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11   Elevated fear responses to threatening cues in rats with early life stress is associated with greater
12   excitability and loss of gamma oscillations in ventral-medial prefrontal cortex
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Abstract

Stress experienced early in development can have profound influences on developmental trajectories and ultimately behaviors in adulthood. Potent stressors during brain maturation can profoundly disrupt prefrontal cortical areas in particular, which can set the stage for prefrontal-dependent alterations in fear regulation and risk of drug abuse in adulthood. Despite these observations, few studies have investigated *in vivo* signaling in prefrontal signals in animals with a history of early life stress (ELS). Here, rats with ELS experienced during the first post-natal week were then tested on a conditioned suppression paradigm during adulthood. During conditioned suppression, electrophysiological recordings were made in the ventral medial prefrontal cortex (vmPFC) during presentations of a fear-associated cues that resolved both single-unit activity and local field potentials (LFPs). Relative to unstressed controls, ELS-experienced rats showed greater fear-related suppression of lever pressing. During presentations of the fear-associated cue (CS+), neurons in the vmPFC of ELS animals showed a significant increase in the probability of excitatory encoding relative to controls, and excitatory phasic responses in the ELS animals were reliably of higher magnitude than Controls. In contrast, vmPFC neurons in ELS subjects better discriminated between the shock-associated CS+ and the neutral ("safe") CS- cue than Controls. LFPs recorded in the same locations revealed that high gamma band (65-95 Hz) oscillations were strongly potentiated in Controls during presentation of the fear-associated CS+ cue, but this potentiation was abolished in ELS subjects. Notably, no other LFP spectra differed between ELS and Controls for either the CS+ or CS-. Collectively, these data suggest that ELS experience alters the neurobehavioral functions of PFC in adulthood that are critical for processing fear regulation. As such, these alterations may also provide insight into to increased susceptibility to other PFC-dependent processes such as risk-based choice, motivation, and regulation of drug use and relapse in ELS populations.

Introduction

Early Life Stress (ELS) has been identified as a particularly potent risk factor due to the insults and stressors occurring during critical developmental time windows in brain maturation. In human populations, ELS is a result of neglect, abuse and/or trauma experienced before the age of 18. Child Protective Services investigated 3.5 million cases of possible child maltreatment in 2018, an 8% increase from 2014 in the United States. However, estimating the prevalence of ELS is difficult as most cases are unreported. Human and animal studies refer to perinatal stress as exposure to acute and/or chronic stressors during prenatal and early postnatal life. While the definition is broad, it aims to highlight a critical period of growth, organogenesis and brain development in which the fetus or child are highly vulnerable to insult. During this period, exposure to stress during early life is associated with higher rates of chronic illness such as diabetes, obesity, cardiovascular, gastrointestinal and respiratory illness as well as autoimmune disorders (McEwen, 2003; Taylor, 2010).

In addition to these somatic responses, ELS can produce profound consequences on the developing central and peripheral nervous system (Lupien et al., 2009; McEwen, 2003). The prevalence of ELS in mental illness is alarmingly high such that 50% to 64% of patients diagnosed with depression, anxiety or substance use disorders report exposure to early life adversity (Dube et al., 2003; Enoch, 2011; Vogt et al., 2016). Clinical studies have also reported a positive correlation between the intensity and duration of ELS and the number of psychopathologies an individual develops, as well as symptom severity (Carr et al., 2013). Indeed, a host of stress and anxiety disorders such as generalized anxiety disorder, panic disorder, post-traumatic stress disorder (PTSD) and obsessive-compulsive disorder have the highest rate of co-occurrence and are comorbid with substance use disorders (SUD) (Regier et al., 1990), all of which have been

92   linked to ELS as a predictor of their development (Brady and Sinha, 2005; Brown and Barlow,

93   1992; Enoch, 2011; McEwen, 2003; Regier et al., 1990).

94   The rodent and human brain follow a highly-conserved sequences of structural

95   development (Rice & Barone, 2000). Rodents exposed to ELS have reported morphological,

96   neurochemical and behavioral alterations that parallel some of the findings reported in humans

97   (McEwen, 2003; Weinstock, 2017, 2008). Unlike subcortical structures, in which early cellular

98   processes such as migration, differentiation, synaptogenesis and gliogenesis are well underway,

99   limbic-connected structures such as hippocampus, amygdala and prefrontal cortex (PFC) are in

100   their early stages of development at birth (Herlenius and Lagercrantz, 2004; VanTieghem and

101   Tottenham, 2018). More importantly, the PFC is the last cortical structure to fully develop as it

102   continues these processes into adulthood making the PFC highly susceptible to environmental

103   insult throughout childhood (Kroon et al., 2019; VanTieghem and Tottenham, 2018). During the

104   early stages of development, cortical and subcortical projections reach the PFC to ensure proper

105   communication between the PFC and the rest of the brain, many of which introduce

106   neuromodulatory activity like dopamine into the region (Kalsbeek et al., 1988). These

107   neuromodulators play critical roles in the development of PFC circuits during the first week of

108   postnatal life. Additionally, increased glutamatergic activity during early development dominates

109   cortical pyramidal cell transmission and is critical in synaptogenesis as well. NMDA activity is

110   predominant and aids in fine tuning synapses and in areas of excessive activity it enables apoptosis

111   (Herlenius and Lagercrantz, 2004; Rice and Barone, 2000).

112   In rodents, the PFC along the medial aspect frontal regions is typically subdivided into two

113   functional divisions, the dorsal medial aspect (*dmPFC*; which includes the prelimbic [PL] cortex)

114   and the ventral medial aspect (*vmPFC*; including the infralimbic [IL] cortex). Though these PFC

115   regions lack direct homology with primate regions (Laubach et al., 2018), evidence exists that

116   functional and developmental overlap between rodent vmPFC and similar human prefrontal

117   regions such as Brodmann's area 25. For example, this region has been implicated in cognitive

118   and behavioral flexibility deficits in patients that suffer from Anxiety Disorders, ASD and SUD

119   (Greenberg et al., 2013; Jackson et al., 2016; Myers-Schulz and Koenigs, 2012), as well as

120   decreased activity during fear generalization tasks (Greenberg et al., 2013). Similar activity

121   patterns were also detected in individuals suffering from PTSD when presented with trauma

122   associated cues (Shin et al., 2004) and extinction recall (Milad et al., 2009).

123   In rodents, vmPFC participates in similar functions (Giustino and Maren, 2015). Via

124   connections with limbic-associated targets such as the amygdala complex, the IL is involved in

125   the consolidation, maintenance and expression of extinction learning as well as habitual behaviors

126   (Quirk and Mueller, 2008). Within the domain of fear conditioning specifically, IL activity is both

127   necessary and sufficient to support fear extinction. Stimulation of IL accelerates fear extinction

128   (Adhikari et al., 2015; Bukalo et al., 2021; Milad and Quirk, 2002) and suppresses spontaneous

129   recovery of fear (Kim et al., 2010), while neurons in this area increase activity during the early

130   stages of extinction learning, with cue-elicited phasic activity emerging only after extinction

131   learning has occurred (Sierra-Mercado et al., 2011a). Conversely, pharmacological or optical

132   silencing of this IL pathway in fear extinction learning results in increased freezing behavior and

133   reduced extinction rates (Adhikari et al., 2015; Gutman et al., 2017; Laurent and Westbrook,

134   2009).

135   However, ELS appears to alter these normal fear and anxiety-related processes, disrupting

136   conditioned fear as well as decreasing exploration of open arms in an elevated plus maze (Nisar et

137   al., 2019; Oldham Green et al., 2021; Toda et al., 2014). While recent work has provided

138   tremendous *ex vivo* insight into the genetic, epigenetic, and molecular bases for these differences

139   particularly in the PFC (Oldham Green et al., 2021; Torres-Berrío et al., 2019), surprisingly little

140   is known about how mature *in vivo* neurons in ELS-experienced animals encode information about

141   threat and safety during behavior. Using limited experience with wet bedding as variant of a limited

142   bedding model of ELS (Léonhardt et al., 2007; Molet et al., 2014; Walker et al., 2017), we assessed

143   behavioral and *in vivo* neural activity in vmPFC in adulthood of these ELS subjects (along with

144   unstressed Controls) in a conditioned suppression task. Conditioned suppression is a higher-order

145   assay where rats must use the learned value of a threat-associated cue to guide conflicting actions:

146   (1) continue to seek reward despite threats, or (2) engage in defensive behaviors (e.g. freeze

147   immobility) despite the opportunity to earn rewards. Here we report that ELS animals showed

148   more persistent fear-suppressed motivation than Controls, and that during this task, vmPFC

149   neurons displayed greater excitatory responding to fear cues, but decreased high gamma

150   oscillations in the local field potential band.

151   **Methods**

152   **Subjects**

153   Subjects for this experiment were initially 26 male and female Long-Evans rats (9 male

154   and 17 female) at the start of the experiment. All animals were bred in-house in the CU Boulder

155   vivarium in an AAALAC-approved facility. Subjects were bred against a TH::Cre background

156   (males were TH::Cre-positive Long-Evans [original line sourced from Rat Resource & Research

157   Center (RRRC)] mated with female standard non-transgenic Long-Evans [Envigo]), though we

158   did not use manipulations to selectively target TH-containing cells in this study, so cre status was

159   not assessed in this study in group assignments. TH::cre rats do not differ in behavior from

160   littermate controls, and are routinely used without manipulation as equivalent to cre-negative

161   littermate (Ferland et al., 2019). All rats were bred in-house and maintained on a 12:12 light/dark

162   cycle (lights on 7:00 A.M.–7:00 P.M.) and all testing occurred during the light phase.

163   The behavioral procedures occurred in two distinct periods. During the first ("early life")

164   phase of the experiment (PND 0 - PND 21), dams were allowed *ad libitum* access to enriched

165   breeder chow (Teklad) and water in the home cage, while pups were allowed *ad libitum* access to

166   nursing. During the second ("adulthood") period for the original pups, rats were first tested under

167   ad libitum conditions (PND180), and afterwards (approximately PND 180-PND 320) restricted to

168   95% of their free feed weight receiving 10-15g of standard laboratory chow (Teklad) provided

169   directly in their home cage after daily test sessions.

170   During the pre-weaning period (PND 0-PND 21), the dam and pups were housed in a

171   plastic container (48cm (l) X 26cm (w) X 20cm (h)) with approximately 2cm of wood shaving

172   bedding and a laboratory paper twists for enrichment and nesting material. For the remainder of

173   the experiment the home cage consisted of a plastic container (48cm (l) X 26cm (w) X 20cm (h))

174   with approximately 2cm of bedding. All procedures were performed in accordance with University

175   of Colorado Institutional Animal Care and Use Committee guidelines for the humane use of

176   laboratory rats in biological research.

177

178   **Behavior**

179   *Early life stress (wet bedding and nesting material)*

180   Male and females were paired, and after given time for mating, pregnant females were

181   isolated to gestate without males present. The early life stressor in this study was an incidental

182   water supply malfunction (Hydropac Alternative Watering System), in which one of the two

183   hydropacs on the cage lid ruptured, resulting in exposure to wet and cool bedding (2 cm) and

184   nesting material for the dam and pups. Note that because cages were provided with two hydropacs,

185   the primary stressor here was related to potential thermal loss in the pups and dam, limited *quality*

186   (dry) bedding to build warm "domed" nests, and potential maternal stress related to these

187   conditions. Indeed, prior models of stress experienced by the dam via exposure to wet-bedding for

188   as little as 10h has been shown to alter concurrent maternal behavior for over a week after exposure

189   (Léonhardt et al., 2007). Importantly, in the present study, the wet bedding stressor was not

190   sufficient to cause any loss of individual pups or litters.

191   Animals were observed on PND1 and PND2 (Thu/Fri) by a lab research staff member, and

192   then ~48h later (PND4; Sun) when the wet bedding was discovered. At this point, animals were

193   immediately removed from the wet bedding and placed in normal (dry) bedding. During this time

194   in the vivarium, it was our intention to decrease dam stress by limiting human (care staff)

195   interactions in the vivarium in the first few days post-delivery to a minimum. This activity

196   nevertheless included rapid checks that dams had at least one intact hydropac and access to food.

197   As such, dams retained access to *ad libitum* water and food during this wet-bedding period.

198   We had initially intended the offspring in this study to be used in our lab to establish future

199   breeding lines for our transgenic colony. Because early life stress is known to potentially induce

200   epigenetic changes in individuals (Torres-Berrío et al., 2019), transgenerational transmission of

201   these change could present a confound, and therefore these animals were not considered viable for

202   future breeding. To avoid euthanasia for these subjects, we instead wondered whether this

203   incidental exposure (while unintended) could provide an opportunity to compare how this

204   developmental stressor during the first week postnatal could alter developmental trajectories,

205   particularly in contrast to controls born and reared in otherwise identical conditions. Indeed,

206 unanticipated stressors can provide valuable insights as to how these events affect brain and

207 development (Dallman et al., 1999), and the lead author on this project has an established record

208 of characterizing perinatal models of stress and development in rats (Bercum et al., 2015).

209 Importantly, as this wet bedding exposure was incidental, we immediately discussed with and

210 obtained approval from our veterinarian and IACUC committee before continuing with these

211 studies. With their support and approval, we designated pups who experienced the wet/cold cage

212 the Early Life Stress (ELS) group, while pups reared under identical conditions in the vivarium at

213 the same time but were raised under normal dry bedding and nesting conditions throughout

214 development comprised the Control group. Animals were pair-housed during the post-weaning

215 period into adulthood, and were only separated following intracranial implants just prior to

216 Conditioned Suppression training.

217

218 *Early Life: Juvenile Social Exploration*

219 Social exploration tests were conducted at PND 180 as described previously (Christianson

220 et al., 2011). For this test, each subject was placed in plastic tub cage (48cm X 26cm X 20cm) that

221 contained 2 cm of fresh bedding located in a designated testing room for a 1-hour habituation

222 period. A novel Long-Evans juvenile rat (target) was then introduced into the cage for 3 min. The

223 behavioral response of the subject animals (ELS/Controls) to the target juvenile was recorded with

224 a video camera placed above the cage. The behavior was then assessed by measuring total duration

225 and frequency of social exploration. All videos were scored in a blinded and randomized manner.

226 *Adulthood: Conditioned Suppression*

227 As adults, rats were trained in a Conditioned Suppression paradigm. This task consisted of

228 a sequence of three phases: instrumental conditioning [Context A], auditory fear conditioning

229   [Context B], and finally, fear-conditioned suppression test [Context A]. The specific approaches

230   are explained in detail below.

231   Instrumental Conditioning: Lever press was established to assess motivation to seek

232   rewards. On the first day of training, rats (n=16) were introduced to a large operant chamber

233   (Context A: 60cm (w) X 56cm (l) X 36cm (h), smooth Plexiglas floors; MED Associates) where

234   they were first magazine trained to obtain food (45mg raspberry-flavored grain pellets, Purina Test

235   Diet) randomly delivered to a centrally-located foodcup on average about every 60s schedule.

236   On the subsequent days, a lever was extended and rats could press the lever and obtain

237   food. Each session ended when the rat pressed the lever enough times to deliver 25 pellets or

238   30min, whichever occurred first. If a rat received the 25 pellets within the 30min session limit, on

239   the following day, the subject was promoted to the next reinforcement schedule. Rats were first

240   trained on an FR1 schedule of reinforcement (each press produces a pellet), followed by variable

241   interval schedules of VI-5 (i.e., the first press in each 5-sec bin reinforced), then VI-15, VI-30 and

242   finally VI-60. Following completion of the VI-60 schedule, rats were implanted with bilateral

243   electrophysiological arrays (see below), then allowed at least 7d to recover. Following recovery,

244   rats were retested in the VI-60 schedule to re-establish stable pressing behavior. For rats who failed

245   to perform adequately at the VI-60 schedule, retraining at denser reinforcement schedules until

246   they could once again advance to stable completion of the VI-60 schedule over three consecutive

247   days.

248   Fear Conditioning: On the day following completion of the last VI-60 schedule of pressing,

249   rats were trained in a standard tone-shock fear conditioning paradigm consisted of a single 49-min

250   session. In this task, subjects were tested in a novel chamber (Context B: 43cm X 43cm X 53cm,

251   stainless steel grid floor; MED Associates) that was located in a different location in the research

252   facility from the original Instrumental Context A. The first 5 min of the session consisted of a

253   habituation phase where no cues were presented followed by a randomized presentation of a total

254   of 14 trials with a 180s ITI (7 CS+ trials: 30s tone (5000Hz, 80dB) co-terminating with a 0.8mA

255   footshock delivered through the stainless floor grid bars, and 7 CS- trials: 30s tone (3000 Hz,

256   80dB) presented without any programmed consequences). Average freezing during the cue

257   presentations and inter-trial intervals (ITI) were automatically scored using MED-Associates

258   software.

259   Conditioned Suppression: The day after Fear Conditioning training, test subjects were

260   returned to the original Instrumental Context A chamber. As in prior instrumental testing sessions,

261   a lever was presented and presses were reinforced with 45mg pellets on a VI-60 schedule. However

262   in the Conditioned Suppression sessions, after an initial 5min habituation phase where no cues

263   were presented, rats received random presentations of either the previously shock-predictive CS+

264   cue (30s high tone; 5000 Hz, 80dB; n=7 presentations) or the neutral CS- cue (30s low tone;

265   3000Hz, 80dB; n=8 presentations). Note that in this Context A, auditory cues were presented

266   without shock under extinction conditions. Cues were presented with an ISI on average of 150s

267   throughout the session. The number of lever presses during baseline period (no cues BL) was

268   compared to pressing during both the CS+ and CS- period to assess the degree of cue-elicited

269   suppression of instrumental activity. Rats received three consecutive Conditioned Suppression

270   sessions to assess the rate of recovery of instrumental pressing with extinction of the fear response.

271

272   **Electrophysiological Recording**

273   Single-unit recordings were acquired during Conditioned Suppression test sessions. Using

274   Plexon Omniplex systems (Plexon, Dallas TX) with a sampling rate of 40 kHz, analog voltages

275   recorded at the site of wires relative to a ground wire were amplified with a unity gain head

276   stage. The other connector on the tethered headstage was connected to a 16-channel electrical

277   commutator (Crist Instruments, Hagerstown, MD) to allow free movement in the chamber. Signals

278   from the commutator were then passed to an A/D converter (MiniDigiAmp) where analog voltages

279   were digitized and filtered to capture spikes and local field potentials (LFPs) on dedicated channels

280   for each wire. OmniPlex received synchronized TTL inputs from the MED Associates (St Albans,

281   NH) system running the test chambers to capture real-time behavioral events (including

282   experimenter-delivered events like cues and reward delivery, and subject-generated inputs like

283   lever presses), allowing perievent analysis of neural activity and LFP power.

284

285   **Surgery**

286   *Electrophysiological Recordings*

287   A subset of subjects (n = 13 rats; 10 female, 3 male) underwent bilateral electrode

288   implantation surgery (n= 7 ELS [6F/1M], 6 CTL [4F/2M]). Stereotaxic surgery was performed

289   under isoflurane anesthesia (2–5%) using aseptic techniques. For each surgery, rats were secured

290   in a stereotaxic apparatus (Kopf, Tijunga CA) using blunt earbars. Hair on the scalp was removed

291   and the underlying skin scrubbed with two sets of alternating washes of Betadine scrub and 70%

292   ethanol. Optical ointment (Vaseline) was applied gently to protect the eyes. A midline incision

293   was made with a scalpel, and the scalp and underlying fascia retracted laterally with hemostats. A

294   probe attached to the stereotaxic was used to measure the DV and ML deviations of Bregma and

295   lambda; deviations of more than 0.1mm were adjusted until the head was level. Coordinates for

296   array implants were generated from an atlas (Paxinos and Watson, 1996) for infralimbic cortex

297   (AP, +2.7; ML, ±0.5), and then holes drilled using a dental burr over the location. At each of these

298   insertion sites, the underlying dura was retracted to ensure the wires were inserted directly into

299   brain tissue. In addition, holes were drilled for skull screws (typically three on each hemisphere)

300   and the location of the ground wire (one each hemisphere).

301   Skull screws were inserted, after which each array was inserted (AP, +2.7; ML, ±0.5; DV,

302   −5.0 mm), with the left inserted first. Each electrode consisted of two 8-wire electrode arrays

303   (circular array surrounding an optical fiber; each wire consisting of a 50-µm dia Teflon-coated

304   stainless-steel wire spaced 500 µm apart; NM Labs, Denison, TX). Arrays were lowered slowly

305   (approximately 0.5mm/min) to the final recording location and fixed in place with dental acrylic.

306   After this, the ground wire was wrapped around the posterior skull screw, then inserted into the

307   brain at the ground wire hole. This was then repeated on the right side, with a specific mount used

308   to ensure the two array connectors were spaced correctly for the headstage tethers that would be

309   used later on the recording rigs. Rats received intramuscular injections of the antibiotic Baytril and

310   the NSAID analgesic Meloxicam-SR at the end of surgery. Rats were given a 7-day post-surgery

311   recovery period before conditioned suppression training began.

312
313
314   *Perfusion and Histology*

315   Following the final behavioral test, rats were deeply anesthetized using isoflurane 4% and

316   transcardially perfused with 0.9% saline followed by 4% paraformaldehyde. Electrode placements

317   were marked by passing current from a 9V battery through each electrode wire. Brains were

318   postfixed in 4% paraformaldehyde for at least 12h, followed by 36-48h in 20% sucrose as a

319   cryoprotectant, then stored at −80°C. Tissue was sectioned at 20 µm and mounted onto SuperFrost

320   Plus slides (Fisher Scientific) using a cryostat at -20°C and imaged using a light microscope

321   (Leica) to confirm electrode placement.

322

323   **Data Analysis**

324   *Behavioral Analysis*

325   During Early Life, behavior was assessed for pup weight and juvenile social exploration.

326   These data were analyzed with a between-subjects (unpaired) t-test using ELS and unstressed

327   Controls as the factors of interest.

328   During Adulthood, behavior for Conditioned Suppression was measured by using a

329   suppression ratio. Presses made during the 30-sec cue presentation (CS) was compared the 30-sec

330   period immediately prior to the cue onset (BL). The suppression ratio for each stimulus type (i.e.,

331   CS+ or CS-) was calculated as:

332
$$Suppression\ Ratio = \frac{(CS - BL)}{(CS + BL)}$$

333   This produces a range of scores from -1 to +1, with -1 being total suppression of pressing during

334   the cue and 0 reflecting no difference in pressing during the cue relative to the baseline.

335   Differences between groups for behavioral suppression were determined using two-way ANOVA

336   using the factors of Day (Days 1-3) and Stress (ELS vs unstressed Controls) as the variables of

337   interest. Tukey's HSD test was used for post hoc comparisons.

338

339   *Single Unit Electrophysiological Analysis*

340   Putative single units were sorted for each channel (wire) using principal component

341   analysis clusters based on waveform similarity (Offline Sorter; Plexon). Unit clusters were then

342   subject to secondary confirmation using auto-correlated firing properties. Auto-correlated firing

343   histograms typically contain a "notch" at the 0 point indicative of a biologically-relevant refractory

344   period for action potential generation (typically at least +/- 4 ms) Putative cells that showed

345   significant numbers of spike events in this refractory period were rejected as units as being

346   biologically implausible, and were not subsequently analyzed.

347   For perievent analysis, data were binned into 200ms blocks and averaged across events

348   within a session. The perievent firing rate was then z-transformed based on mean and standard

349   deviation of the average perievent activity. Thus, the z-normalized firing rate for each bin was

350   calculated as:

351
$$z_{Bin} = \frac{(FiringRate_{Bin} - Mean\ BL\ Firing\ Rate)}{StDev\ BL\ Firing\ Rate}$$

352   To ensure relatively uniform distributions of z-normalized firing, units with activity of less

353   than 0.5Hz were excluded from subsequent analysis. Within the remaining units, averaged

354   populations were grouped by generally excitatory (firing rate greater than 0.5z within 1s after cue

355   onset) or generally inhibitory (firing less than -0.5z within 2s after cue onset). Based on this, we

356   assessed two components of perievent cue firing. The first is the relative proportion of cells that

357   exhibited generally excitatory (>0.5z), generally inhibitory (< -0.5z) or non-phasic relative to cue

358   onset. These proportions were compared using chi square analysis. The second quantifies the peak

359   firing during these onset periods for each cell. These were assessed using three-way ANOVAs

360   with Stress (CTL vs ELS), Cue (CS+ vs CS-) and Epoch (Baseline vs Cue Onset) as factors.

361

362   *Local Field Potential Analysis*

363   LFP data generated spectrograms from 1-120 Hz perievent aligned to the CS+ and CS-

364   cues. Spectrograms included a 5sec baseline followed by a 30s cue presentation and a 5-sec post-

365   cue period, averaged into 200ms bins. Prior to fast Fourier transform (FFT), spectrograms were

366   mean background subtracted, then normalized by the log of the Power Spectral Density (dB). From

367   these spectrograms, specific frequencies were selected based on their established importance in

368   circuit signaling: Delta (1-4 Hz), Low Theta (5-8 Hz), High Theta (9-14 Hz), Beta (15-22 Hz),

369   Low Gamma (23-55 Hz), and High Gamma (65-95 Hz). The average power in these bands were

370   then z-normalized by the average and standard deviation of the 5sec baseline period prior to cue

371   onset, then applied to each 200ms bin throughout the perievent trace (similar to that described

372   above for neural activity normalization).

373   For stress-related comparisons, for each spectrum, the subject's baseline and average

374   power during the cues (CS+ and CS-) was assessed separately for all days of the Conditioned

375   Suppression tasks. Note that the averaged power during the cue period excluded the first 400ms

376   of activity, but did include the rest of the cue period. These were assessed using three-way

377   ANOVAs with Stress (CTL vs ELS), Cue (CS+ vs CS-) and Epoch (Baseline vs Cue Onset) as

378   factors.

379

380

381   **Results**

382   **Behavior**

383

384   *Early Life Stress Impairs Development*

385

386   Pups were weighed six days following birth (PND6). We found that stress had a significant

387   negative impact on weight gain, with animals in the ELS group showing reliably lower weights

388   than Controls, $t_{24} = 15.85$, $p< 0.0001$ (Figure 1A).

389

390   *ELS Reduces Social Behaviors*

391   After growing to adulthood in the vivarium (but prior to any experimental conditioning), adult

392   rats were assessed in a juvenile social interaction (JSI) task on PND180 to assess social

393   behaviors and anxiety-related phenotypes. Rats in the ELS group generally showed a decrease in

394   social behaviors compared to Controls. While the total time spent sniffing the juvenile

395   conspecific was marginally decreased in the ELS group ($t_7 = 2.32$, $p=0.052$; Figure 1B, left), the

396   number of interaction bouts initiated by the ELS subjects were significantly lower than

397   unstressed Controls, $t_7 = 3.14$, $p=0.017$; Figure 1B, right).

398
399   --
                                        Figure 1 about here
400   --

401   *Instrumental Learning and Fear Conditioning are unaffected by ELS status*

402   In the acquisition phase of instrumental learning, ELS appeared to have no effect on the

403   motivation to press for food. Rats in the ELS group showed a similar ability as Controls on the last

404   three days of each schedule to press for the food, and likewise to increase the number of presses

405   per reward delivered based on the schedule requirements (Figure 1C). These observations

406   produced a significant main effect of Schedule, $F_{2,223} = 200.7$, $p < 0.001$. This effect was almost

407   exclusively due to a linear increase in press rate per reward earned across the decreasing

408   reinforcement schedule, as a linear contrast on these data was significant, $F_{1,223} = 274.1$, $p < 0.001$

409   and accounted for 82% of the main effect variance. However, there were no effects of Stress or

410   any interactions of Stress by any other factor (Schedule, Day) (all $F < 1$).

411   Following instrumental conditioning, rats learned fear conditioning to the CS+ and CS-

412   tones in a novel context (Figure 1D). While both groups showed rapid acquisition of fear to the

413   cues and context (main effect Trial, $F_{12,168} = 48.72$, $p < 0.0001$) and Cue (ITI vs Cue, $F_{1,14} = 15.61$,

414     $p = 0.001$; greater freezing during cue), we did not see any Stress ($F_{1,14} = 0.01$, $p < 0.91$) or Stress

415   X Cue X Trial interactions ($F_{12,168} = 1.25$, $p = 0.25$). Thus, there were no differences in the

416   acquisition of conditioned fear between groups.

417

418 *ELS Abnormally Suppresses Motivated Seeking Under Threat*

419   Following this, rats were returned to the original context for the Conditioned Suppression

420 task. Here, rats were reinforced on a VI60 schedule while receiving presentations of the CS+ (fear-

421 associated cue; n=8) or neutral CS- (n=8). Because no shocks were delivered in this context, we

422 repeated this Suppression paradigm for three consecutive days to assess the rate of fear extinction.

423 For average pressing within each session, both groups showed suppression of lever presses during

424 the presentation of the CS+, though ELS subjects were more suppressed than Controls (main effect

425 Stress, $F_{1,16} = 5.21$, $p=0.047$; Figure 1E). This suppressive effect in the ELS animals was limited

426 to the CS+ (interaction of Stress X Cue, $F_{1,16} = 10.54$, $p=0.005$), with ELS showing reliably greater

427 suppression than controls during the CS+ (Tukey, $p=0.005$), but no differences between the CS-

428 (Tukey, $p=0.99$).

429   Finally, we assessed the degree of successful extinction by assessing whether the

430 suppression ratio was reliably negative (i.e., still suppressed) on each session. Controlling for

431 multiple comparisons (Bonferroni), we found that in Controls, suppression for the CS+ was

432 reliably below 0 on Day 1 ($p<0.001$), but not on Day 2 or Day 3. In contrast, ELS rats showed

433 suppression during the CS+ that was reliably below 0 on all three days (Day 1: $p<0.0001$; Day 2:

434 $p=0.005$; Day 3: $p=0.01$). Overall, these data indicate that relative to Controls, ELS animals display

435 greater suppression of motivated behavior to fear-related stimuli that is more resistant to extinction

436 than in Controls. However, ELS rats do not appear to show generalized fear, as they are adept at

437 discriminating between fearful and "safe" stimuli; indeed, even better than Controls.

438
439 *ELS increases the rate of excitatory responses to fear cues in vmPFC neurons*

440   Recordings of single unit activity were conducted during Conditioned Suppression in both

441   Controls (n=6) and ELS (n=7) subjects. From these recordings, we identified n=129 neural units

442   in the Controls and n=191 in the ELS subjects in histologically-confirmed locations in the vmPFC

443   based on boundaries found in (Paxinos and Watson, 1996) (Figure 2).

444

445   ---

446   **Figure 2 about here**

447   ---

448

449   Z-normalized firing rates were then aligned by their phasic response to the onset of the

450   fear-associated cues (CS+ and CS-) by taking the average Z score during the first 1sec following

451   cue onset. Data were considered generally excitatory if they exhibited an increase in firing greater

452   than +0.5z relative to baseline, while inhibitions were those with a phasic response less than -0.5z.

453   Data from all recorded cells relative to CS+ onset are shown in Figure 3A (Control) and Figure 3B

454   (ELS). Population responses to the CS+ cue in the Controls were biased towards inhibitory

455   signaling with 36.7% of cells demonstrating phasic inhibitions and 28.9% displaying excitations;

456   34.3% of cells were non-phasic in either direction. In contrast, ELS neurons showed the opposite

457   pattern, as these units were almost twice as likely to display an excitatory response (50.5%) than

458   an inhibitory response (25.5%) to the CS+ cue. ELS neurons also had slightly fewer cells that were

459   non-phasic (24.0%). This shift from inhibitory to excitatory response to the CS+ between groups

460   was significantly different, $\chi^2_1 = 10.96$, $p = 0.0009$ (Figure 3B). Notably, these EXC/INH/non

461   proportions were quite stable by groups over days, with Controls showing generally more

462   inhibitory responses than excitations to the CS+, and ELS showing the opposite pattern (*Controls*:

463   Day 1 - 29% EXC, 35% INH; Day 2 – 32% EXC, 53% INH; Day 3 – 33% EXC, 27% INH; *ELS*:

464   Day 1 - 56% EXC, 20% INH; Day 2 – 48% EXC, 31% INH; Day 3 – 40% EXC, 21% INH; all χ^2

465   comparisons between day, p>0.20). In contrast to the fear-associated CS+, the relative proportion

466   of excitatory and inhibitory response to the CS- cues was not different between groups (Control:

467   29.2% excitatory vs 32.1% inhibitory; ELS: 41.7% excitatory vs 34.9% inhibitory; $\chi^2_1 = 0.57$, $p =$

468   0.45). These data suggest that ELS experience alters the function of the vmPFC to bias neurons

469   towards an abnormally excitatory response to threatening (but not neutral, or "safe") cues.

470   ---
471   **Figure 3 about here**
472   ---

473
474   *ELS impairs normal shifts in extinction-related firing to the CS+ cue*

475   Prior investigations have reliably demonstrated that vmPFC neurons are critical for

476   mediating extinction of fear via connectivity with amygdalar structures (Adhikari et al., 2015;

477   Maren and Quirk, 2004; Milad and Quirk, 2002; Sierra-Mercado et al., 2011b). Phasic perievent

478   excitatory activity relative to the CS+ in the Controls was consistent with this established

479   finding, demonstrating a slight increase in the magnitude of phasic activity over days. In

480   contrast, vmPFC neurons in ELS animals showed the opposite pattern, with the greatest level of

481   excitatory activity during the CS+ occurring on the first day and decreasing magnitude of this

482   response over repeated days of extinction (Figure 4A). In general, the ELS animals showed a

483   reliably higher overall excitatory phasic response to the fear cues (main effect Stress, $F_{1,133} =$

484   4.77=8, $p = 0.03$) and an interaction of Cue (CS+ vs CS-) X Stress (ELS vs Control) X

485   Extinction Day (1-3), $F_{2,133} = 3.34$, $p = 0.04$. This interaction showed that the phasic response to

486   the CS+ was greater in ELS than Controls on Day 1 ($p = 0.005$) but not on subsequent days.

487   There were no differences to the CS- between groups on any day (Figure 4B). However, in

488   general, CS+ elicited significantly greater activity than the CS- in both the Controls ($p = 0.02$)

489   and in the ELS group ($p < 0.001$).

490   In contrast to the excitatory phasic responses, for inhibitory responses, there were no

491   stress-related main effect, $F_{1,104} = 3.07$, $p = 0.08$, though there was an interaction of Stress X

492   Day, $F_{2,104} = 3.18$, $p = 0.046$, and Stress X Cue, $F_{1,104} = 5.90$, $p = 0.017$, but not Stress X Cue X

493   Day, $F_{2,104} = 0.75$, $p = 0.47$ (Figure 4C). Indeed, for planned comparisons, we found no

494   differences in the magnitude of the inhibitory response between Controls and ELS for the CS+

495   cues (all $p > 0.28$), though there were differences between ELS and Controls for the "safe" CS-

496   on Day 1 ($p < 0.001$) and on Day 2 ($p = 0.04$), but not on Day 3 ($p = 0.75$). On those same days,

497   ELS animals showed a better ability to discriminate neural firing responses between the CS+ and

498   CS- on Day 1 ($p = 0.005$), Day 2 ($p = 0.001$) and Day 3 ($p < 0.001$), whereas Controls only

499   successfully discriminated between CS+ and CS- cues on Day 3 (p = 0.01) but not on Day 1 ($p =$

500   0.75) or Day 2 ($p = 0.76$), Figure 4D.

501   ---
502   **Figure 4 about here**
503   ---

504   *ELS abolishes High Gamma LFP responses to threat cues in vmPFC*

505   LFPs have been proposed to reflect the coherence of the aggregate voltage in a region.

506   Given the large amount of surface area on dendritic arbors in a region relative to somatic

507   activity, one potential interpretation of LFP oscillations is that it reflects a significant component

508   of input to those local arbors from afferent regions. We recorded LFPs on the same wires and

509   locations in the vmPFC as for the single-unit activity described above, and generated perievent

510   spectrographs for defined frequency bands relative to CS+ and CS- onset during the same

511   Conditioned Suppression sessions (West et al., 2021).

512   We found that ELS experience had little effect on changes in LFPs in most spectra

513   (Figure 5). For example, in the Delta, Beta and Low Gamma frequencies, the response to the cue

514   onset reliably decreased the power of these frequencies relative to baseline for the CS+ but not

515   CS- (Cue [CS+ vs CS-] X Onset [baseline vs cue periods]: Delta, $F_{1,53} = 14.52$, $p = 0.0004$; Beta,

516    $F_{1,53} = 8.90$, $p = 0.004$; Low Gamma, $F_{1,53} = 11.66$, $p = 0.001$). However, while the LFP

517   response to the CS+ decreased LFP power in these spectra below baseline for the CS+ (all

518   p<0.003) but not CS-, there were no differences between Controls or ELS for either CS+ or CS-

519   in any of these spectra (all $p > 0.25$). Notably, there were no main effects of Stress or interactions

520   of Stress with other factors in the Delta, Low Theta, High Theta, Beta, or Low Gamma

521   frequencies.

522   However, ELS appeared to selectively impair the High Gamma frequency response

523   (Figure 5, *bottom right*). In Controls, this response was a large and sustained increase in power

524   for the duration of the CS+ cue, which then returned to baseline after cue offset. In contrast, this

525   frequency response showed only a brief <1sec response to cue onset before rapidly returning to

526   baseline for the rest of the CS+ cue. Quantifying the mean response during the cue period for

527   both CS+ and CS- (Cue) for ELS and Controls (Stress) for the baseline period vs cue period

528   (Onset), an ANOVA found a main effect of Stress, $F_{1,53} = 6.89$, $p = 0.011$, and an interaction of

529   Stress X Cue, $F_{1,53} = 6.76$, $p = 0.012$, Stress X Onset, $F_{1,53} = 6.90$, $p = 0.011$, and Stress X Cue

530   X Onset, $F_{1,53} = 6.76$, $p = 0.012$. Posthoc comparisons of this 3-way interaction indicated that in

531   Controls, the High Gamma response to the CS+ was reliably higher than both its own baseline

532   (p=0.0001) and the CS- cue (p=0.0001). The power for the CS- cue in Controls was, however, no

533   different than its preceding baseline (p=0.99).

534   --
535   **Figure 5 about here**

536   ---

537   For ELS animals, this selective CS+ related increase in power was eliminated; the

538   average power of the CS+ was no different from baseline (p=0.40) nor from the CS- period

539   (p=0.99). Consistent with this loss of High Gamma power in the ELS group, there was a

540   significant decrease for the High Gamma band for the CS+ cue between groups (p=0.0008), but

541   there were no differences in power between groups for the CS- cue (p=0.99). As such, ELS

542   appears to selectively abolish a discrete component of the LFP spectra, while leaving other

543   lower-frequency components relatively unaffected.

544

545   **Discussion**

546   Fear is an adaptive response to potentially threatening stimuli, though the brain must be

547   adaptive enough that fear can extinguish when threats are no longer present. Consistent with

548   prior observations, we found that ELS experience increases the fear-related suppression of

549   reward seeking during the fear-associated CS+ compared to Controls. However, ELS animals did

550   not differ from controls in their responses to the "safe" CS- cue. During these conditioned

551   suppression sessions, recordings were made in the vmPFC that permitted the recording of both

552   single unit and LFP activity in the same location. Neurons in the vmPFC of ELS rats showed

553   both an increase in the proportion of excitatory responses to the fear-associated CS+ cue

554   compared to Controls, as well as an increase in overall magnitude of the excitatory phasic

555   response to cue. In contrast, while there were no differences between ELS and Controls in

556   inhibitory encoding of the CS+, ELS neurons were better able to discriminate between CS+ and

557   CS- stimuli than those in Controls. Finally, LFP oscillations in the vmPFC were consistent with a

558   selective loss of the high gamma band in ELS-experienced rats. This loss is notable in that this is

559   the only frequency where Controls showed a phasic change in activity that discriminated

560   between CS+ and CS- stimuli, suggesting this signal plays a potentially important role in

561   facilitating fear discrimination and feedback during extinction. Collectively, these data are

562   among the first to demonstrate ELS-related functional alterations in vmPFC activity and resultant

563   changes in fear-related behavior.

564   In general, our finding in Controls are congruent with prior findings in neurotypical adult

565   rats undergoing fear conditioning and extinction. Controls showed initial fear to the CS+

566   stimulus that resulted in a robust cessation of motivated pressing for food. However, these fear-

567   suppressed behaviors rapidly returned to pre-suppression levels by the second day of extinction.

568   In these same Control subjects, vmPFC activity showed an appreciable increase in phasic

569   excitatory activity in response to the CS+ commensurate with the resumption in motivated

570   seeking behavior and extinction of the fear-related suppression, while showing reliably less

571   activity to the safer CS- cue. These data are consistent with prior work demonstrating the role of

572   IL and the vmPFC in mediating extinction through new learning (i.e., that the CS+ is now

573   associated with no-shock), and increases in excitatory activity in these regions to permit this

574   plasticity. Prior work, for example, has shown that excitatory stimulation of IL via electrical

575   current or channelrhodopsin is sufficient to expedite fear suppression and extinction (Adhikari et

576   al., 2015; Giustino and Maren, 2015; Milad and Quirk, 2002), and which persists in subsequent

577   days without the stimulation present.

578   The ELS animals showed a different pattern of results; unlike Controls, ELS animals

579   showed greater overall amounts of fear suppression, while at the same time showing increased

580   levels of excitatory responding in single units. It is essential to note that ELS animals face

581   developmental alterations compared to unstressed neurotypical controls. For example, PFC

582   regions continue to develop and integrate neuromodulatory afferents for several days (at least

583   PND16) after birth, including mesocortical dopaminergic wiring and integration with amygdalar

584   nuclei (Cunningham et al., 2008; Kalsbeek et al., 1988; Kroon et al., 2019; Yuan et al., 2021),

585   producing lasting changes in excitability and functional properties of these networks

586   (Muhammad et al., 2012; Zhang, 2004). Thus, for these ELS animals whose stress experience

587   happened during this critical developmental window, functional responses of these neurons may

588   not mirror those in neurotypical individuals. Indeed, the robust and consistent increase in

589   excitability in these neurons suggests that for ELS animals, the typical relationship between

590   greater excitability and faster extinction seen in neurotypical controls no longer holds.

591   These observations argue against an interpretation of ELS inducing a hypofrontal state

592   where extinction of threats are unable to be extinguished by descending prefrontal networks.

593   This outcome would be consistent with some prior work showing decreased activity in human

594   populations during reward and risk processing (Birn et al., 2017), and frankly with our *a priori*

595   predictions for this study. However, the increased excitability suggests instead that vmPFC

596   neurons are appropriately responding to the threat posed by the CS+ cue and are appropriately

597   increasing activity to drive extinction, but that this activity is not sufficient to dampen fear-

598   induced suppression as in controls. A possible interpretation for this set of results is that

599   extinction is a process that requires both new learning (CS+ no longer predicts threat) as well as

600   feedback to stamp in those new associations. Recent findings and models are consistent with the

601   importance of these potential feedback mechanisms to the PFC in normal fear learning and

602   extinction (McNally et al., 2011). For example, during processing of fear stimuli, mesocortical

603   dopaminergic input to the PFC (Vander Weele et al., 2018) as well as amygdalar input (Burgos-

604   Robles et al., 2017) provide event-related information about fear threats to prefrontal networks.

605   Indeed, recent work has demonstrated that pathway-specific inputs from intercalated neurons in

606   the basolateral amygdala to discrete components of dorsal and ventral PFC may differentially

607   regulate feedback to gate continued fear or its extinction (Hagihara et al., 2021). If this is the

608   case, then persistent increases in fear-associated excitability in vmPFC of ELS animals may not

609   be due to an inability to detect threats, but rather for a PFC-amygdala network to cooperatively

610   use error-related feedback to update cues to a new and less-threatening state. If so, then evidence

611   should exist that ELS animals are missing arising information that could be relevant for this

612   learning.

613   Consistent with this interpretation, LFPs in the high gamma band were largely abolished

614   in ELS compared to controls. LFPs reflect aggregate voltage in a region, and given the density of

615   dendritic arbors relative to somas, these changes in voltages in a region may biased towards

616   reflecting afferent inputs to a region, via depolarization and hyperpolarization of dendrites

617   receiving those signals. Support for this perspective was recently provided in models of calcium

618   transient activity with GCaMP sensors in the dorsal striatum (Legaria et al., 2021). Given this,

619   one hypothesis consistent with our data is that vmPFC in ELS animals is lacking relevant

620   feedback on the efficacy of extinction learning, and this information may be provided via gamma

621   band oscillations.

622   This loss may be important for several reasons for interpreting our results. First, gamma

623   oscillations have been thought to reflect in part the activity of GABAergic interneurons (Buzsáki

624   and Wang, 2012; Cho et al., 2020; Sohal et al., 2009), and thus the ELS neurons displaying a

625   heightened excitability in this study may reflect the loss of this GABAergic regulation.

626   Compellingly, BLA afferents preferentially target PFC GABA interneurons during early

627   postnatal development (Cunningham et al., 2008), suggesting these pathways may be particularly

628   vulnerable to insult during early life. Furthermore, disruption of this pathways during early life

629   development appears to functionally alter and impair these arising BLA-PFC pathways, well-

630   characterized dysfunction of this pathway in ELS individuals and animal models (Fan et al.,

631   2014; Guadagno et al., 2018; Ishikawa et al., 2015; VanTieghem and Tottenham, 2018). Another

632   potential source of input may arise from the hippocampus, which has likewise been implicated in

633   fear-related changes in behavior in ELS-experienced animals (Reincke and Hanganu-Opatz,

634   2017). Consistent with this interpretation, high-gamma electrical stimulations in the fibria-fornix

635   preferentially enhanced coordination between PFC and hippocampus, suggesting a likely route of

636   communication on this frequency (Helbing and Angenstein, 2020). Future investigations will

637   need to investigate these pathways, and in particular, why this frequency is uniquely disrupted

638   while others are relatively unaffected.

639   Finally, recent work has focused not only on responses to threats, but also how animals

640   come to learn about stimuli explicitly predictive of no-threat (i.e., safety). For our task, the CS-

641   cue served as a neutral stimulus without consequence, but also signaled the explicit absence of

642   any possibility of shock. This information about this safe cue appears to be reflected quite

643   differentially in the vmPFC of ELS and Control subjects. In general, vmPFC neurons in Controls

644   did surprisingly worse at discriminating between the CS+ and CS- than in ELS animals. While

645   both ELS and Controls were adequate at discriminating between CS+ and CS- stimuli, this was

646   not the case in inhibitory responses. Controls only showed discrimination between CS+ and CS-

647   on the third day of fear extinction, while ELS animals showed robust and reliable discrimination

648   between the CS+ and CS- throughout all days of extinction. These findings suggest the

649   possibility that ELS animals may be more vigilant and ascribe a greater salience to potentially

650   threatening stimuli, while therefore also better able to ascribe safety to non-threatening cues in

651   the same context. This interpretation suggests that separate signals and neurons in the vmPFC

652   may participate in the detection and significance of threat cues and their extinction (excitatory

653   responses), while another participates in the learned safety of explicitly neutral stimuli

654   (inhibitory responses). In this sense, ELS neurons were relatively impaired relative to Controls in

655   excitatory signaling about threats and extinction, while they were relatively enhanced relative to

656   controls in inhibitory signaling about safety signals. This intriguing dichotomy suggests discrete

657   pathways that may coordinate complex responses to environments with ambiguous and

658   competing information.

659   In conclusion, these data demonstrate that ELS is a potent modulator of brain networks

660   that are essential for mediating appropriate and adaptive responses to a host of cognitive tasks

661   including relief from fear, abstinence from drugs of abuse, and adequate assessment of risk in

662   decision making. ELS experience, particularly early in development while the PFC and related

663   limbic network are still in the process of developing functional connectivity, can have lasting

664   effects on stimulus processing and behavioral responses to motivational stimuli. These data

665   present new insights into how ELS-related dysfunction may contribute to the wide variety of

666   mental health disorders that are precipitated by ELS and contribute to risk factors for disorders

667   like addiction and PTSD.

668   **Limitations**

669   The design of the ELS experience was not designed *a priori* as an early life stress model.

670   As noted, the animals were originally destined for another project in our lab, and due to new

671   animal care procedures in a new vivarium, the stressors that were presented were due to

672   incidental damp bedding during a critical development period for both dams and pups. Prior

673   work has demonstrated that unfortunate (but routine) vivarium stressors such as nearby

674   construction can strongly alter animal behavior (Dallman et al., 1999). As such, we feel that

675   these observations should be of interest not only to researchers interested in neural mechanisms

676   of ELS, but also to veterinary and care staff who are interested in which disturbances in care

677   environment can induce lasting changes in subject animals.

678   Second, we are aware that there are different and more standard ELS models of limited

679   bedding, fragmented maternal care and others which have more extensive use in the field (Molet

680   et al., 2014), although as noted, wet bedding as a stressor has some application in the field as

681   well (Léonhardt et al., 2007). Understanding the conditions under more controlled experimental

682   settings will provide a more comprehensive understanding on the impact of this ELS

683   manipulation.

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906   Figure Legends
907
908   **Figure 1. A.** On PND6, weights of the ELS rats were lower than the unstressed Controls. **B.** In
909   adulthood (PND180), ELS subjects showed less time (*left*) and fewer initiated contacts with a
910   novel conspecific juvenile rat (*right*) in a JSI assessment. **C.** During Instrumental acquisition,
911   rats in the ELS group showed similar levels of motivation to press as controls across decreasing
912   schedules of reinforcement. **D.** Rats in both ELS and Control groups rapidly learned conditioned
913   freezing to the presentations of CS+ (cue terminating in 0.8mA shock) and the "safe" CS- (no
914   shock). Orange bars indicate freezing during the CS+ cue, green bars indicate freezing during the
915   CS-. Data between colored bars indicate freezing during the intertrial interval between cues (i.e.,
916   contextual freezing). **E.** In a conditioned suppression task, the fear-associated CS+ cue
917   suppressed lever pressing for food (VI60) more in ELS rats than Controls across three days of
918   fear extinction. *$p<0.05$, main effect, ELS vs Control; #$p<0.06$, main effect, ELS vs Control;
919   &$p<0.05$, ELS vs Control CS+ on that Day.
920
921   **Figure 2.** Placements of array wires in the PFC. Controls (black/gray circles) and ELS animals
922   (red/orange circles) are shown primarily in the infralimbic cortex, with some wires extending
923   ventrally into the medial orbital and dorsal peduncular cortex, and some dorsally into prelimbic
924   cortex. Diagrams of brain and boundaries adapted from Paxinos & Watson, 1996.
925
926   **Figure 3.** Heat plot representation of the population of recorded neural activity in the IL in
927   Controls (**A**) and ELS subjects (**B**) relative to the onset of the CS+ cue in the Conditioned
928   Suppression task. Color reflects magnitude of z-normalized firing with lighter colors indicating
929   greater firing rates ($z > 1$), while darker colors indicate inhibitory activity ($z < 1$). Cells on the plot
930   were sorted by the magnitude of the average firing rate during the first 1000ms after cue onset.
931   Brackets on the left of each plot indicate the range of cells for which the phasic response was at
932   least +0.5z above baseline ("excitatory"; *black top bracket*) or at least -0.5z below baseline
933   ("inhibitory"; *gray bottom bracket*). Bar to the right of each heatplot indicates the scale to translate
934   z score (from +4 to -4z) for each plot. **C.** Relative proportion of excitatory (EXC; greater than
935   +0.5z), inhibitory (INH; less than -0.5z), and non-phasic units relative to the first 1sec of cue onset.
936   ELS animals showed a significant increase in the proportion of EXC cells relative to Controls, χ^2_1
937    $= 10.96, p = 0.0009$
938
939   **Figure 4.** Phasic responses of vmPFC neurons to cue onset over repeated sessions of Conditioned
940   Suppression. Identified excitatory (**A-B**) and inhibitory (**C-D**) units were analyzed separately. **A.**
941   Both ELS (warm colors) and Control subjects (gray colors) showed rapid phasic responses to
942   presentations of the fear-associated CS+ that typically lasted less than 1sec following cue onset.
943   **B**. The average firing rate during the first 1sec following cue onset for each EXC cell for the CS+
944   (solid line) and the CS- (dashed line). C-D. Same as for A-B, but for maximum inhibitions (lowest
945   firing point). *$p<0.05$, Control vs ELS (CS+);[‡]$p<0.05$, CS+ vs CS- (Controls); [β]$p<0.05$, CS+ vs
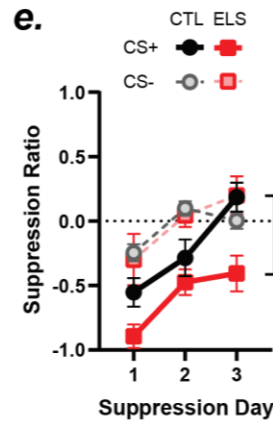946   CS- (ELS).
947
948   **Figure 5.** Perievent spectrograms generated for each of the frequencies identified in each title.
949   Data are z-normalized by the average power in the baseline for each subject. At left in each
950   subfigure is the mean response in 200ms bins over the duration of the CS+ cue presentations
951   (Control: *black*; ELS: *red*). Vertical dotted line indicate cue onset and offset respectively. At right

952 in each subfigure is the average (excluding the first 400ms, which may reflect a non-associative

953 artifact). At left in black/gray are controls, and at right in red/pink are the ELS averages. In each

954 pair, the darker/left bar is the CS+, while the lighter/right bar is the CS-. *$p<0.05$, Control v ELS;

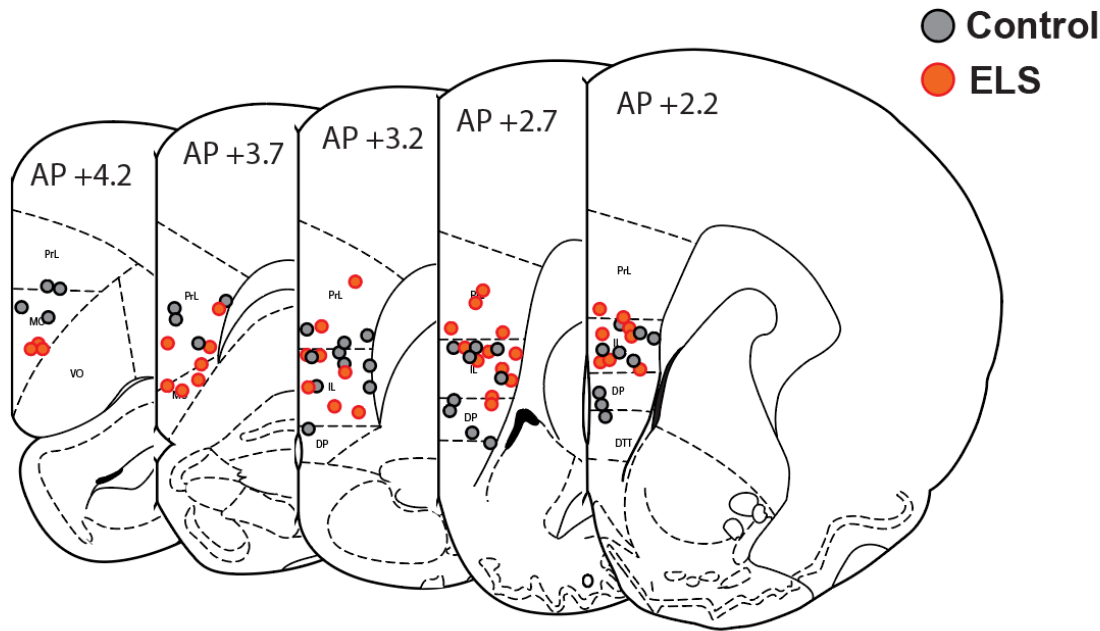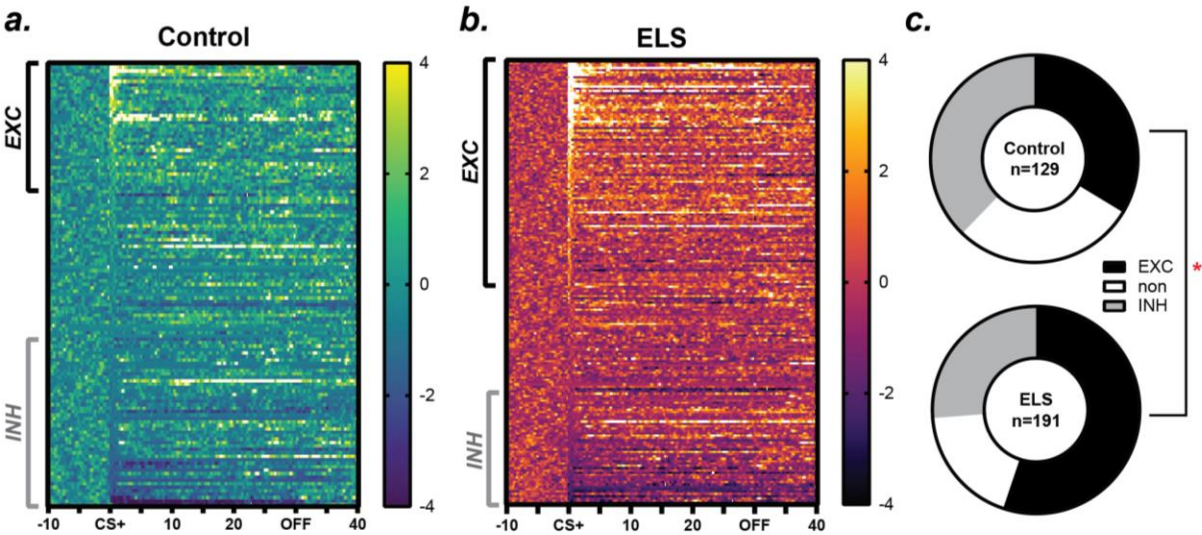955 $^{\$}p<0.05$, Baseline period vs Cue period; $^{\&}p<0.05$, CS+ vs CS-.

956

957   Figure 1.

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