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Serotonergic transcriptional programming determines maternal behavior and offspring survival

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Central serotonergic signaling influences many physiological processes, but a requirement for reproductive success has not been demonstrated. Using mouse dams with a specific disruption in serotonin neuron development, we found that serotonergic function is required for the nurturing and survival of offspring. Full rescue of survival depended on the mother's expression level of the upstream serotonergic transcriptional cascade. Thus, intrinsic transcriptional programming of maternal serotonergic activity determines the quality of nurturing and whether or not the organism survives.

Serotonergic signaling in the CNS is critical for proper maturation and homeostatic modulation of neural circuits that shape emotions and many physiological responses[1,2]. Perturbations in the level of serotonergic gene expression have a substantial effect on behavior and have been implicated in the pathogenesis of several neuropsychiatric diseases, including disorders of anxiety, mood and appetite[1,2]. Although serotonin (5HT) has been implicated in the regulation of female sexual behavior[3], present evidence does not demonstrate an essential role for the central serotonergic transmitter system in reproductive success.

Expression of the *Pet-1* (also known as *Fev*) ETS transcription factor in the brain is restricted to 5HT neurons[4]. Normal numbers of serotonergic precursors are generated in *Pet-1*$^{-/-}$ hindbrain, but expression of serotonergic gene expression and 5HT synthesis are greatly reduced[5]. We used *Pet-1*$^{-/-}$ mouse dams to investigate the effect of their arrested 5HT neuron development on offspring viability. Virgin wild-type and *Pet-1*$^{-/-}$ females were bred with wild-type or *Pet-1*$^{+/-}$ males. Birth rates and offspring body weights were normal for primiparous *Pet-1*$^{-/-}$ dams (**Supplementary Table 1** and **Supplementary Fig. 1** online). However, although 99% of pups survived when they were born and nurtured by wild-type dams, no pups survived when they were born to *Pet-1*$^{-/-}$ dams, and the majority of these offspring were found dead without placentas and were not cannibalized 3–4 days after birth (**Fig. 1a**). Cross fostering at postnatal day 1 (P1) showed that pups born to *Pet-1*$^{-/-}$ dams survived when nurtured by wild-type dams (**Fig. 1a** and **Supplementary Table 1**).

The profound deficit in survival of offspring born to *Pet-1*$^{-/-}$ mice suggested that the dams had a deficit in their maternal behavior. Successful nurturing requires the coordinate expression of several discrete behaviors, including nest building, pup retrieval, cleaning and nursing[6]. Pups born to *Pet-1*$^{-/-}$ dams were consistently observed to have milk in their stomachs each postnatal day before death, suggesting that normal lactation was occurring (**Fig. 1b**). However, the percentage of time spent crouching was significantly less for *Pet-1*$^{-/-}$ dams than for wild-type dams (*Pet-1*$^{-/-}$ dams: 53%, 8.7% s.d., $n = 6$; wild-type dams: 73%, 10.1% s.d., $n = 13$; $P < 0.05$). Moreover, *Pet-1*$^{-/-}$ dams often would not build suitable nests (**Fig. 1b**,**c**). Organizing pups in a huddle is essential for neonate survival, as it facilitates feeding and maintains pup body temperature. Pups born to wild-type dams were always found to be organized in huddles (**Fig. 1b**,**d**), as were cross-fostered pups. In marked contrast, offspring of *Pet-1*$^{-/-}$ dams were never organized in huddles (**Fig. 1b**,**d**). To carefully evaluate maternal behavior, we continuously video monitored dams in the home cage (**Supplementary Methods** online). Both wild-type and *Pet-1*$^{-/-}$ dams gave birth in the nesting area. Immediately postpartum, wild-type dams were crouched over their pups and actively maintained them in an organized huddle in a well-constructed nest. *Pet-1*$^{-/-}$ dams were also present in the vicinity of nesting material during much of the night and did not appear hyperactive. However, the offspring of *Pet-1*$^{-/-}$ dams were not huddled beneath their mother and were often seen to be buried beneath disheveled bedding material or completely exposed at a distance from the material. Pup death occurred near the birth location or at some distance from this site as a result of the pups spontaneous tossing and turning. When we pre-huddled the pups in the evening, we found that *Pet-1*$^{-/-}$ dams failed to maintain the huddle throughout the night and pups were scattered in as little as 30 min after pre-huddling (**Supplementary Fig. 2** online). In addition, providing *Pet-1*$^{-/-}$ dams with a pre-huddle did not increase offspring survival (**Fig. 1a** and **Supplementary Table 1**).

We then asked whether a deficit in retrieval might account for the dispersion of pups. *Pet-1*$^{-/-}$ dams failed to retrieve the majority of their pups in assays in which new bedding material was supplied (**Fig. 1e** and **Supplementary Methods**). Instead, *Pet-1*$^{-/-}$ dams alternated between frequent digging behavior and active traversal of the cage (**Supplementary Video 1** online), and therefore appeared to be inattentive to their young. In contrast, wild-type dams were actively engaged in pup retrieval to a well-constructed nest (**Supplementary Video 2** online). To determine whether the addition of new bedding material contributed to the retrieval deficits, we carried out retrieval assays without disturbing the bedding material for at least 5 d before the assay. Although retrieval improved slightly, the number of pups retrieved by *Pet-1*$^{-/-}$ dams was still lower than the number retrieved by wild-type dams (**Fig. 1e**). In addition, the average time to retrieve pups was threefold longer for *Pet-1*$^{-/-}$ dams (wild-type dams: 22.1 s, 12.9 s

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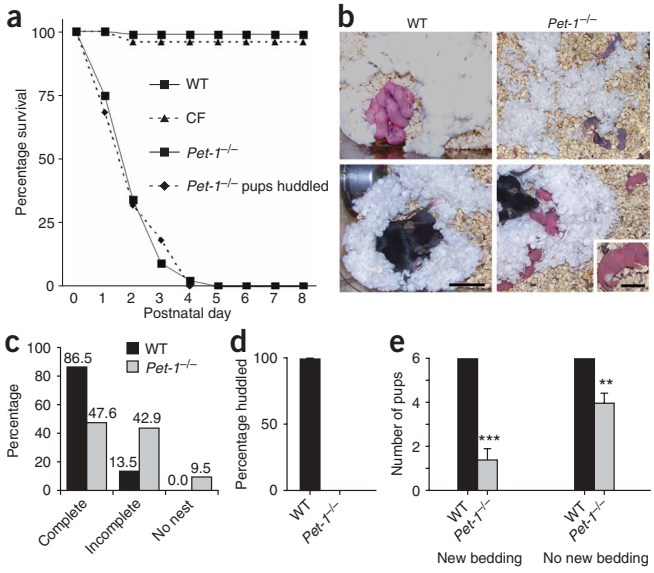

Figure 1 *Pet-1* is required for reproductive success. (**a**) Survival of litters born to wild-type (WT, $n = 13$) and *Pet-1*$^{-/-}$ dams ($n = 9$), litters born to *Pet-1*$^{-/-}$ dams cross-fostered to wild-type dams (CF, $n = 4$) and litters born to *Pet-1*$^{-/-}$ dams with pre-huddled pups ($n = 4$). (**b**) Representative views of nests and huddles for wild-type and *Pet-1*$^{-/-}$ dams. Scale bar represents 4 cm. Inset, pup on P1 with milk band; scale bar represents 1 cm. (**c**) Nest quality of wild-type versus *Pet-1*$^{-/-}$ dams. (**d**) Litters of *Pet-1*$^{-/-}$ dams were never seen grouped in huddles. Wild type, $n = 13$; *Pet-1*$^{-/-}$, $n = 6$ (**c**,**d**). (**e**) *Pet-1*$^{-/-}$ dams retrieved fewer pups than wild-type dams when new bedding was added ($n = 6$ for each genotype, $^{***}P < 0.0001$) and when no new bedding was added ($n = 4$ for each genotype, $^{**}P < 0.005$). Wild-type dams retrieved all six pups in all tests. Error bars represent s.e.m.

s.d.; *Pet-1*$^{-/-}$ dams: 72.17 s, 2.6 s s.d.; $n = 4$ for each genotype, $P < 0.005$). *Pet-1*$^{-/-}$ dams did not show different elevated-plus maze activity compared to wild-type dams, indicating that anxiety-like and locomotor activity were normal (**Supplementary Fig. 3** online). In addition, *Pet-1*$^{-/-}$ dams showed normal latencies to detect various odors, indicating normal olfaction (**Supplementary Fig. 4** online).

We next investigated whether *Pet-1* loss of function could be corrected with a bacterial artificial chromosome (BAC) encoding *FEV*, the human ortholog of *Pet-1* (**Supplementary Fig. 5** online). *FEV* expression in several *FEV* BAC founder lines, *Tg(FEV); Pet-1*$^{-/-}$, was restricted to raphe nuclei (**Supplementary Fig. 5**), consistent with exclusive serotonergic expression of *Pet-1* in the brain[4]. The numbers of 5HT-positive cells and fibers in the various mouse rescue lines were not different from those of wild-type mice (**Supplementary Fig. 5**). All of the classic membrane properties of 5HT neurons (**Supplementary Table 2** online), as well as anxiety and home-cage locomotor behavior (**Supplementary Fig. 6** online), were the same as in wild type, indicating that there was no demonstrable disruption of these characteristics by BAC insertion.

We then quantitated the absolute levels of *FEV* transcripts in the dorsal raphe of the *Tg(FEV); Pet-1*$^{-/-}$ lines. This analysis revealed significantly different transcript levels above and below the level of wild-type *Pet-1* transcripts in the postnatal period ($P < 0.05$; **Fig. 2a** and **Supplementary Table 3** online). Therefore, we sought to determine whether reduced *FEV* expression in *Tg(FEV)3; Pet-1*$^{-/-}$ (41% of wild type) or increased expression in *Tg(FEV)4; Pet-1*$^{-/-}$ (ninefold greater than wild type) would program early postnatal changes in the levels of brain serotonergic characteristics. 5HT was at wild-type levels in the *Tg(FEV)1; Pet-1*$^{-/-}$ and *Tg(FEV)4; Pet-1*$^{-/-}$ lines, but the level of 5HT in the *Tg(FEV)3; Pet-1*$^{-/-}$ line was intermediate to that of the wild-type and *Pet-1*$^{-/-}$ brains (**Fig. 2b**). Overexpression of mouse *Pet-1* in wild-type brain did not result in changes in brain 5HT (**Supplementary Fig. 7** online). Consistent with reduced 5HT levels in *Tg(FEV)3; Pet-1*$^{-/-}$ mice, the level of tryptophan hydroxylase 2 (TPH2) RNA, as well as that of the 5HT transporter (SERT), in the midbrain raphe of *Tg(FEV)3; Pet-1*$^{-/-}$ was reduced relative to their levels in wild-type mice (**Fig. 2c**). In contrast, their levels were not different from wild type in either the *Tg(FEV)1; Pet-1*$^{-/-}$ and *Tg(FEV)4; Pet-1*$^{-/-}$ lines (**Supplementary Fig. 8** online). Dopamine levels did not differ (**Supplementary Fig. 9** online). Together, these findings show that *FEV* can rescue serotonergic defects in *Pet-1*$^{-/-}$ mice, but full rescue of 5HT levels and serotonergic gene expression depends on the level of expression of the upstream transcriptional cascade directed by *FEV*. However, increased *FEV* or *Pet-1* expression above that of endogenous *Pet-1* had no impact on 5HT, indicating a possible homeostatic transcriptional constraint on serotonergic character.

We then investigated the survival of offspring born to *Tg(FEV)3; Pet-1*$^{-/-}$ dams. Notably, these offspring showed reduced survival compared with the nearly 100% survival of pups born to wild-type, *Tg(FEV)1; Pet-1*$^{-/-}$ and *Tg(FEV)4; Pet-1*$^{-/-}$ dams (**Fig. 2d** and **Supplementary Table 1**). However, overall survival of *Tg(FEV)3; Pet-1*$^{-/-}$ offspring was better than that observed for offspring born to *Pet-1*$^{-/-}$ dams. Decreased survival was observed in about 50% of litters born to *Tg(FEV)3; Pet-1*$^{-/-}$ dams (**Fig. 2e**). Pup survival was normal in litters born to *Tg(FEV)3; Pet-1*$^{+/-}$ dams in which one intact *Pet-1* allele was present, showing that poor survival could be corrected in the *Tg(FEV)3; Pet-1*$^{-/-}$ line by increasing serotonergic characteristics (**Fig. 2d** and **Supplementary Table 1**). The partial restoration of *Tg(FEV)3; Pet-1*$^{-/-}$ offspring survival suggested that there was a partial rescue of maternal care. Indeed, although the time engaged in crouching and nest building were normal in *Tg(FEV)3; Pet-1*$^{-/-}$ mothers (**Supplementary Fig. 10** online), the number of offspring born to *Tg(FEV)3; Pet-1*$^{-/-}$ dams found outside of the nest was greatly increased over the numbers seen for pups born to wild-type dams, but was less than the number of offspring seen out of the nest that were born to *Pet-1*$^{-/-}$ dams (**Fig. 2f**). Pups were found outside of the nests in nearly two-thirds of litters born to *Tg(FEV)3; Pet-1*$^{-/-}$ dams (**Fig. 2g**). The number of pups born to *Tg(FEV)4/Pet-1*$^{-/-}$ mothers that were found outside of the nest was not different from the number of pups found out of the nest that were born to and nurtured by wild-type dams. A Kendall Tau-b statistical analysis (tau-b = –0.66, $P < 0.0001$; **Supplementary Methods** and **Supplementary Table 4** online) suggested that an intermediate level of maternal behavior was occurring in the *Tg(FEV)3/Pet-1*$^{-/-}$ line.

Despite the prevalence of postpartum depression and the frequent use of selective 5HT reuptake inhibitors to treat this disorder[7], a requirement for maternal serotonergic function in the mother's behavior toward her offspring or offspring survival has not been convincingly demonstrated with either pharmacological depletion of 5HT or targeting of 5HT receptors[8,9]. Our findings directly demonstrate an important role for the serotonergic system in reproductive fitness by showing that a specific disruption of maternal 5HT neuron differentiation causes pup mortality, the likely consequence of pup exposure resulting from a failure of the dam to maintain pups in an organized huddle in a properly constructed nest. Although *Pet-1*–deficient adults that were not in a reproductive period showed increased anxiety-like behavior[5], *Pet-1*–deficient dams did not. This difference in emotional state is consistent with the known reduction in anxiety and fear that accompanies lactation in mice and rats[6], and suggests that the deficit in maternal behavior in *Pet-1*$^{-/-}$ mice is not the result of a generalized anxiety phenotype.

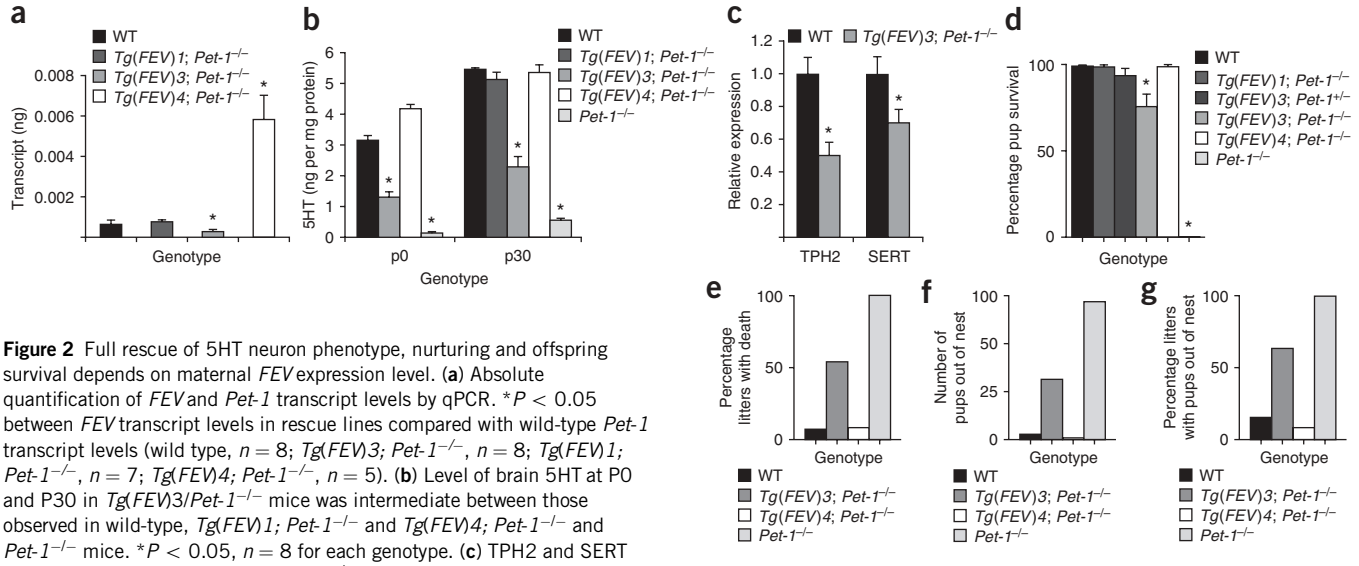

Figure 2 Full rescue of 5HT neuron phenotype, nurturing and offspring survival depends on maternal *FEV* expression level. (**a**) Absolute quantification of *FEV* and *Pet-1* transcript levels by qPCR. $*P < 0.05$ between *FEV* transcript levels in rescue lines compared with wild-type *Pet-1* transcript levels (wild type, $n = 8$; *Tg(FEV)3; Pet-1*$^{-/-}$, $n = 8$; *Tg(FEV)1; Pet-1*$^{-/-}$, $n = 7$; *Tg(FEV)4; Pet-1*$^{-/-}$, $n = 5$). (**b**) Level of brain 5HT at P0 and P30 in *Tg(FEV)3/Pet-1*$^{-/-}$ mice was intermediate between those observed in wild-type, *Tg(FEV)1; Pet-1*$^{-/-}$ and *Tg(FEV)4; Pet-1*$^{-/-}$ and *Pet-1*$^{-/-}$ mice. $*P < 0.05$, $n = 8$ for each genotype. (**c**) TPH2 and SERT RNA were reduced in *Tg(FEV)3; Pet-1*$^{-/-}$ brains relative to wild type (wild type, $n = 11$; *Tg(FEV)3; Pet-1*$^{-/-}$, $n = 14$; $*P < 0.05$). (**d**) Percentage of surviving pups born to wild-type ($n = 13$), *Tg(FEV)1; Pet-1*$^{-/-}$ ($n = 10$), *Tg(FEV)3; Pet-1*$^{+/-}$ ($n = 9$), *Tg(FEV)3; Pet-1*$^{-/-}$ ($n = 11$), *Tg(FEV)4; Pet-1*$^{-/-}$ ($n = 12$) and *Pet-1*$^{-/-}$ ($n = 13$) dams. ANOVA with Dunnett's post test, $*P < 0.05$. (**e**) Percentage of litters with offspring death. (**f**) Number of alive and dead pups found outside of the nest. (**g**) Percentage of litters with alive or dead pups out of the nest. (wild type, $n = 13$; *Tg(FEV)3; Pet-1*$^{-/-}$, $n = 11$; *Tg(FEV)4; Pet-1*$^{-/-}$, $n = 12$; *Pet-1*$^{-/-}$, $n = 6$). Error bars represent s.e.m.

The neural circuitry that gives rise to the various behavioral components of nurturing comprise numerous nuclei and their interconnections situated in many different brain regions, such as the medial preoptic area and the bed nucleus of the stria terminalis[6]. Dopaminergic modulation arising in the ventral tegmental area has been implicated in maternal retrieval of pups[9] and studies of dopamine β hydroxylase–deficient mice have demonstrated that it is important for noradrenergic modulation of pup retrieval, nursing and offspring survival[10]. Several transcription factor genes have been implicated in maternal behavior, but, unlike *Pet-1*, in most instances the specific CNS neuronal cell types in which these factors are intrinsically needed to enable nurturing have not yet been determined[6].

The maternal neglect shown by *Pet-1*–deficient dams may be caused by an acute insufficiency of 5HT or other serotonergic neuron–derived signals in, for example, the bed nucleus of the stria terminalis and medial preoptic area, which are normally innervated by serotonergic afferents arising in the dorsal raphe, median raphe and B9 nucleus[11]. Alternatively, disruption of serotonergic signaling earlier in the life of *Pet-1*–deficient females might account for the neglect of offspring. Proper levels of the SERT[12,13], TPH[13,14] and 5HT-1a receptors[15] during the early postnatal period are probably required for the normal maturation of circuitry governing emotional behaviors. Pet-1 is required specifically to coordinate serotonergic transcription of TPH2, SERT, aromatic amino acid decarboxylase and vesicular monoamine transporter 2 (ref. 5). This raises the possibility that the abnormal nurturing shown by *Pet-1*–deficient dams is the result of the altered formation of circuitry governing maternal motivation and reward.

Our findings illustrate a potential mechanism whereby genetically or environmentally directed decreases, but not increases, in the expression level of intrinsic transcriptional control genes, developmentally program graded changes in the levels of serotonergic neuron characteristics. These changes may in turn alter the quality of adult 5HT-modulated behaviors such as those needed for offspring nurturing and survival.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

This study was designed, interpreted and written by J.K.L.-H. and E.S.D. J.K.L.-H. carried out all of the experiments except for the electrophysiology. D.F. advised and performed statistical analyses on maternal care data. L.K.C. and S.G.B. carried out and analyzed the electrophysiological data.

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