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Serotonergic transcriptional programming determines maternal behavior and offspring survival

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Abstract

Central serotonergic signaling influences many physiological processes but a requirement for reproductive success has not been demonstrated. Using dams with a specific disruption in serotonin neuron development we show that serotonergic function is required for nurturing and survival of offspring. Full rescue of survival depends 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 organism survival.

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 significant impact 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* ETS transcription factor in the brain is restricted to 5HT neurons ⁴. Normal numbers of serotonergic precursors are generated in *Pet-1*^{-/-} midbrain but expression of serotonergic gene expression and 5HT synthesis are greatly reduced ⁵. We used *Pet-1* deficient dams to investigate the impact 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, online; Supplementary Fig. 1, online). Yet, while 99% of pups survived when born and nurtured by wild type dams, no pups survived when born to *Pet-1*^{-/-} dams and the majority of these offspring were found dead without placentas and not cannibalized 3–4 days after birth (Fig. 1a). Cross fostering at postnatal day 1 showed that pups born to *Pet-1*^{-/-} dams survived when nurtured by wild type dams (Fig. 1a, Supplementary Table 1 online).

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The profound deficit in survival of offspring born to *Pet-I*^{-/-} dams suggested deficient maternal behavior. Successful nurturing requires the coordinate expression of several discrete behaviors including nest building, pup retrieval, cleaning, and nursing⁶. Pups born to *Pet-I*^{-/-} dams were consistently observed to have milk in their stomachs each postnatal day before death suggesting normal lactation (Fig. 1b, inset). However, the percent time crouching was significantly less for *Pet-I*^{-/-} dams compared to wild type dams (*Pet-I*^{-/-} dams: 53%, stdev 8.7%, n=6; wild type dams: 73%, stdev 10.1%, n=13; p<0.05). Moreover, *Pet-I*^{-/-} 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 organized in huddles (Fig 1b, d) as were cross-fostered pups. In striking contrast, offspring of *Pet-I*^{-/-} 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-I*^{-/-} dams gave birth within the nesting area. Immediately postpartum wild type dams were crouched over their pups and actively maintained them in an organized huddle within a well-constructed nest. *Pet-I*^{-/-} 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-I*^{-/-} dams were not huddled underneath their mother and were often seen 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-I*^{-/-} dams failed to maintain the huddle through the night and pups were scattered in as little as thirty minutes after pre-huddling (Supplemental Fig. 2 online). Additionally, providing *Pet-I*^{-/-} dams with a pre-huddle did not increase offspring survival (Fig. 1a, Supplementary Table 1 online).

We then asked whether a deficit in retrieval might account for the dispersion of pups. *Pet-I*^{-/-} dams failed to retrieve the majority of their pups in assays in which new bedding material was supplied (Fig. 1e, Supplementary methods online). Instead, *Pet-I*^{-/-} dams alternated between frequent digging behavior and active traversal of the cage (Supplementary Video online) and therefore appeared inattentive to their young. To determine whether the addition of new bedding material contributed to the retrieval deficits we performed retrieval assays without disturbing the bedding material for at least 5 days prior to the assay. While retrieval improved slightly, the number of pups retrieved was still lower than the number retrieved by wild type dams (Fig. 1e). Additionally, the average time to retrieve pups was three times longer for *Pet-I*^{-/-} dams (wild type dams: 22.1s, stdev 12.9s; *Pet-I*^{-/-} dams: 72.17s, stdev 2.6s; n=4 for each genotype, p<0.005). Elevated-plus maze activity was not different from wild type indicating normal anxiety-like and locomotor activity in *Pet-I*^{-/-} dams (Supplementary Fig. 3 online). Additionally, *Pet-I*^{-/-} dams exhibited normal latencies to detect various odors, indicating normal olfaction (Supplementary Fig. 4 online).

We next investigated whether *Pet-I* loss of function could be corrected with a bacterial artificial chromosome (BAC) encoding *Fev*, the human orthologue of *Pet-I* (Supplementary Fig. 5 online). *Fev* expression in several *Fev* BAC founder lines, *Tg(Fev)/Pet-I*^{-/-}, was restricted to raphe nuclei (Supplementary Fig. 5a online), consistent with exclusive serotonergic expression of *Pet-I* in the brain⁴. The number of 5HT⁺ cells and fibers in the various rescue lines were not different from wild type (Supplementary Fig. 5b-f online). 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 wild type, indicating no demonstrable disruption of these characteristics by BAC insertion.

We then quantitated absolute levels of *Fev* transcripts in the dorsal raphe of the *Tg(Fev)/Pet-I*^{-/-} lines. This analysis revealed significantly different transcript levels above and below the level of wild type *Pet-I* transcripts in the postnatal period (Fig. 2a, Supplementary Table

3 online). Therefore, we sought to determine whether reduced *Fev* expression in *Tg(Fev)3/Pet-1^{-/-}* or increased expression in *Tg(Fev)4/Pet-1^{-/-}* 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 *Tg(Fev)3/Pet-1^{-/-}* line was intermediate to the levels in wild type and *Pet-1^{-/-}* brain (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^{-/-}* were 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 levels of endogenous *Pet-1* had no impact on 5HT indicating a possible homeostatic transcriptional constraint on serotonergic character.

We then investigated survival of offspring born to *Tg(Fev)3/Pet-1^{-/-}* dams. Strikingly, these offspring showed reduced survival compared to the nearly 100% survival of pups born to wild type, *Tg(Fev)1/Pet-1^{-/-}* and *Tg(Fev)4/Pet-1^{-/-}* (Fig 2d, Supplementary Table 1 online). Yet overall survival of *Tg(Fev)3/Pet-1^{-/-}* offspring was better than survival seen 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 online). The partial restoration of *Tg(Fev)3/Pet-1^{-/-}* offspring survival suggested a partial rescue of maternal care. Indeed, 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, Supplementary Fig. 10 online). Pups were found outside of 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 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 Table 4 online), suggested an intermediate level of maternal behavior 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 disorder⁷, 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}. The findings reported here directly demonstrate a critical 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 caused by a failure of the dam to maintain pups in an organized huddle within a properly constructed nest. Although *Pet-1* deficient adults not in a reproductive period exhibited increased anxiety-like behavior⁵, *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 rats⁶ and suggests that the deficit in *Pet-1^{-/-}* maternal behavior is not the result of a generalized anxiety phenotype.

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 (MPA) and the bed nucleus of the stria terminalis (BST)⁶.

Dopaminergic modulation arising within the ventral tegmental area has been implicated in maternal retrieval of pups⁹ and studies of dopamine beta hydroxylase deficient mice have demonstrated a critical role for noradrenergic modulation of pup retrieval, nursing and offspring survival¹⁰. 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 needed intrinsically to enable nurturing have not yet been determined⁶.

Maternal neglect exhibited by *Pet-1* deficient dams may be caused by an acute insufficiency of 5HT or other serotonergic neuron derived signals in, for example the BST and MPA, which are normally innervated by serotonergic afferents arising in the dorsal raphe, median raphe and B9 nucleus¹¹. 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 SERT¹², TPH^{13, 14}, and 5HT 1a receptors¹⁵ during the early postnatal period are likely to be required for normal maturation of circuitry governing emotional behaviors. This raises the intriguing possibility that abnormal nurturing exhibited by *Pet-1* deficient dams is the result of 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 in turn may alter the quality of adult 5HT modulated behaviors such as those needed for offspring nurturing and survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. *Pet-1* is required for reproductive success

(a) Survival of litters born to dams with genotypes and conditions: wild type, $n=13$; *Pet-1*^{-/-} $n=9$; litters born to *Pet-1*^{-/-} dams cross fostered to wild type dams (+/+, CF), $n=4$; *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=4cm. Inset, pup on postnatal day 1 with milk band, scale bar=1cm. (c) Nest quality of wild type versus *Pet-1*^{-/-} dams. (d) Litters of *Pet-1*^{-/-} dams were never seen grouped in huddles. (e-d) dams observed: wild type, $n=13$; *Pet-1*^{-/-}, $n=6$. (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.

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. Asterisks indicate significantly ($P < 0.05$) different *Fev* transcript levels in *Tg(Fev)/Pet-1^{-/-}* rescue lines compared to 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 postnatal day 0 and 30 in *Tg(Fev)3/Pet-1^{-/-}* mice was intermediate between the level 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^{-/-}* brain relative to wild type level. 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. (e-g): Number of litters observed: wild type, n=13; Tg(*Fev*)3/*Pet-I*^{-/-}, n=11; Tg(*Fev*)4/*Pet-I*^{-/-}, n=12; *Pet-I*^{-/-}, n=6. Error bars represent s.e.m.