GPCR signaling: Role in mediating the effects of early adversity

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Abbreviations:

5-HT : serotonin
5HIAA : 5-hydroxyindoleacetic acid (5-HIAA)
BDNF : Brain Derived Neurotrophic factor
cAMP : cyclic AMP
CB : Cannabinoid
CREB :cAMP response element-binding
CRF :Corticotropin release factor
DA/D :Dopamine
DOI :1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane
DRN :Dorsal Raphe Nuclei
EPSC :excitatory postsynaptic potential
GABA :Gamma aminobutyric acid
Glu : Glutamate
GPCR : G-protein coupled receptors
GS : Gestational stress
IP3 : inositol 1,4,5-trisphosphate
LGABN : Licking grooming and arch back nursing
LBN : Limited Bedding and Nesting
M1 : Muscarinic receptor 1
mGluR : metabotropic Glutamate receptor
MIA : Maternal immune activation
mPFC : Medial Prefrontal cortex
mRNA : messenger Ribonucleic acid
MS : Maternal Separation
MSUS: Maternal Stress combined with Unpredictable Stress to the dam
NE: Norepinephrine
PFC: Prefrontal Cortex
PKA: Protein Kinase A
PNFlx: Postnatal Fluoxetine
SSRI: Selective Serotonin reuptake inhibitor

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Abstract:

Early adversity is a key risk factor for the development of several psychiatric disorders, including anxiety and depression. During early life, neurocircuits that regulate emotionality undergo substantial structural remodelling and functional maturation, and are thus particularly susceptible to modification by environmental experience. Preclinical evidence indicates that early stress enhances adult anxio-depressive behaviors. A commonality noted across diverse early stress models, is life-long alterations in neuroendocrine stress responses and monoaminergic neurotransmission in key limbic circuits. Dysregulation of G-protein coupled receptor (GPCR) signaling is noted across multiple early stress models, and is hypothesized to be an important player in the programming of aberrant emotionality. This raises the possibility that disruption of GPCR signaling in key limbic regions during critical temporal windows could establish a substrate for enhanced risk for adult psychopathology. Here we review literature, predominantly from preclinical models, which supports the building hypothesis that a disruption of GPCR signaling could play a central role in programming persistent molecular, cellular, functional and behavioral changes as a consequence of early adversity.

Introduction:

Early adversity is a common risk factor for psychopathology in adulthood, contributing substantially to global disease burden [1,2]. Clinical evidence indicates that individuals with a history of early stress exhibit enhanced vulnerability for multiple psychiatric disorders, including but not restricted to anxiety, depression, schizophrenia and bipolar disorder [3,4]. The early life window is a sensitive temporal epoch, wherein neurocircuitry that regulates emotional behavior and is laid down using genetic blueprints, is fine-tuned and modified by experience thus setting up the neurocircuitry that drives emotionality later in life [5,6]. Neurocircuits undergo significant structural and functional maturation in response to environmental stimuli during these critical periods [7–11]. Fine-tuning of developing neurocircuitry, based on incorporating cues from early environmental experience, contributes to the establishment of mature circuit function in adulthood, so as to achieve optimally adapted behavioral responses [12,13]. The brain in this critical window is thus particularly malleable and responsive to modification by experience. Depending on the nature of early stress (trauma /abuse /poverty /neglect /poor parental care), the time of onset, the duration and number of traumatic events, these varied experiences of early adversity drive structural and functional changes in key neurocircuits thus programming enhanced risk for psychiatric disorders [3,13–15]. Several preclinical models of early adversity have attempted to delineate the persistent behavioral changes of early stress, and to mechanistically decipher the underlying molecular, cellular and functional changes that may contribute to life-long alterations in mood behavior [5,6,16].

Pathways implicated as central mediators of the persistent effects of early stress, include a disrupted hormonal stress response pathway involving perturbed glucocorticoid signaling [17–19] as well as altered monoaminergic neurotransmission through a large family of G-protein coupled receptors (GPCRs), as key mediators of establishing circuit dysfunction that could program enhanced risk for adult psychopathology. Early adversity also impinges on signaling through other neurotransmitter receptors including the excitatory and inhibitory neurotransmitters, glutamate (Glu) and γ-aminobutyric acid (GABA) [9,20], endocannabinoids [21] and neuropeptides [22–24] several of which signal via GPCRs. This has raised the intriguing hypothesis that perturbed GPCR signaling could serve as a convergent target across diverse models of early adversity, and mechanistically mediate specific consequences of early

trauma. The primary focus of our review is to provide a perspective on the evidence relevant to the hypothesis that perturbed GPCR signaling in key neurocircuits may play a major role in mediating some of the lasting molecular, cellular, functional and behavioral consequences of early stress. Our review will be primarily restricted to a discussion of evidence based on preclinical studies using rodent animal models.

Animal models of early stress: Behavioral consequences

The quantity and quality of nurture received by pups in the early postnatal window of life can exert a profound influence on shaping of the pup's stress response pathways, and the programming of mood-related behavior in adulthood [5,25] (Figure 1). Rodent studies using naturalistic models based on variation in maternal care, indicate that pups that receive poor quality of maternal care, associated with perturbed licking, grooming and arched back nursing behavior (LGABN) from the dam, exhibit enhanced anxiety- and despair-like behavior as well as cognitive impairments in adulthood, and an accelerated trajectory for aging-associated dysfunction [26–28]. These alterations in mood-related behaviors noted in low LGABN animals are hypothesized to involve epigenetic modifications that drive sustained changes in gene expression within key limbic neurocircuits [29], thus contributing to structural and functional changes in brain regions such as the hippocampus, prefrontal cortex (PFC) and amygdala, and a disruption of monoaminergic neurotransmission [27,30,31].

Several preclinical models of early stress are based on perturbations that disrupt dam-pup relationships, fragmenting the nature and quality of maternal care. The classical model of maternal separation (MS), involves separation of the pups from their dam for 180 minutes daily commencing from postnatal day 2 to 14 [32,33]. Animals with a history of MS exhibit enhanced anxiety- and despair-like behavior, altered cognition, perturbed stress responses, an accelerated aging profile, and altered structural and functional changes in multiple limbic neurocircuits [34]. The limited bedding and nesting (LBN) model [35,36], as well as the maternal separation model combined with additional restraint stress to the dam (MSUS) [37], also evoke fragmented maternal care. Common across all three models (MS, MSUS, LBN) are enhanced anxio-depressive behaviors noted in adulthood, however these models differ both in the nature and magnitude of effect noted on cognition, fear conditioning and social avoidance behavior. It is important to note that prenatal perturbations, including gestational stress (GS) and maternal immune activation (MIA) that serve as models for schizo-affective and

neurodevelopmental disorders [38,39] also exhibit many similar long-lasting anxio-depressive behavioral phenotypes as noted in models of postnatal perturbations like MS, MSUS and LBN [40–42]. In common across the gamut of early stress models is enhanced "trait anxiety" which refers to the increased anxiety-like behavior across diverse behavioral tests observed across the life-span, and noted often in the absence of overt anxiogenic stimuli [43].

The striking commonalities noted in behavioral and physiological deficits across these early stress models (Figure 1), despite key differences in the nature, timing and severity of perturbation, argue for shared neurobiological mechanisms for the manner in which early adversity programs persistent anxio-depressive behavioral changes. Although there are several working hypotheses for the specific behavioral outcomes of early stress, amongst the relevant integrative signatures noted across these diverse models is the disruption of neurotransmitter coupled, GPCR signaling pathways implicated in mediating the effects of early trauma. Direct pharmacological perturbation of monoaminergic neurotransmission has led to an understanding of distinct developmental epochs wherein monoaminergic signaling disruption can program changes in affective behavioral states [44,45]. The role of disrupted serotonergic neurotransmission within postnatal windows has been best studied, wherein administration of the selective serotonin reuptake inhibitor (SSRI) fluoxetine during postnatal day 2-21 (PNFlx) results in the establishment of life-long increases in anxiety and despair-like behavior, phenocopying the behavioral alterations noted in early stress models [46,47]. These paradoxical anxiogenic and pro-depressive effects of postnatal SSRI treatment are hypothesized to involve a role for elevated serotonin in the developmental programming, modulation and fine-tuning of plasticity in key brain regions, including but not restricted to the prefrontal cortex (PFC) and the dorsal raphe nucleus (DRN) [48,49]. This early developmental window is characterized by the transient expression of the serotonin transporter (SERT), the molecular target of SSRI drugs, in non-serotonergic neurons of the neocortex, including DRN-projecting PFC neurons [48,49]. The DRN is a key source of serotonergic input to the PFC, which in turn provides top-down control of DRN neurons through glutamatergic excitatory feedback projections [50]. The PFC-DRN pathway has been implicated in evoking antidepressant-like behavioral effects and a percept of "controllability" in stress responses [50–52]. Given that the PFC has a prolonged period of circuit maturation and expresses SERT during a critical developmental epoch [53], it is hypothesized that one of the mechanisms via which postnatal SSRI administration may result in life-long altered emotionality is via impinging on the maturation of this key PFC-DRN circuit. Further, the autoreceptor feedback

control of the DRN, as well as the fine-tuning of excitatory and inhibitory inputs onto DRN neurons, matures during the early postnatal time window [54]. It has been speculated that the development of differential sensitivity of serotonergic neurons to feedback inhibition, and altered excitability of the DRN could contribute to the paradoxical pro-depressive and anxiogenic effects of postnatal SSRI administration. This opens up a central idea that common across models of early stress may be changes in monoamine neurotransmitter signaling, in particular serotonergic pathways, which are known to recruit GPCR signaling cascades.

Early stress and the regulation of G-protein coupled receptors

Monoaminergic neurotransmission plays a central role in the fine-tuning and sculpting of key limbic circuits that program adult mood-related behaviors [55]. Monoaminergic receptors predominantly coupled to G-protein linked signaling cascades, are often functionally coupled and expressed at the earliest stages of embryonic brain development. The perinatal window is also a period in which monoaminergic receptor composition, density, and functional coupling undergoes major dynamic changes in the rodent brain prior to the establishment of adult-like expression levels and function attained usually by the third to the sixth postnatal week [55]. Serotonin (5-HT), dopamine (DA) and norepinephrine (NE), the three major monoaminergic neurotransmitters, contribute substantially in distinct perinatal temporal windows to the shaping of circuits that modulate emotionality, thus providing a neural substrate through which environmental perturbations such as early trauma can disrupt the programming of mood-related behaviors [56–58]. Although our review is focussed predominantly on monoaminergic receptors, in particular 5-HT receptors, it is of importance to note that other G-protein signaling coupled neurotransmitter and neuropeptide receptors, including the metabotropic glutamate receptors (mGluRs) [38,59,60], GABA-B receptors [61], muscarinic acetylcholine receptors [62], cannabinoid receptors (CB) [21,63] and the corticotropin releasing factor (CRF) receptors [47,64,65] have also been implicated in contributing to the effects of early stress on establishing perturbed emotionality (Figure 2).

Across diverse early life models of adversity, is noted altered functionality and in specific cases increased receptor expression and binding, for specific Gq-coupled receptors, in particular within the neocortex. Animals with a life-history of MS, MIA or GS exhibit enhanced Gq-coupled serotonin$_{2A}$ receptor (5-HT$_{2A}$R) functionality, with enhanced receptor

function revealed via potentiated 5-HT_{2A}R mediated head twitch responses, increased EPSCs and augmented cortical immediate-early gene regulation evoked by 2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT_{2A}R agonist [38,66–69]. MS animals differ from those with an MIA or GS life-history in the nature of influence of early stress on 5-HT_{2A}R binding and expression. While MS animals exhibit a transient, small increase in prefrontal 5-HT_{2A}R binding [67], MIA and GS are associated with robust increases in cortical 5-HT_{2A}R binding, accompanied by significant increases in 5-HT_{2A}R gene expression noted in adulthood[38]. Our unpublished results indicate that PNFlx treatment also evokes a transient increase in 5-HT_{2A}R mediated head twitch responses during the juvenile period suggestive of increased 5-HT_{2A}R function. Collectively, this suggests that the normal process of developmental progression for 5-HT_{2A}R responses within key cortical brain regions, including the PFC, is disrupted in multiple models of early perturbation (MS, MIA, GS, PNFlx). 5-HT_{2A}R mediated excitatory responses within cortical pyramidal neurons are thought to peak in the first two weeks and attenuate significantly by adulthood[70]. MS disrupts this ontogenic process and results in heightened Gq-coupled 5-HT_{2A}R mediated excitatory drive of prefrontal pyramidal neurons noted well into adulthood, associated with robust increases in spontaneous network activity[49,67,71]. Interestingly, GS is also associated with enhanced EPSC frequency and amplitude of local field potentials in the PFC [72]. Further, MS animals also exhibit sustained alterations in prefrontal gene expression of several Gαq-protein coupled and phospholipase C-associated genes, such as calcium-calmodulin kinase 1, guanine nucleotide binding proteins, a network of proteins that interact with the IP3 receptor, calpain, and calcineurin in MS animals [68]. The notion that early stress may disrupt Gq-coupled signaling cascades is further supported by evidence of perturbed signaling via the Gq-coupled M1 acetylcholine receptor noted in the PFC in adult animals with a history of MS [62]. Early stress results in a disruption of the developmental ontogeny of muscarinic signaling, resulting in the continued expression of a more adolescent-like state for downstream calcium signaling linked to the Gq-coupled M1 acetylcholine receptor in the PFC [62]. This would have important implications for PFC regulation of executive function, and top-down control of anxio-depressive behavior states by the PFC. Taken together, the evidence thus far suggests perturbed Gq-coupled signaling downstream of the 5-HT_{2A}R and the M1 acetylcholine receptor within the PFC following early adversity, suggestive of enhanced Gq-coupled receptor driven responses and an adolescent-like functional phenotype possibly linked to a delayed maturation of key prefrontal circuits.

In addition to the building evidence that specific receptors coupled to Gq-mediated signaling pathways are altered in functional responses as a consequence of early stress, there are also several reports of early stress-evoked disruption of Gi-coupled receptor signaling pathways. In particular, the Gi-coupled serotonin$_{1A}$ receptor (5-HT$_{1A}$R) mediated currents are known to be transiently enhanced in the PFC of animals undergoing MS during postnatal life [73]. Strikingly, when adult-onset stress is overlaid on a history of MS, it results in a steep decline in 5-HT$_{1A}$R driven currents within the PFC [73]. This is suggestive of the fact that a combination of early trauma and the second-hit of adult stress can create a potent disruption of signaling via the Gi-coupled 5-HT$_{1A}$R in the neocortex. Adult animals with a history of MS and MSUS also show altered 5-HT$_{1A}$R mRNA levels in several limbic neurocircuits that modulate emotional and fear-related responses, as well as in the dorsal raphe nucleus (DRN) [74–78]. Changes in 5-HT$_{1A}$R levels in the DRN are of particular importance given that the 5-HT$_{1A}$R acts as an autoreceptor regulating 5-HT release via a feedback mechanism [79]. Reduced levels of 5-HT$_{1A}$R in the DRN hint towards a dysregulation of serotonergic neurotransmission, altering the levels of 5-HT release and firing patterns of serotonergic neurons, thereby influencing affective behaviors. This is corroborated by the observation of altered 5-HT turnover, measured via 5-HIAA to 5-HT ratio, in the brainstem and multiple target brain regions of MS animals [80–82]. GS and MIA animals also show reduced expression of 5-HT$_{1A}$R in the DRN and in the GABAergic neurons of the PFC, and the 5-HT$_{1A}$R binding was also reduced in the ventral hippocampus of male animals [83,84], which points towards a serotonergic pathway dysregulation in limbic circuits directly involved in the programming of mood-related behaviors in adulthood.

Early stress also modulates expression and function of the Gi-coupled group II and group III mGlu receptors both at the level of receptor binding and gene expression. The Gi-coupled mGlu$_4$ receptor expression is significantly decreased in the hippocampi of animals with a history of MS [85], and prenatal stress of GS and MIA decreases mGlu$_2$R expression in the frontal cortex [38]. Given that the group II and group III mGluRs are involved in regulating glutamate release via a negative feedback mechanism, a reduction in levels and function of these receptors could cause enhanced glutamatergic tone, skewing the excitation-inhibition balance towards increased excitability in these limbic regions, which is suggested to be one of the possible underlying mechanisms programming psychopathology of mood-related disorders. This is also validated by studies that report deficits in GABAergic signaling in prenatal stress models, which are known to program schizoaffective behavioral states possibly

via Glu/GABA dysfunction [86–88]. The heteromerization of Gi-coupled $mGlu_2$ receptors and Gq-coupled 5-HT_{2A}R is implicated in modulating psychosis-like states, and a disrupted $mGlu_2$R signaling could perturb biased agonism via 5-HT_{2A}R [38,39,89–91]. This is also corroborated by clinical studies from post-mortem human brains of schizophrenic patients showing increased levels of 5-HT_{2A}R but reduced levels of $mGlu_{2/3}$ receptors in the frontal cortex [90,92,93]. The Gi-coupled CB_1 receptor also functionally interacts with the Gq-coupled 5-HT_{2A}R to modulate hallucinogenic responses and cognitive processing [94,95], and perinatal stress causes reduced CB_1 receptor binding in multiple limbic regions, including the PFC, the hippocampus and the amygdala [63,96,97]. Collectively, emerging evidence points towards a perturbed expression of several Gi-coupled receptors in limbic brain regions, with the preponderance of literature suggestive of decreased signaling via specific Gi-signaling coupled receptors, namely the 5-HT_{1A}, $mGlu_{2/3/4}$ and CB_1 receptors.

Gs-coupled neurotransmitter and neurohormone receptor pathways have also been the focus of study, both in regards to their regulation by early stress, as well as their mechanistic contribution to the effects of early stress. In particular, there is extensive literature detailing the regulation of the Gs-coupled receptors for the stress-associated neurohormone, corticotrophin releasing factor (CRF), CRFR1 and CRFR2, as well as their role in mediating the persistent effects of early stress. The role of CRF receptors in the effects of early stress has been the focus of several recent reviews [98,99], and hence we have restricted ourselves to describing the literature focused on the effects of early stress on Gs-coupled neurotransmitter receptors. A recent report indicates that in GS animals the modulatory effects of the Gs-coupled 5-HT_7 receptors on the frequency of sEPSC/sIPSCs in DRN projection neurons appears to be lost [100]. This raises the intriguing possibility that the disruption of excitatory and inhibitory input onto DRN projection neurons could directly modulate 5-HT release in key target brain regions, and thus impinge on stress-response neurocircuitry and affective behavioral states. Animals with a history of MS also exhibit reduced mRNA levels of Gs-coupled $D_{1/5}$ receptors in many brain regions, and also result in reduced $D_{1/5}$ agonist dependent grooming behavior [101]. Dopaminergic signaling is known to impact phosphorylation of CREB via the $D_{1/5}$ receptors coupled through the recruitment of a cyclic AMP- protein kinase A (cAMP-PKA) signaling cascade [102]. CREB is known to impinge on the regulation of BDNF signaling, which exerts a highly circuit-specific influence on mood-related behaviors [103,104]. Both CREB and BDNF expression are known to be altered by early stress, and the directionality of change and spatio-temporal pattern of regulation are reported to vary across models in a circuit-specific

manner[105]. The findings thus far motivate a detailed study of the role of Gs-coupled receptors that recruit a PKA-CREB-BDNF pathway, in the programming of altered mood behavior that arise due to early adversity. It is noteworthy that the Gs-coupled receptors for the stress-associated neuropeptide CRF, $CRFR_1$ and $CRFR_2$, have been extensively studied in the context of early stress, and their function and expression is altered in multiple limbic brain regions, including but not restricted to the PFC, hippocampus and amygdala [64].

The existent literature indicates that early stress may serve to bias signaling response in favour of excitatory Gq-coupled signaling with a concomitant reduction in inhibitory Gi-coupled tone within neocortical circuits, suggestive of a dysregulation in the excitation-inhibition balance within these early critical windows. The data so far support the notion of enhanced Gq-coupled 5-HT_{2A}R mediated drive in multiple limbic circuits in particular the PFC, thought to contribute mechanistically to the programming of anxio-depressive behavioral states. This is accompanied by a reduction in inhibitory Gi-coupled 5-HT_{1A}R signaling in animals that have adult-onset stress overlaid on a history of early adversity. This suggests a tipping in balance towards enhanced Gq-coupled excitation within key limbic circuits, and is indeed supported by enhanced network activity in the neocortex of MS animals. However, one caveat to keep in mind is that the increased 5-HT_{2A}R mediated functional and electrophysiological responses are noted in layer V PFC neurons in MS animals [67], whereas the reduced Gi-coupled 5-HT_{1A}R IPSCs are observed in layer II/III of the PFC [73]. There is a paucity of data directly addressing the impact of early stress on the balance of Gq- versus Gi-coupled serotonergic receptor signaling in specific neuronal subtypes in key limbic circuitry. There remains an urgent need for detailed study of the impact of early stress on GPCR coupled signaling pathways in specific neuronal populations. Nevertheless, the evidence thus far raises an intriguing hypothesis that early stress via regulation of the fine balance between the Gq- and Gi-coupled signaling pathways in key brain regions such as the PFC could regulate the emergence of appropriate excitation-inhibition balance, and serve to program alterations in mood-related behavior.

Early stress could impinge on GPCR signaling cascades at multiple levels, programming persistent changes in GPCR mRNA and protein expression levels, ligand affinity, functional coupling to G proteins (Gq/Gi/Gs), receptor internalization, recycling dynamics, perturbations in downstream signaling cascades and second messenger pathways, as well as biased agonism with preferential recruitment of specific signaling pathways. While

modulation at several of these levels could contribute to an early stress-evoked disruption of GPCR signaling, the preponderance of the literature has reported effects of early stress on GPCR mRNA and protein expression, and on downstream signaling pathways. The underlying mechanisms that mediate the effects of early stress in disrupting GPCR signaling pathways remain poorly understood. The overlap of early stress with developmental time windows wherein ontogenic changes in receptor expression and function take place, raises the intriguing possibility that stressful experience disrupts normal ontogeny and establishes persistent disruption of multiple GPCR pathways. This could arise through an influence of early stress on neurotransmitters and neurohormones, trophic factor signaling pathways, and a modulation of plasticity-associated mechanisms which may further impinge upon GPCR signaling cascades.

Contribution of G-protein coupled receptors to the effects of early stress

In this section of our review we focus on the evidence that addresses the contribution of specific GPCR-linked pathways to the molecular, cellular, functional and behavioral consequences of early stress. Our focus is primarily on the Gq-coupled 5-HT_{2A}R and Gi-coupled 5-HT_{1A}R pathways, and we will only briefly discuss the contributions of Gs-coupled $CRFR_1$ and $CRFR_2$ receptors which have been the focus of other reviews. Early stress causes alterations at multiple levels of organization spanning from molecular changes that include altered gene expression sustained via epigenetic modifications [30,37,106], cellular changes spanning from altered spine density to global dendritic architecture [46], functional and network level alterations measured predominantly via electrophysiological studies [67,73,100], and behavioral changes that are noted across a gamut of anxio-depressive behaviors [35,107–109], fear conditioning responses [35,110–112], hallucinogen-mediated head twitch response (HTR) [67,113,114], sensorimotor gating [38,59,60], social approach-avoidance behavior [35,115,116] and cognitive performance [35,65,73] (Figure 2).

Several studies have attempted to rescue the effects of early stress by modulating GPCR signaling in the early postnatal window, thus attempting to address causal contributions of specific GPCR receptors in programming the persistent consequences of early adversity. Substantial evidence over the past decade demonstrates enhanced Gq-coupled 5-HT_{2A}R function as a common alteration across diverse models of early stress including MS, PNFlx and MIA [38,66,67,117]. Pharmacological blockade of 5-HT_{2A} receptors during the postnatal window overlapping with the MS paradigm prevents the emergence of anxiety behavior,

ameliorates the adult stress-induced dysregulated immediate early gene expression pattern, and normalizes the transcriptional dysregulation of specific G-protein signaling associated pathways [68]. These findings implicate perturbed postnatal 5-HT_{2A}R function and signaling in programming the long-lasting affective dysfunction in MS animals. Further, tactile stimulation during the postnatal window, which represents an important component of maternal care, has been suggested to directed modulate the transcriptional regulation of the stress-responsive glucocorticoid receptor within the hippocampus via the Gq-coupled 5-HT_{2A}R [26]. In this regard, it is interesting to note that altered serotonergic dysfunction in the early postnatal window has itself been implicated in setting up altered anxio-depressive behaviors in adulthood. Pharmacological elevation of serotonin in the postnatal window (PNFlx) leads to enhanced anxiety and despair-like behavior in adulthood [3,46,117]. Intriguingly, postnatal 5-HT_{2A}R blockade overlapping with the administration of fluoxetine prevents the emergence of anxiety and despair-like behavior in adulthood, while conversely 5-HT_{2A}R activation in the early postnatal window mimics the anxiogenic effects of PNFlx and MS [117]. Collectively, these observations indicate that systemic pharmacological activation of the 5-HT_{2A}R in the early postnatal developmental window, is sufficient to program enhanced anxiety-like behavior, whereas blocking of the 5-HT_{2A}R overlapping with either MS or PNFlx can prevent the emergence of adult anxio-depressive behavioral states [68,117]. Interestingly, pharmacological blockade of the 5-HT_{2A}R also reverses a component of the gene dysregulation noted in the MS and PNFlx-associated transcriptome in the PFC [68,117], and unpublished data from our lab indicates that it can also prevent the changes noted in hippocampal neurogenesis in MS animals. In the MIA model enhanced placental serotonin, thought to arise due to heightened inflammation, results in enhanced 5-HT exposure in the fetal forebrain which in turn impacts axon outgrowth of the fetal serotonergic pathways, that could contribute to the anxio-depressive outcomes in MIA pups [118]. MIA animals also exhibit upregulated 5-HT_{2A}R expression and function in the frontal cortex, concomitant with schizo-affective behaviors that emerge in adulthood [38]. It will be interesting to delineate the contribution of Gq-coupled 5-HT_{2A}R which are expressed in multiple limbic forebrain regions, at relatively early embryonic temporal windows, in programming the behavioral consequences of the effects of MIA. Further evidence for a critical role of the 5-HT_{2A}R in contributing to the modulation of anxio-depressive states, following early life perturbations, comes from genetic loss of function studies which indicate that the forebrain Gq-coupled 5-HT_{2A}R is essential to the establishment of trait anxiety behavior [119]. 5-HT_{2A}R knock-outs exhibit a significant reduction in anxiety-like behavior, which is mediated via the cortical 5-HT_{2A}R and can be restored via genetic rescue

experiments that reinstate forebrain 5-HT_{2A}R expression. Collectively, this evidence suggests that enhanced signaling via the Gq-coupled 5-HT_{2A}R could play a critical role in mediating a component of the enhanced anxio-depressive and schizo-affective behaviors noted in adulthood following early stress.

In context of specific Gi-coupled receptors, the contribution of the 5-HT_{1A}R has been extensively studied with regards to the programming of anxio-depressive behavioral states. Systemic pharmacological blockade of 5-HT_{1A}R in the postnatal window is sufficient to induce enhanced anxiety-like behavior in adulthood [107]. It is also interesting to note that adult acute administration of a 5-HT_{1A}R agonist can attenuate the social interaction deficits that are noted to arise in a transgenerational manner in offspring of MSUS animals [37,115]. Genetic loss of function of the 5-HT_{1A}R also results in significant increases in anxiety-like behavior in adulthood [120,121]. This raises the possibility that signaling via the Gi-coupled 5-HT_{1A}R serves to reduce trait anxiety behavior. Genetic rescue strategies indicate that postsynaptic 5-HT_{1A}R in the forebrain during the early postnatal window may play a key role in the programming of trait anxiety behavior [121]. However, it is important to note that additional studies also implicate the presynaptic 5-HT_{1A} autoreceptor in contributing to the establishment of anxiety-like behavioral states [122,123]. Currently, the precise contribution of pre versus postsynaptic 5-HT_{1A}R in programming anxiety-like behavior during the postnatal critical period window is unclear and requires detailed future study. The evidence thus far raises the possibility that a balance between the Gi-coupled 5-HT_{1A}R and the Gq-coupled 5-HT_{2A}R in key forebrain circuitry plays an important role in the establishment of trait anxiety states. This motivates further investigation into whether early stress experience, which could enhance forebrain serotonin levels, may shift the balance in signaling from the high affinity Gi-coupled 5-HT_{1A}R to the low-affinity Gq-coupled 5-HT_{2A}R [124] thus increasing the predisposition for stress-evoked disruption of anxio-depressive behaviors. The notion that a disruption of the balance between Gi-coupled 5-HT_{1A}R and Gq-coupled 5-HT_{2A}R signaling impacts anxiety-like behavior is supported by studies wherein the anxiogenic effects of systemic chronic pharmacological blockade of the 5-HT_{1A}R in postnatal life can be attenuated by a concomitant blockade of the 5-HT_{2A}R [117]. This provides support for the idea that a fine-tuned balance between Gq and Gi-coupled signaling pathways within key forebrain circuits during postnatal developmental windows could contribute to the shaping of trait anxiety. This hypothesis will require detailed and careful experimentation to parse out the contribution of specific GPCRs, the time-window and specific neuronal populations that may shape trait

anxiety behavior, and could be targeted by early stress to program increased anxiety-like behavioral states that persist across the life-span.

Thus far, few studies have addressed the contribution of Gs-protein coupled serotonin receptors, in the developmental influence of 5-HT on anxiety-like behavior and in mediating the effects of early stress. The Gs-coupled 5-HT_7R has been shown to play a role in modulating specific behavioral consequences of PNFlx treatment. Early life blockade of 5-HT_7R, as well as 5-HT_7R knockout mice, do not exhibit the enhanced anxiety and despair-like behavior induced by PNFlx administration [125,126]. Pharmacological blockade of the 5-HT_7R in adulthood could remediate the GS-evoked disruption of sEPSC/sIPSC frequency in the DRN [100]. Conversely, overexpression of 5-HT_7R in early life results in altered development of the PFC, resulting in increased despair like behavior[125]. Given that the 5-HT_7R expresses transiently in the serotonergic positive neurons in the DRN during development, the maturation of circuits involved in mood-related behavior programming, especially the PFC to DRN circuit, is highly influenced by 5-HT_7R-mediated signaling[125]. These findings suggest an important role for 5-HT_7R in mediating the effects of ES, and motivate future experiments to delineate the role of the Gs-coupled 5-HT_7R. While the focus of our review has been to predominantly discuss the influence of specific serotonergic receptors, it is important to draw attention to the large body of literature that addresses the contribution of Gs-coupled $CRFR_1$ and $CRFR_2$ receptors in mediating the effects of early stress. Forebrain $CRFR_1$ is strongly implicated in mediating the effects of early stress on both anxio-depressive behavior states, as well as on cognitive behavior [65]. Forebrain-specific $CRFR_1$ knockout mice do not exhibit the robust effects of unstable maternal care on anxiety-like behavior and memory deficits [65]. Maternal deprivation evokes perturbed excitation-inhibition balance within the lateral habenula (LHb) and enhances LHb excitability, an effect thought to involve a key role for CRF-$CRFR_1$-PKA signaling [127]. While beyond the scope of the present review, it is critical to emphasize that when discussing GPCR signaling pathways there is a central role for Gs-coupled CRF receptors in programming altered emotionality arising from early adversity [47,128,129].

The emerging consensus suggests that a critical balance between forebrain Gq-coupled 5-HT_{2A}R and Gi-coupled 5-HT_{1A}R driven signaling in the postnatal window could contribute to the establishment of trait anxiety. Diverse models of early adversity may serve to disrupt this fine balance, and thus tip the scales towards enhanced Gq-coupled 5-HT_{2A}R drive in key forebrain circuits such as the PFC, thus setting up a neural substrate for increased risk for adult

psychopathology. While systemic pharmacological perturbations and genetic knockout studies provide support for this hypothesis, they fail to delineate the role of specific neuronal populations within discrete forebrain neurocircuits wherein the disruption of a Gq- versus Gi-signaling balance may be critical to programming altered affective behaviors.

When considering the contribution of GPCR pathways to the effects of early stress, it is also of interest to take into account possible roles of orphan GPCRs, which have recently been implicated in the modulation of affective dysfunction. Several studies, including genetic loss of function studies, indicate a role for orphan GPCRs, namely, ADGRB2 (BAI2) [130], GPR3 [131], GPR26 [132], GPR37 [133,134] and GPR158 [135] in the regulation of emotional behavior. The contribution of orphan GPCRs to the modulation of mood behavior has been extensively reviewed recently [136]. However, the influence of early stress on orphan GPCR expression and function, as well as the role of orphan GPCRs in mechanistically programming persistent alterations in emotionality that arise following early stress remains poorly understood.

DREADD-based approaches to address the role of GPCRS in the effects of early stress

Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are genetically engineered human muscarinic receptors that bind synthetic ligands and can facilitate chemogenetic stimulation of Gq (hM3Dq), Gi (hM4Di) or Gs-coupled downstream signaling cascades [137]. DREADD-based chemogenetic approaches using both transgenic and viral strategies, allows for spatio-temporal regulation of select G-protein coupled signaling cascades in discrete neuronal populations [138]. It is only relatively recently that DREADD-mediated chemogenetic strategies have been exploited to address the contribution of Gq and Gi-mediated signaling pathways in mediating the effects of early stress in programming persistent changes in anxio-depressive behavioral states [48,139,140].

Firstly, DREADD based modulation of G-protein coupled signaling cascades during early life can be used to manipulate specific neural circuits for chronic periods of time. This has distinct advantages over optogenetic strategies as it does not involve chronic heating-related damage to the brain [141]. and the ligand can be orally administered reducing the requirement for repeated injections of DREADD ligands which could be stressful in this early life window. In this regard, a few studies have utilized chronic DREADD-mediated

perturbations during postnatal life in a cell type/circuit-specific manner to shed light on the role of GPCR-signaling mediated neuronal activation or inhibition in contributing to the long-lasting behavioral effects of early stress (Figure 3). Chronic inhibition of the PFC from postnatal day 2-14 using the Gi-coupled inhibitory DREADD hM4Di driven via a pan-neuronal hSyn promoter, which would drive expression in both excitatory and inhibitory neurons, mimics the effects of MS [139]. Further, a reduction of PFC activity during the first two postnatal weeks using hM4Di DREADD also results in a premature differentiation of oligodendrocytes, a cellular phenotype reported to be observed with MS [139]. In contrast, the Gq-coupled hM3Dq-based DREADD activation of PFC neurons with a pan-neuronal hSyn promoter in pups subjected to the MS paradigm can prevent the emergence of MS-evoked short-term memory impairments and enhanced despair-like behavior [139]. Work from our lab has recently demonstrated that chronic chemogenetic activation of Gq signaling in CaMKIIα positive forebrain excitatory neurons during the early postnatal window (P2-14) is sufficient to program a persistent increase in anxiety and despair-like behavior, accompanied by sensorimotor gating deficits [140]. Adult animals with a history of hM3Dq-based DREADD activation of excitatory forebrain neurons display an altered excitatory/ inhibitory balance in cortical circuits as revealed through metabolomic and electrophysiological signatures, phenocopying some of the functional changes associated with pre-clinical models of psychiatric disorders [140]. The differences noted across the studies described above could arise as a consequence of the specific population of neurons targeted and the neurocircuit that is being regulated (Figure 3). In the first study, the genetic driver would target hM3Dq to both inhibitory and excitatory neurons in the PFC, with hM3Dq DREADD activation reported to ameliorate the consequences of MS. In contrast, the second study targets hM3Dq expression to CamKIIα-positive excitatory neurons across the entire forebrain, and results in behavioral phenotypes that mimic the effects of early stress. These studies highlight the critical importance of parcellating the role of specific neuronal subpopulations within key limbic brain regions, and motivates further studies of this nature to gain a deeper mechanistic insight into the role of G-protein coupled signaling cascades in programming mood-related behavior. These studies also give rise to several key open questions: (1) What are the differences in role of GPCR signaling pathways across circuits (mesoscale) and cell types (microscale) in mediating persistent behavioral changes during the perinatal window? (2) What are the downstream molecular, cellular, and network events that lead to dysfunction in specific neural circuits in adulthood, contributing to aberrant mood behavior? (3) Is it possible to have specific early or late interventions (environmental/ pharmacological) that remediate or reverse the long-lasting

effects of early stress? The current tools designed to measure and manipulate neuronal activity are designed keeping adult rodent models in mind, thus can be challenging to use in rodent pup studies. These questions call for both a creative use of existing tools and building newer tools that can be equally useful in pups as well.

Secondly, DREADD-mediated activation of GPCR coupled pathways in adult animals with a history of early stress can be used to address approaches to ameliorate the effects of early trauma, thus uncovering approaches to normalize function in dysregulated neural circuits and identify possible therapeutic interventions. In this regard, acutely activating the Gq-coupled hM3Dq signaling in the mPFC of adult animals with a history of PNFlx administration attenuated the pro-depressive phenotypes associated with PNFlx [48]. Conversely, Gi-coupled hM4Di-mediated inhibition in adult animals with PNFlx history further aggravated the adverse behavioral phenotypes evoked by early fluoxetine administration [48] (Figure 3). In summary, these chemogenetic studies provide key insights into the contribution of GPCR signaling pathways in specific forebrain neurocircuits that may play a central role in the modulation of mood-related behavior in critical postnatal developmental windows, and that could serve as key targets for early stress.

Concluding remarks

The current literature in the field leads to an intriguing hypothesis that there may be a balance between the Gq-coupled and Gi-coupled pathways, especially within the serotonergic system between the Gq-coupled 5-HT_{2A}R and Gi-coupled 5-HT_{1A}R, within forebrain circuits that drive specific behavioral consequences of early stress. While there is compelling evidence that suggests that this hypothesis merits further investigation, there is still a substantial dearth of information on whether specific GPCRs downstream of monoamines are involved in the disruption caused by early adversity, or whether multiple distinct receptors spanning the spectrum from glutamatergic, GABAergic and neuropeptide receptors may contribute to mediating the effects of early stress. Further, it is important to parcellate the role of GPCR pathways in distinct neuronal subpopulations within discrete neurocircuits, that may serve as important targets to program the consequences of early stress. In conclusion, the goal of our review has been to summarise the important contributions of specific GPCRs in the behavioral changes evoked by early stress, and to motivate future experiments that directly

address the mechanistic role of specific Gq-, Gi- and Gs-coupled receptors in stress-mediated sequelae.

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Figure Legends

Figure 1. The figure summarizes the influence of various preclinical models of early adversity on mood and cognition-related behaviors in adulthood, as well as highlights the specific GPCRs that are reported to be dysregulated in these models of early stress. Shown in the panels are diverse models of early adversity, namely A. Low Maternal Care as denoted by low Licking Grooming Arched back Nursing (LGABN) behavior; B. Maternal Separation; C. Maternal Separation + Unpredictable stress to the dam during the period of separation from the pups; D. Limited Bedding and Nesting which fragments maternal care; E. Postnatal administration of Selective Serotonin Reuptake Inhibitor (SSRI) Fluoxetine; F. Maternal Immune Activation; and G. Gestational Stress. These diverse models impinge upon a wide array of anxiety [35,48,84,107–109,112,120,125,142–149] and despair-like behaviors [35,48,61,108,109,125,143,146,148,150–152] and also impact cognition [22,35,65,73,149,152–154], fear conditioning [35,110–112,152,155], social interaction [35,61,115,116,156–158], sensorimotor gating [38,59,60,91,93] and hallucinogen evoked 5-HT_{2A}R mediated head twitch response (HTR) [67,113,114]. Amongst the G-Protein Coupled Receptors (GPCRs) reported to be dysregulated in these models of early adversity are the Gq-, Gi- and Gs-coupled receptors involved in the signaling downstream of monoamines like serotonin (5-HT_{1A}R, 5-HT_{2A}R, 5-HT_{7}R) [26,38,49,67,73–78,83,84,100,107,109,110,113–115,117,120,125,146,147,149,150,159,160], dopamine (D_1R, D_2R, D_3R, D_4R, D_5R) [31,101,161–164] and norepinephrine (α_2AR, β_3AR) [117,155] glutamate ($mGluR_2$, $mGluR_4$) [38,59,60,85,91,93,113,117,151,161,165–168] endocannabinoids (CB_1R) [21,63,162,169], and Acetylcholine (M_1) [62], corticotropin-releasing factor ($CRFR_1$, $CRFR_2$) [22,37,64,84,112,142,145,154,158,170–175].

Figure 2. In this figure, we summarise the specific GPCRs that have been implicated in mediating the effects of early stress at multiple levels of organizations modulating the molecular, cellular, functional-network, and behavioral consequences. Distinct groups of Gq-, Gi- and Gs-coupled receptors have been suggested to contribute to specific effects of early stress at the molecular level [59,66,68,87,97,102,176], modulating the epigenome, transcriptome and translatome, at the cellular level [175,177,178] influencing neuronal architecture, spine density and dendritic arborisation, at the functional and network level [62,67,72,73,127,150,168] by impacting the electroencephalogram (EEG) pattern, excitation-inhibition balance and electrophysiological responses, and at the behavioral level [38,39,60–

62,65,66,68,94,117,119,125,179], impacting anxiety and despair-like behavior, cognition, attention, reward and social behaviors.

Figure 3. DREADD-based chemogenetic strategies to address the contribution of specific G-protein signaling pathways in mediating or mimicking the behavioral effects of early stress. Shown in panel A is a summary of the behavioral effects of hM3Dq-DREADD activation of CaMKIIα forebrain excitatory neurons in the postnatal window (P2-14), which results in enhanced anxiety and despair-like behaviors, and reduced sensorimotor gating responses revealed via prepulse inhibition deficits noted in adulthood, phenocopying the behavioral changes evoke by early stress. Panel B shows that hM4Di DREADD-mediated inhibition, driven via a hSyn promoter in excitatory and inhibitory neurons of the PFC in postnatal life, promotes increased anxiety and despair-like behaviors in adulthood, accompanied by a reduction in object recognition memory mimicking the effects of early stress. Whereas, hM3Dq DREADD-mediated activation, virally expressed downstream of a hSyn promoter in excitatory and inhibitory neurons of the PFC in postnatal life, overlapping with the maternal separation (MS) paradigm results in the attenuation of enhanced despair-like behavior and reduced object recognition memory associated with MS. Panel C depicts the hM4Di inhibition or hM3Dq DREADD-activation of CaMKIIα-positive excitatory neurons or Serotonin Transporter (SERT+) expressing neurons in the PFC in animals subjected to postnatal fluoxetine (PNFlx) administration. hM4Di DREADD inhibition of CaMKIIα-positive excitatory neurons or SERT+-positive neurons in the PFC in the background of PNFlx treatment exacerbates the anxiogenic and despair-like behavioral effects of PNFlx. hM3Dq DREADD activation of CaMKIIα-positive excitatory neurons or SERT+-positive neurons in the PFC in the background of PNFlx treatment attenuates the anxiogenic and despair-like behavioral effects of PNFlx.

Figure 1

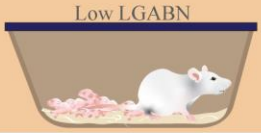
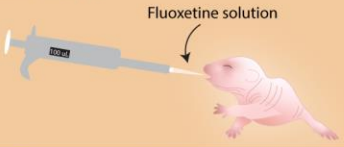
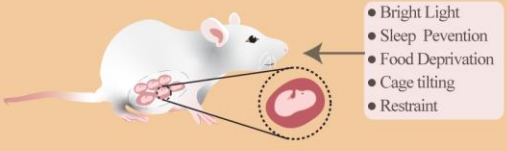
Models of Early Adversity	Behaviours perturbed in Adulthood	GPCRs regulated in models of early adversity		
		Gq	Gi	Gs
A. Low Maternal Care 	Anxiety Despair Cognition Social Interaction Fear Conditioning	5-HT_{2A} $mGluR_1$	α_2AR	CRFR1
B. Maternal Separation 	Anxiety Despair Cognition Social Interaction Fear Conditioning Head Twitch Response	5-HT_{2A} M_1R	5-HT_{1A} CB_1R $mGluR_4$ $mGluR_7$ $GABA_B$ D_2R D_3R D_4R	5-HT_7 D_1R D_5R β_3AR CRFR1 CRFR2
C. Maternal Separation + Unpredictable Stress 	Anxiety Despair Social Interaction Cognition Social Interaction		5-HT_{1A}	5-HT_7 CRFR2
D. Limited Bedding and Nesting 	Anxiety Despair Cognition Social interaction Fear Conditioning		5-HT_{1A} D_4R	CRFR1
E. Postnatal SSRIs 	Anxiety Despair Cognition Social Interaction Fear Conditioning Head Twitch Response	5-HT_{2A} $mGluR_1$ $mGluR_5$	5-HT_{1A} 5-HT_{1B} $mGluR_2$	5-HT_7
F. Maternal Immune Activation 	Anxiety Despair Cognition Fear conditioning Sensorimotor Gating Head Twitch Response	5-HT_{2A} $mGluR_1$ $mGluR_5$	CB_1R $mGluR_2$ $mGluR_3$ $mGluR_4$ D_2R D_3R	CRFR1 CRFR2
G. Gestational Stress 	Anxiety Despair Cognition Social Interaction Fear Conditioning Sensorimotor Gating Head Twitch Response	5-HT_{2A} 5-HT_{2C} $mGluR_1$ $mGluR_5$	5-HT_{1A} $mGluR_2$ $mGluR_3$ D_2R	5-HT_7 CRFR1 CRFR2

Figure 2

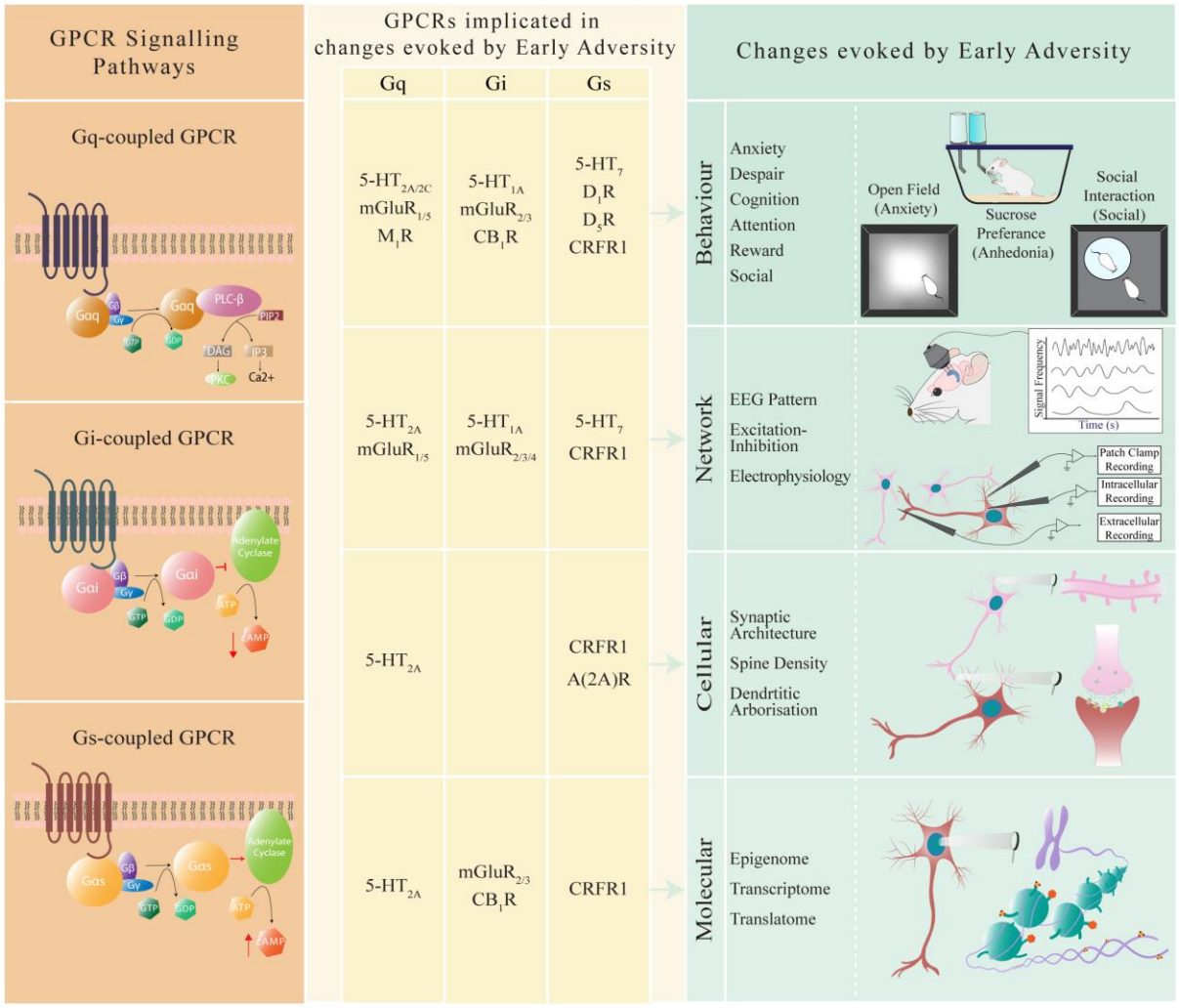
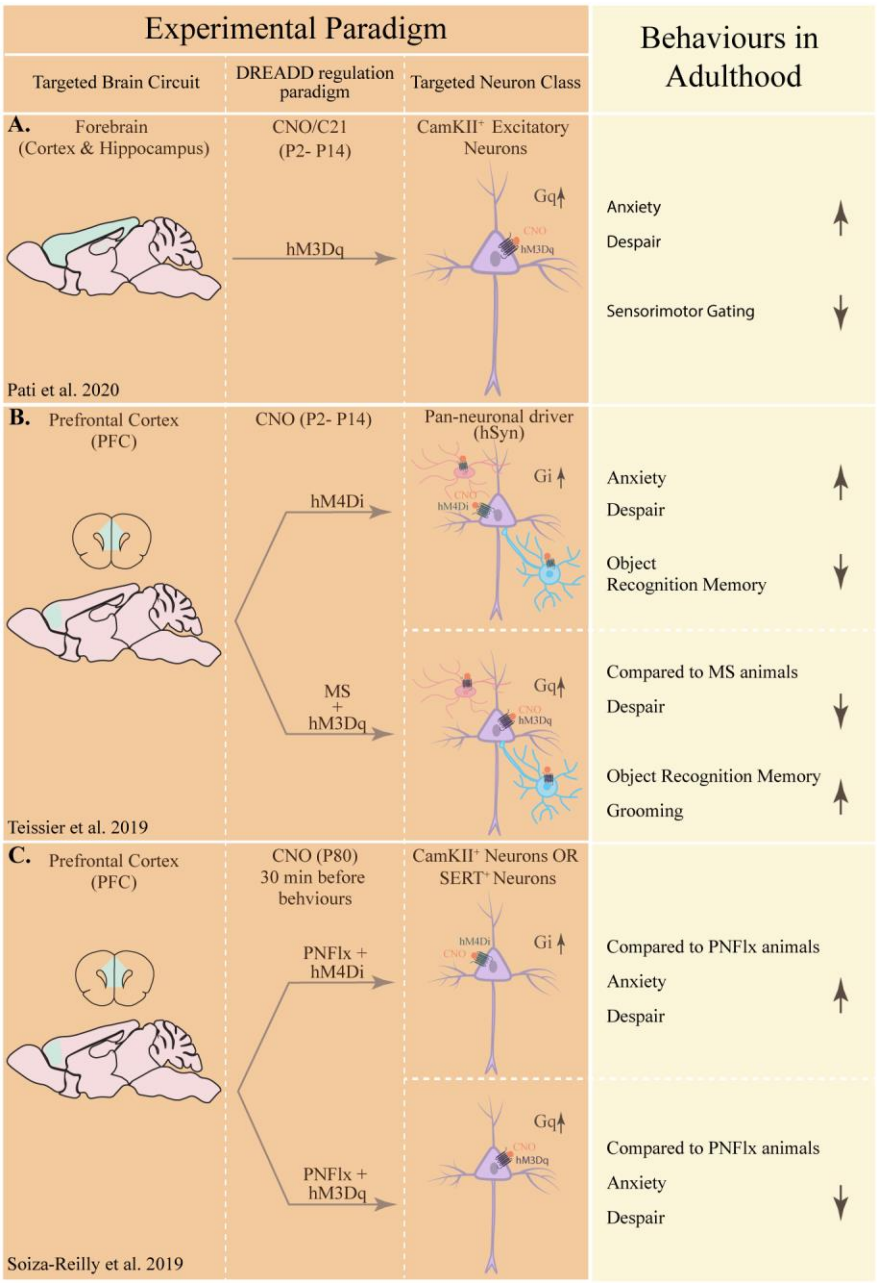

GPCR Signalling Pathways
Gq-coupled GPCR
Gi-coupled GPCR
Gs-coupled GPCR
GPCRs implicated in changes evoked by Early Adversity
Gq
Gi
Gs
5-HT2A/2C mGluR1/5 M1R
5-HT1A mGluR2/3 CB1R
5-HT7 D1R D5R CRFR1
5-HT2A mGluR1/5
5-HT1A mGluR2/3/4
5-HT7 CRFR1
5-HT2A
CRFR1 A(2A)R
5-HT2A
mGluR2/3 CB1R
CRFR1
Changes evoked by Early Adversity
Behaviour
Anxiety
Despair
Cognition
Attention
Reward
Social
Open Field (Anxiety)
Sucrose Preferance (Anhedonia)
Social Interaction (Social)
Network
EEG Pattern
Excitation-Inhibition
Electrophysiology
Signal Frequency
Time (s)
Patch Clamp Recording
Intracellular Recording
Extracellular Recording
Cellular
Synaptic Architecture
Spine Density
Dendrtitic Arborisation
Molecular
Epigenome
Transcriptome
Translatome

Figure 3


Experimental Paradigm
Behaviours in Adulthood
Targeted Brain Circuit
DREADD regulation paradigm
Targeted Neuron Class
A. Forebrain (Cortex & Hippocampus)
CNO/C21 (P2- P14)
CamKII+ Excitatory Neurons
hM3Dq
Gq
Anxiety
Despair
Sensorimotor Gating
Pati et al. 2020
B. Prefrontal Cortex (PFC)
CNO (P2- P14)
Pan-neuronal driver (hSyn)
hM4Di
Gi
Anxiety
Despair
Object Recognition Memory
MS + hM3Dq
Gq
Compared to MS animals
Despair
Object Recognition Memory
Grooming
Teissier et al. 2019
C. Prefrontal Cortex (PFC)
CNO (P80) 30 min before behviours
CamKII+ Neurons OR SERT+ Neurons
PNFlx + hM4Di
Gi
Compared to PNFlx animals
Anxiety
Despair
PNFlx + hM3Dq
Gq
Compared to PNFlx animals
Anxiety
Despair
Soiza-Reilly et al. 2019