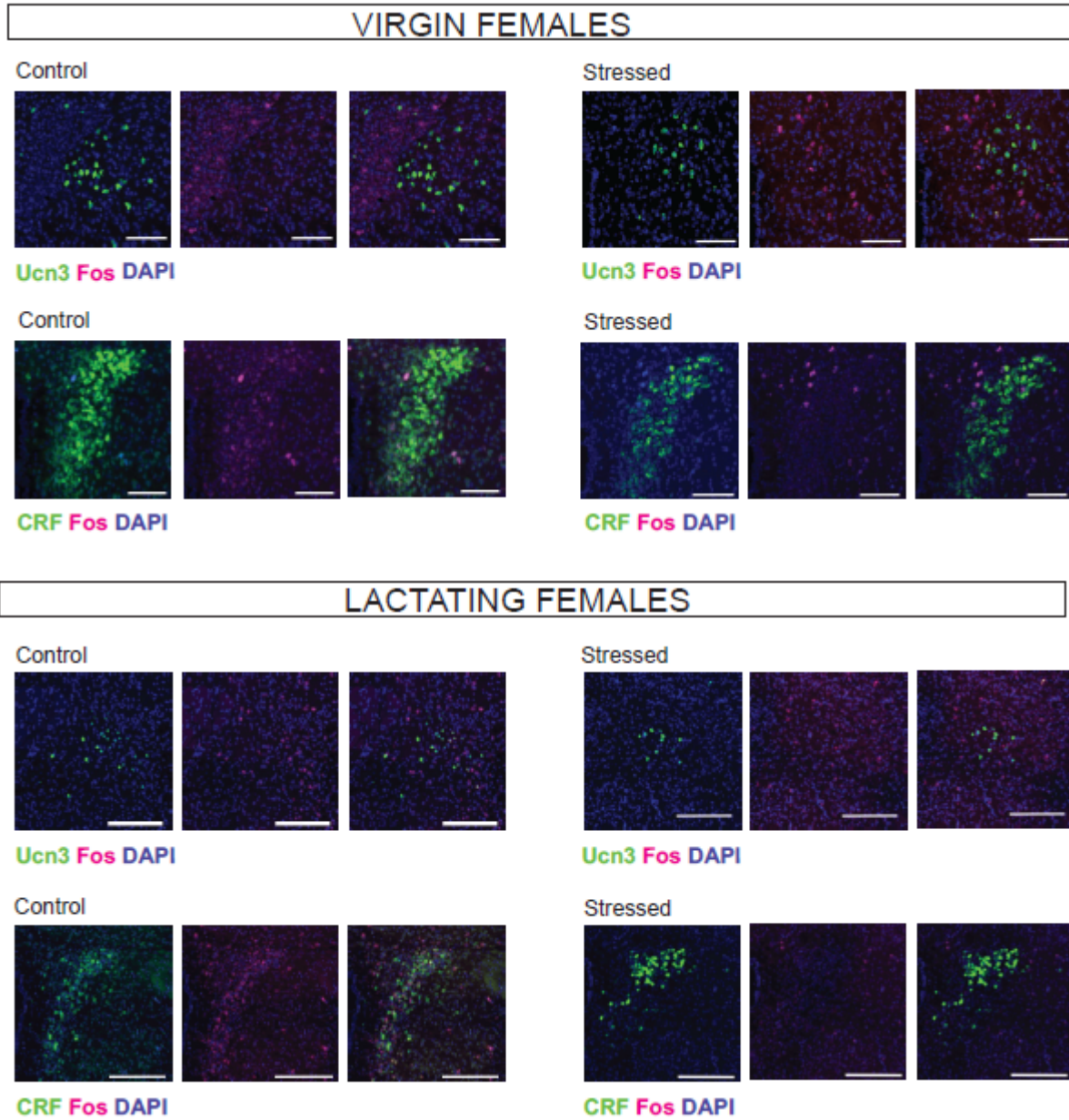
Supplemental Figure 5-1. Colocalization of Ucn3 or CRF and Fos in virgin or lactating females exposed to pups in control or chronic restraint stress conditions. Representative images from Figure 5 with Ucn3 or CRF and Fos channels separated (Ucn3 or CRF green channel; Fos magenta channel; scale bar 100 μm).

879

880
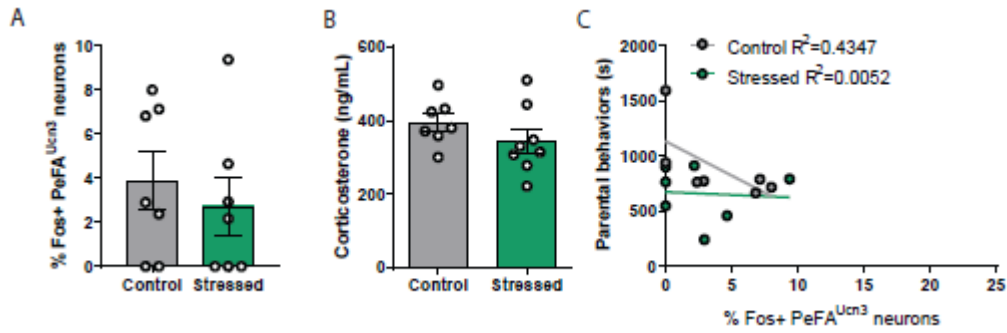
Supplemental Figure 5-2. Intruder stress in lactating females does not affect PeFA^{Ucn3} neuronal activation levels or circulating corticosterone. (A) Percentage of Fos+ PeFA^{Ucn3} neurons is not significantly different between mothers with or without intruder stress. **(B)** Corticosterone levels are indistinguishable between mothers with or without intruder stress. **(C)** Linear regression analysis reveals a trend towards negative correlation between time spent parenting and PeFA^{Ucn3} activation levels in control lactating females but not in mothers with intruder stress (Control R^2=0.4347 Stressed R^2=0.0052).

888
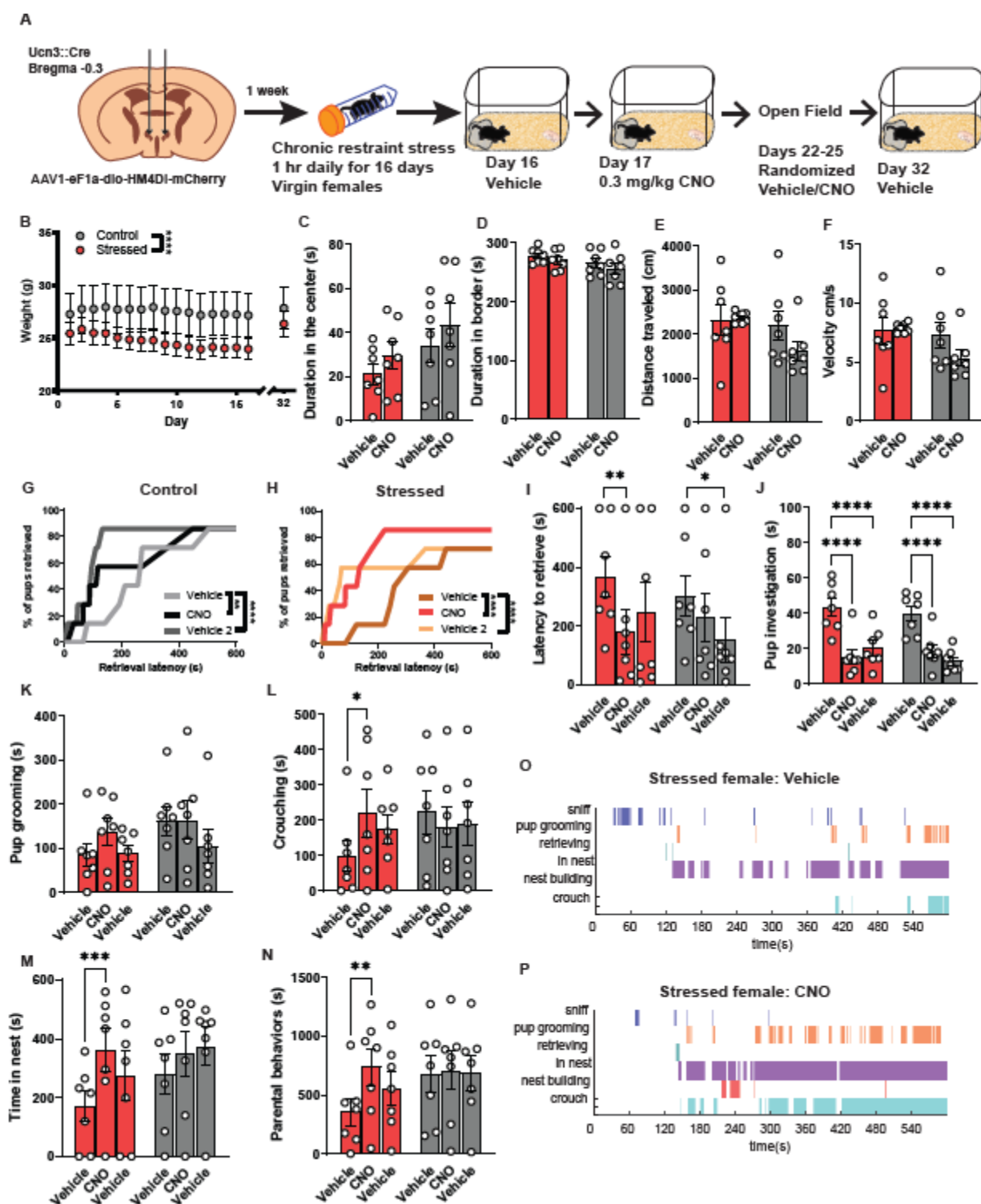
**Figure 6. PeFA^{Ucn3} inhibition ameliorates parenting deficits in stressed virgin females. (A)**
Schematic of viral injection strategy and behavior timeline (n=7 stressed Cre+ females; n=7

893   control Cre+ females). **(B)** Stressed females had a significant difference in weight compared to

894   control females (2-way repeated measures ANOVA, main effect of stress x time p<0.0001). **(C-**

895   **F)** Open field results show no difference in time spent in center, border, distance moved, or

896   velocity in control or stressed mice with or without CNO treatment. **(G)** Cumulative pup retrieval

897   in control animals improves significantly with CNO injection (Friedman's test; p=0.0013). **(H)**

898   Cumulative pup retrieval in stressed animals improve with CNO administration (Friedman's test;

899   p=0.0003). **(I)** CNO treatment decreases latency to retrieve in stressed females (Two-way

900   repeated measures ANOVA, main effect time $F_{(2,24)}$=8.978 p<0.01; Sidak's multiple comparisons

901   test) but not in control animals. **(J)** Pup investigation dramatically reduces in both animal groups

902   with CNO (Two-way repeated measures ANOVA, main effect of time $F_{(2,24)}$=82.86 p<0.0001;

903   Sidak's multiple comparisons test). **(K)** Pup grooming is unchanged with CNO treatment. **(L)**

904   Time spent crouching increases with CNO administration in stressed females (Two-way

905   repeated measures ANOVA, main interaction effect of stress and drug $F_{(2,24)}$=3.506 p<0.0462$_)$;

906   Tukey's multiple comparisons). **(M)** Stressed females spent significantly more time in the nest

907   with CNO administration (Two-way repeated measures ANOVA, main effect of time $F_{(2,24)}$=9.399

908   p=0.001; Sidak's multiple comparison's test). **(N)** Cumulative time spent parenting increases

909   with CNO treatment in stressed females (Two-way ANOVA, main effect of time $F_{(2,24)}$=3.942

910   p=0.0331; Sidak's multiple comparisons). **(O-P)** Representative behavior traces showing

911   induction of more parenting behaviors with CNO in stressed females. (Significant post-hoc

912   comparisons noted as follows: *p=0.05; **p=0.01; ***p=0.001; ****p=0.0001).

913

914
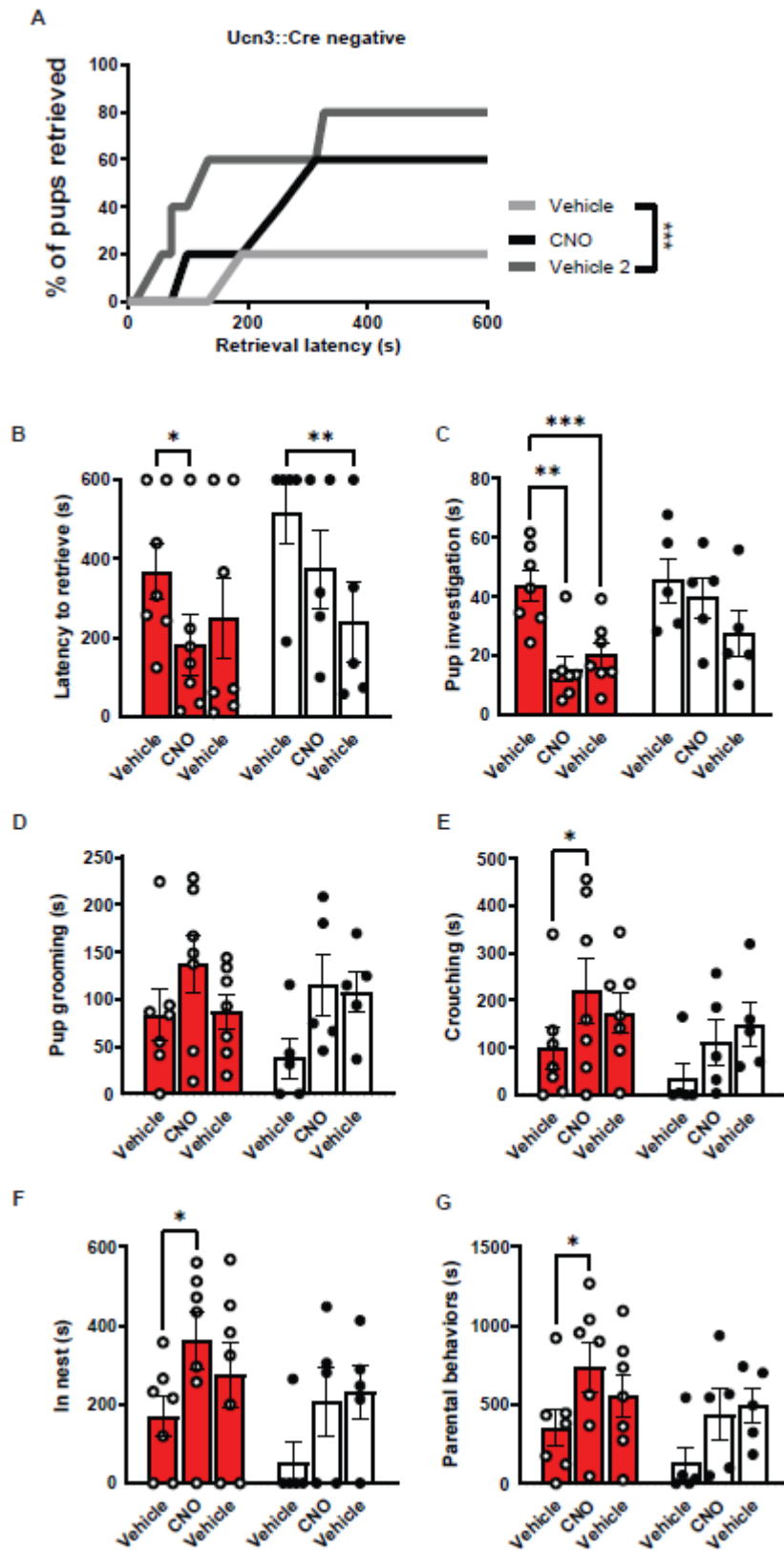
915 **Supplemental Figure 6-1. CNO treatment does not impact parenting behavior in**

916 **Ucn3::Cre- stressed virgin females. (A)** Cumulative pup retrieval in control animals improves

917 significantly with CNO injection (Friedman's test; p=0.0002 followed by Dunn's posthoc

918 comparisons). **(B)** CNO reduces latency to retrieve pups in stressed Ucn3::Cre+ females but not

919 in stressed Cre- females (Two-way repeated measures ANOVA main effect of drug treatment

920 $F_{(2,20)}$=9.667 p<0.0012, Sidak multiple comparisons post-hoc effect significant for Cre+ stressed

921 group vehicle versus CNO p<0.0224 and Cre- stressed group vehicle vs. vehicle 2 p<0.0036).

922 **(C)** Pup investigation is impacted in the stressed Cre+ group but not the stressed Cre- group

923 (Two-way repeated measures ANOVA main effect of drug treatment $F_{(2,20)}$=10.94 p<0.0006,

924 Sidak multiple comparisons post-hoc effect significant for Cre+ stressed group vehicle versus

925 CNO p<0.0010 and vehicle versus vehicle 2 p<0.0009). **(D)** Pup grooming is unaffected. **(E)**

926 CNO treatment increases crouching significantly in Ucn3::Cre positive females (Two-way

927 repeated measures ANOVA main effect of drug treatment $F_{(2,20)}$=4.898, p<0.0186, Tukey

928 multiple comparisons post-hoc effect significant for Cre+ stress group vehicle versus CNO

929 p<0.05) **(F)** as well as duration in nest (Two-way repeated measures ANOVA main effect of

930 drug treatment $F_{(2,20)}$=8.098, p<0.0037, Sidak multiple comparisons post-hoc effect significant

931 for Cre+ stress group vehicle versus CNO p<0.05) **(G)** and cumulative time spent parenting

932 (Two-way repeated measures ANOVA main effect of drug treatment $F_{(2,20)}$=7.728 p<0.0033,

933 Sidak multiple comparisons post-hoc effect significant for Cre+ stress group vehicle versus CNO

934 (p<0.05).

935

48

936

937

938
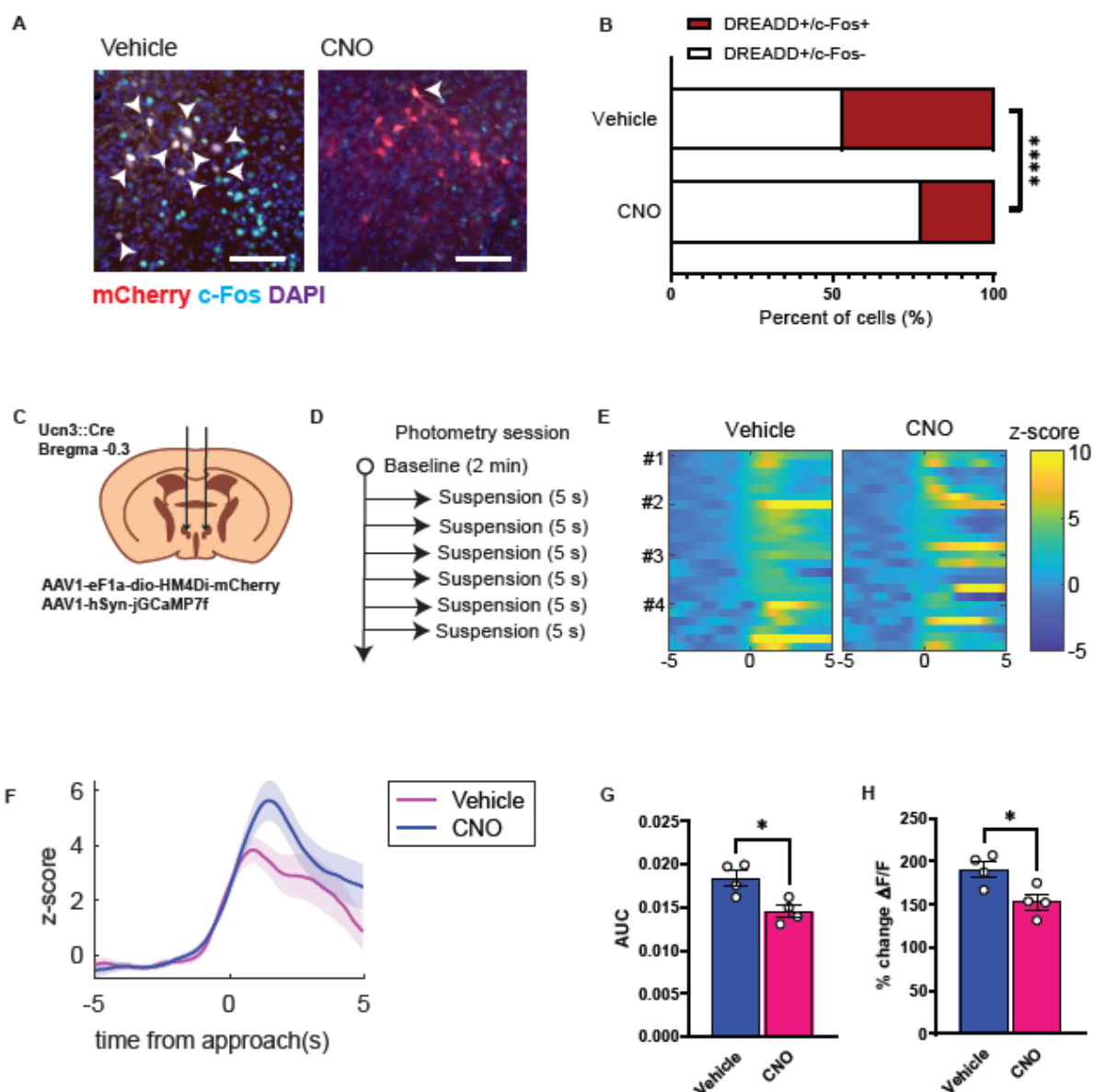
Supplemental Figure 6-2. Confirmation of CNO reduction of PeFA^{Ucn3} on AAV-mediated hM4di in Ucn3::Cre females. (A) Representative image of changes in PeFA^{Ucn3}/c-Fos levels in AAV-hM4di DREADD injected Ucn3::Cre females when CNO is injected intraperitoneally compared to vehicle after 30 minutes of pup interaction (scale bar 100 μm). Arrow indicates

943   Ucn3/c-Fos colocalization. **(B)** Quantification of DREADD expressing neurons co-expressing c-

944   Fos is significantly reduced in Ucn3::Cre females injected with CNO. (Fisher exact test: Vehicle

945   n=180 N=2; Stressed n=101 N=2; p<0.0001). **(C)** Schematic of experimental design to record

946   photometric signals from the PeFA with expression of inhibitory DREADD hM4Di construct in

947   the PeFA^{Ucn3} neurons. **(D)** Schematic of tail suspension session design during photometry

948   recording. **(E)** Z-score of the normalized fluorescence signal (ΔF/F) for each individual trial

949   during the 10s peri-event window centered around the onset of tail suspension. Left: vehicle;

950   right: CNO treatment (n=6 trials, N= 4 female mice) **(F)** Average Z-score of ΔF/F averaged

951   across the 6 trials for each of the four females (dark line represents the mean and the shaded

952   area represents the SEM). **(G)** Area under the curve calculated on the average ΔF/F signal for

953   the tail suspension events (n=6 trials, N=4 female mice). AUC of the 5s segment following the

954   suspension is significantly reduced in the CNO trial compared to vehicle treatment (Paired t-test

955   p<0.0281, N=4 female mice, average of 6 trials for each). **(H)** Percent change of ΔF/F relative

956   to baseline reveals significant reduction of fluorescence evoked by tail suspension with CNO

957   treatment relative to vehicle treatment (Paired t-test p<0.0025, N=4 female mice, average of 6

958   trials for each).

959

960
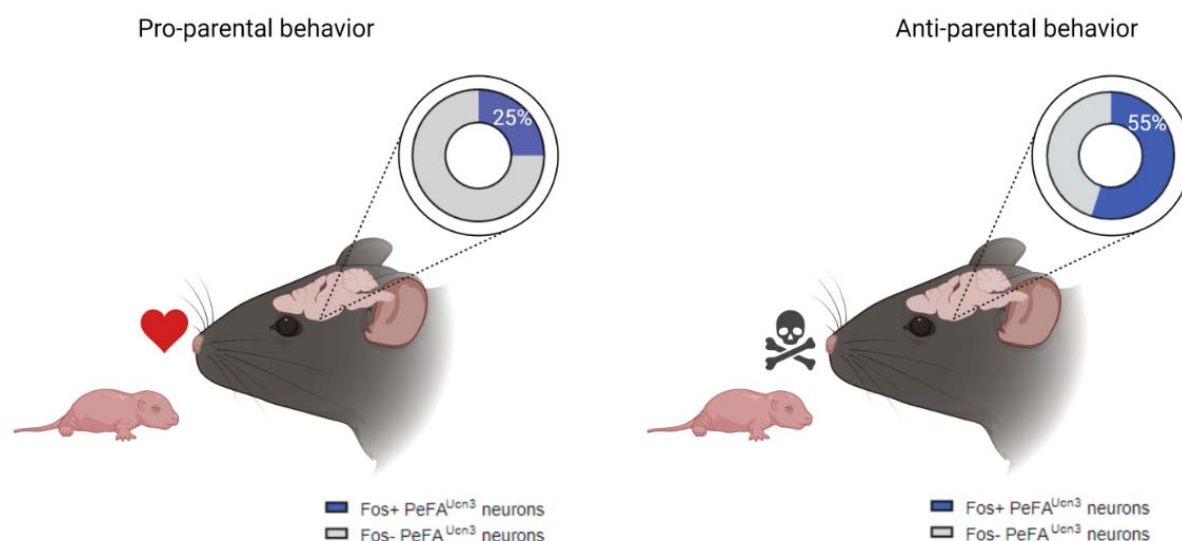
Supplemental Figure 6-3. Model for PeFA^{Ucn3} neuronal activation in pup-directed behavior across sexes and physiological states. (Left) Pro-parental behavior such as grooming, retrieving, and crouching over pups in the nest is accompanied by low-level PeFA^{Ucn3} neuronal activation (`25%). Mice in this category include alloparental virgin females, unstressed mothers, and stress-resistant mothers, as demonstrated in this study and Autry et al. 2021. We predict that this level of activation of PeFA^{Ucn3} neurons may also be observed in other categories of mice that display pro-parental behavior including alloparental virgin males, unstressed fathers, and stress-resistant fathers. **(Right)** On the other hand, anti-parental behavior such infant-directed aggression or neglect is accompanied by increased activation of PeFA^{Ucn3} neurons (~55%). Mice in this category include stressed virgin females and infanticidal males as demonstrated in this study and Autry et al., 2021. We predict that this level of activation of PeFA^{Ucn3} neurons may also be observed in other categories of mice that display anti-parental behavior including stress-susceptible mothers, stressed virgin males, and stress-susceptible fathers. Model created with Biorender.com.

975

976