Steroid hormone-mediated regulation of sexual and aggressive behaviour by non-genomic signalling

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Figure 1

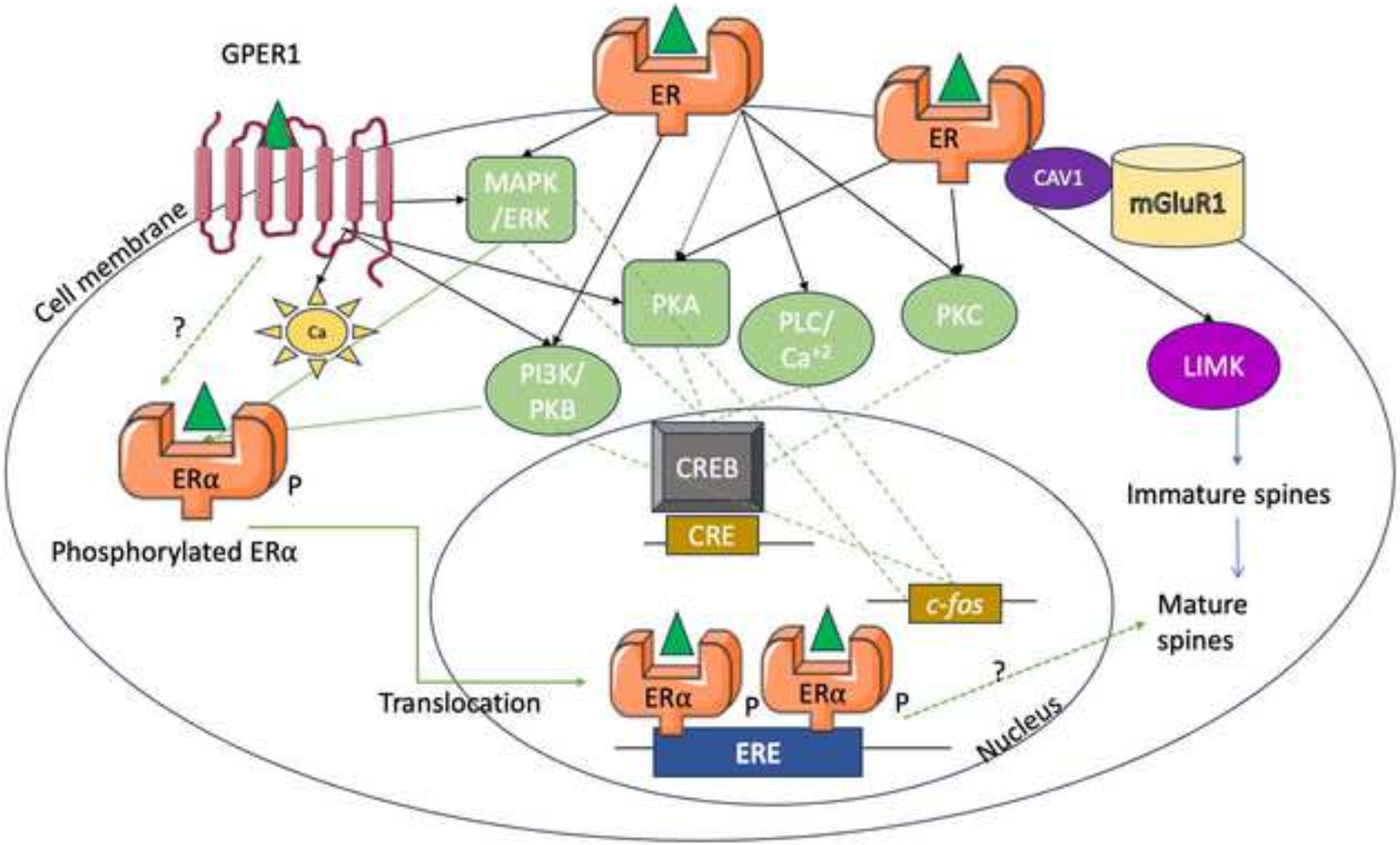


Figure 2

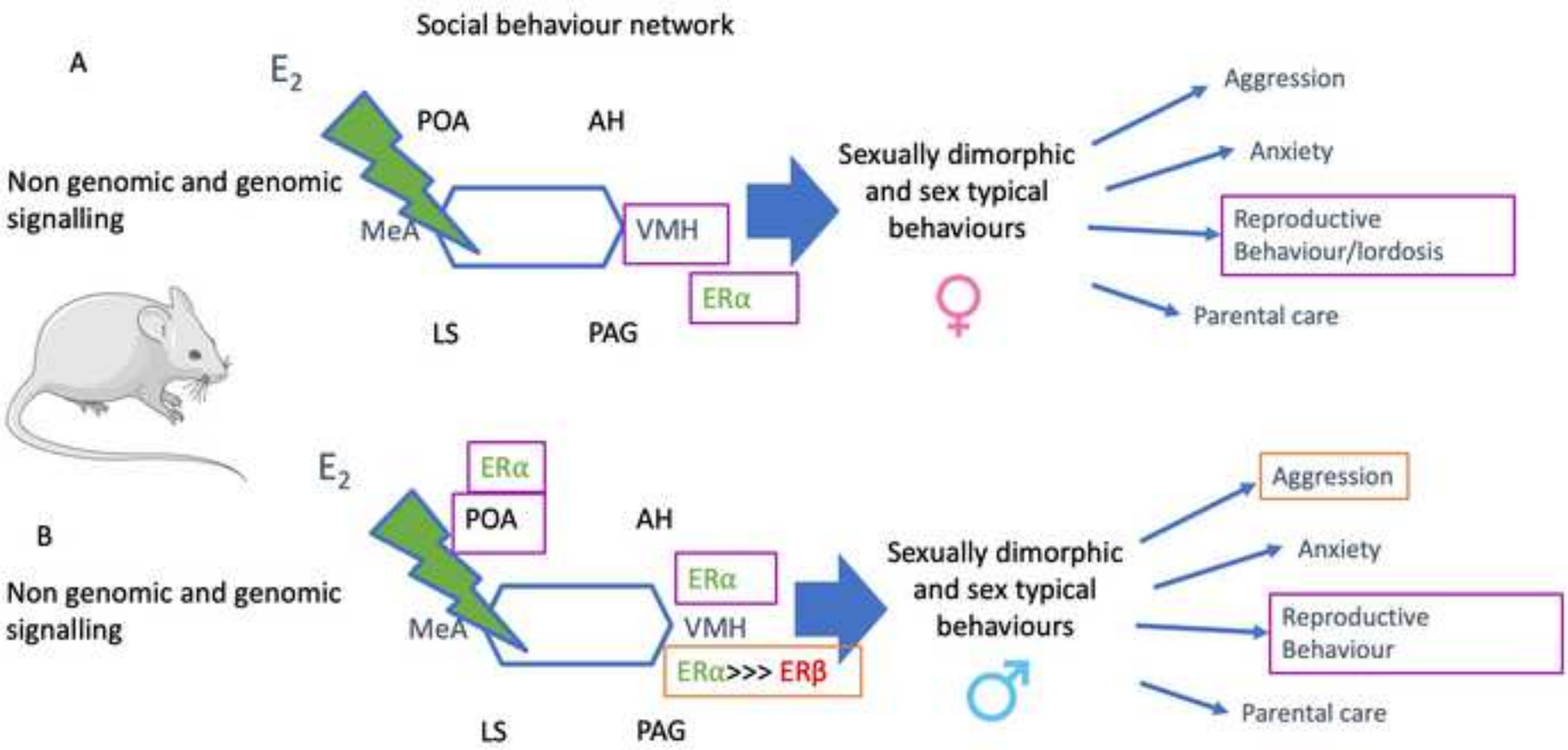
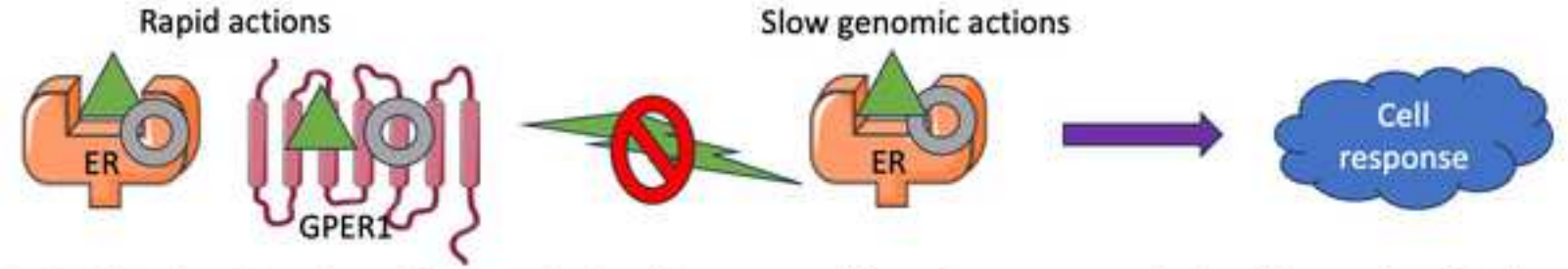
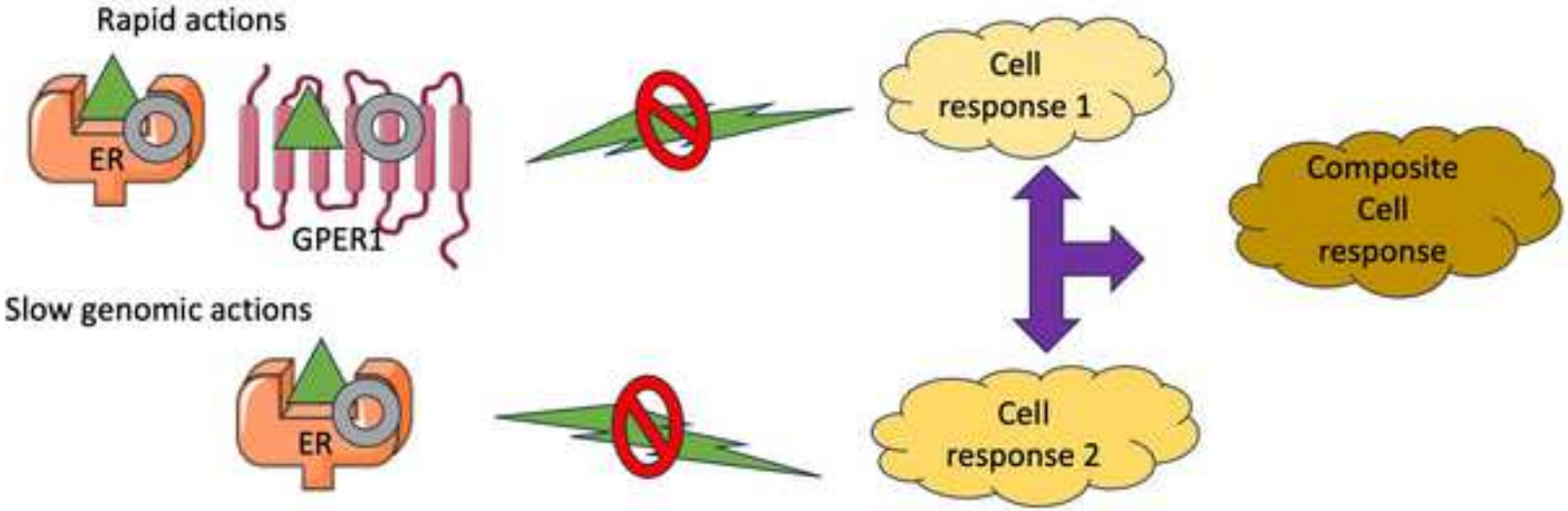


Figure 3



A: Rapid actions in series with genomic signalling can amplify or dampen genomic signalling-mediated cell responses



B: Rapid actions acting in parallel with slow genomic actions can generate an additive composite cell response

Steroid hormone-mediated regulation of sexual and aggressive behaviour by non-genomic signalling.

Authors
DeAsia Davis[1], Janine Dovey[1], Shoko Sagoshi[2,3], Kongkidakorn Thaweepanyaporn[1], Sonoko Ogawa[3] and Nandini Vasudevan[1].

1: School of Biological Sciences, University of Reading, United Kingdom.
2: Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, United States.
3: Laboratory of Behavioural Neuroendocrinology, University of Tsukuba, Tsukuba, Japan.

Corresponding author
Dr Nandini Vasudevan
School of Biological Sciences
University of Reading, WhiteKnights Campus, Reading RG13LL
Email: n.vasudevan@reading.ac.uk

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Abstract (250 words)

Sex and aggression are well studied examples of social behaviours that are common to most animals and are mediated by an evolutionary conserved group of interconnected nuclei in the brain called the social behaviour network. Though glucocorticoids and in particular estrogen regulate these social behaviours, their effects in the brain are generally thought to be mediated by genomic signalling, a slow transcriptional regulation mediated by nuclear hormone receptors. In the last decade or so, there has been renewed interest in understanding the physiological significance of rapid, non-genomic signalling mediated by steroids. Though the identity of the membrane hormone receptors that mediate this signalling is not clearly understood and appears to be different in different cell types, such signalling contributes to physiologically relevant behaviours such as sex and aggression. In this short review, we summarise the evidence for this phenomenon in the rodent, by focusing on estrogen and to some extent, glucocorticoid signalling. The use of these signals, in relation to genomic signalling is manifold and ranges from potentiation of transcription to the possible transduction of environmental signals.

Highlights

- Non-genomic signalling contributes to sexually dimorphic social behaviours.
- Estrogen receptor isoforms and variants may contribute differentially both in magnitude and in mode of signalling to social behaviours.
- Crosstalk between nuclear hormone receptors could result in differential outputs by cells to the same ligand.

Keywords: social behaviour, aggression, sex behaviour, rapid signalling

Introduction

Steroid hormones are lipophilic small molecules derived from cholesterol that control reproductive physiology and parallel social behaviours. Social behaviours such as sex and aggression are critically dependent on estrogen signalling in the brain (1-4). The predominant endogenous ligand, 17β-oestradiol (E2), derived from testosterone by the action of the enzyme aromatase, transduces these effects by binding specific nuclear hormone receptors, estrogen receptor (ER)α and ERβ that act as ligand dependent transcription factors. These receptors are part of a large nuclear hormone receptor family with conserved domain structures with modules for transcriptional activation, ligand and coactivator binding and dimerization (5-7). Genomic signalling occurs when E2 binds ERα and ERβ within cells and the liganded receptor binds to DNA sequences and modulates gene expression in the nucleus. This could be either via direct binding to specific EREs (estrogen response elements) or by the binding of the ER to DNA-bound transcription factors such as AP-1 which may then bind to non-ERE-containing enhancer elements (8-10). The first evidence of non-genomic signalling was reported by Szego and Davis in 1967 as a rapid increase in uterine cAMP of ovariectomized female mice within 15 mins of E2 stimulation (11), presumably by a receptor that is at the plasma membrane. Though mostly intracellular, a small fraction of ERα and ERβ, are also found in the plasma membrane and can rapidly activate signalling cascades similar to G-protein coupled receptors (GPCRs) (12,13). In addition, a former orphan GPCR called GPR30/GPER1 is another putative membrane ER (mER) that can bind E2 and rapidly activate extracellular regulated kinase (ERK), calcium and protein kinase A (PKA) cascades (14-17). Like the ER, the glucocorticoid receptor (GR) is a nuclear hormone receptor that binds corticosteroids but can also be localized to the plasma membrane (18,19). In many cases, signaling from the plasma membrane has been shown by the use of a membrane-limited steroid-bovine serum albumin (BSA) conjugate that cannot enter cells and/or outputs that can be regulated within short time frames of 40 minutes or less (12).

Though transcriptional signalling was originally studied in more detail, in recent decades, membrane-initiated signalling has been investigated in several hormone-responsive tissues. In this review, we focus on nongenomic signalling by estrogens and to a lesser extent, glucocorticoids in areas of the brain that drive sex-specific sexual and aggressive behaviour in the rodent. Though many studies have shown membrane steroid receptors at the membrane, the identity, the mode of anchoring of the mER or mGR to the plasma membrane as well as their association with Gα or Gβγ subunits remain controversial (extensively reviewed in (20). The intracellular ERα, ERβ and GR has been shown to be localized to the cell membrane, in association with caveolin proteins (21) (22) (23,24) in several cell types (Figure 1). Though the data demonstrating that the GPER1 is a membrane protein due to its

GPCR classification is not surprising, GPER1 subcellular localization and trafficking from the membrane is unclear, with various studies localizing it on the plasma membrane (17,25), endoplasmic reticulum (14) (26) and perinuclear space (27). The subcellular localization of the GPER1 and localisation of ERs in different areas of the brain relevant to social behaviours has been discussed extensively in (28) (29).

Since adrenal steroids link stress to reproduction (30), it is worth briefly mentioning their non-genomic effects on social behavior. Membrane glucocorticoid receptors (mGR) were shown in the plasma membrane fractions of rat brain synapses via [3H]-corticosterone binding assays with higher levels in the hypothalamus than the cortex or hippocampus (31). Immunocytochemistry revealed the presence of GR at the plasma membrane of hypothalamic neurons in the rat (32). Rapid actions of glucocorticoids via a putative mGR in the hypothalamus are instrumental in suppressing glutamatergic input onto the CRH neuron by stimulating endocannabinoid release from the postsynaptic CRH neuron via a Gαs-dependent mechanism. In addition, rapid release of nitric oxide by the putative mGR stimulated the presynaptic GABAergic neurons. Hence, the combination of decreased excitatory input and increased inhibitory input onto the CRH neurons by membrane-initiated glucocorticoid signalling via a mGR results in rapid negative feedback at the level of the hypothalamus (33,34) to maintain homeostatic control the hypothalamo-pituitary-adrenal (HPA) axis.

2. SEXUAL DIMORPHISM IN THE SOCIAL BEHAVIOUR NETWORK

The social behavior network (SBN) was first introduced by Newman 1999, who identified a 'core' functional and connective construct in mammalian brains that is key to the regulation of common sexually dimorphic reproductive and aggressive behaviors (35) (36) . The SBN consists of six bidirectional, reciprocally connected nodes that are listed here a) the extended medial amygdala and the bed nucleus of stria terminalis (meA/BNST) b) lateral septum (LS), c) medial preoptic area (mPOA), d) the anterior hypothalamus (AH) e) the ventromedial hypothalamus (VMH) and the midbrain sections that include the periaqueductal gray (PAG) (35) (36) (Figure 2). The role of each node was first inferred by the presence of lesions that resulted in behavioral deficits during social interactions (35,37,38) (36). Comparative neuroendocrine studies demonstrate these nuclei are a) hormonally sensitive and therefore possess hormone receptors b) present in all vertebrate species c) often sexually dimorphic in size. For example, in the rodent the male mPOA and the BNST are larger than the female mPOA due to an increased number of neurons (39-41) while the male meA is larger than the female meA due to the increased soma size of the neurons (42).

The differential localisation and signalling of androgen and estrogen receptors in the SBN (summarized in (28)) plays an important role in both the organizational effects of steroid hormones during critical periods of development and the activational effect of these hormones,

in order to drive sexually dimorphic phenotypes such as nuclei size and/or reproductive and agonistic behaviours in adulthood. Typically, studies utilize either deletions or reductions of specific receptors or pharmacological approaches to delineate the contribution of receptors to these phenotypes (20). Male mice that are knockouts for ERα (αERKO) showed a demasculinized BNST i.e., smaller volume and lower number of neurons compared to wildtype (WT) males but there was no difference seen when comparing αERKO females to αWT females (43). In the male mPOA, some of this phenotype could be presumably via non-genomic signalling since an ERα-mutant incapable of membrane-initiated signalling also showed a demasculinized phenotype that was intermediate between males and females. This phenotype was characterized by an increased number of kisspeptin neurons but decreased number of calbindin-positive neurons in males, that was consistent with the intermediate phenotype obtained when neonatal estradiol injections were given to females (44), suggesting that ERα signalling is critical in the organization of the male BNST and mPOA. However, ERβ activation may also play a role in the female BNSTp since both ERα and ERβ agonists administered during the perinatal period to females increased the volume (45); the longer timeframes used here does not permit identification of the mode of signalling. Sexually dimorphic function of these SBN brain regions appears to be dependent on the relative levels of these receptors for e.g., phospho-CREB in the mPOA could be increased by either ERα or ERβ since pCREB increases were normal upon EB administration in either the ovariectomized αERKO or βERKO female mice. However, in the VMH where ERα is predominant, there was no pCREB increase in the αERKO female though the βERKO female was unaffected when compared to βWT mice (46,47). Hence, sexual dimorphism in the SBN may depend on differential contributions of ER isoforms that may also further be characterized by the mode of signalling. As far as we are aware, the effect of GPER1 activation on the volume of sexual dimorphic SBN nuclei is not known.

3. NON-GENOMIC SIGNALLING CONTRIBUTES TO LORDOSIS BEHAVIOUR IN THE FEMALE

In females, the VMH is considered essential for the expression of female reproductive behaviour or lordosis behavior. Pfaff and Keiner (1973) found that the VMH nuclei contained estrogen concentrating cells (48) and that estrogen injected directly into the VMH of female rats facilitated lordosis behavior (49). In female mice, the decreased level of ERα in the VMH using silencing RNA greatly decreased lordosis (50), suggesting that E2 acting via ERα in the VMH is necessary for lordosis to occur. A number of genes such as oxytocin, the oxytocin receptor and the enkephalins are transcriptionally regulated by E2 to drive lordosis in the rodent (51-55). In estradiol benzoate (EB)-primed ovariectomized (OVX) female rats that were infused i.c.v with PPT and DPN (ERα and ERβ agonist respectively), there was an increase

in lordosis behavior compared with DMSO-infused controls at 30 mins, 120 mins and 240 mins. Furthermore, injections of selective antagonists of ERα (MPP) and ERβ (PHTPP) significantly decreased lordosis behavior that was induced by subsequent E2 administration (56). These data suggest that activation of both ERα and ERβ are required for rapid facilitation for lordosis behavior, implying that non-genomic signalling by these receptors also contributes to lordosis. Consistent with this inference, PKA, ERK and *c-src* antagonists infused into the VMH 30 mins before testing inhibited EB-primed lordosis behaviour in female rats (57) while application of ligands that bind G-protein coupled receptors (GPCR) such as oxytocin, vasopressin or acetylcholine increased frequency of action potentials in the VMH (58,59). In ovariectomized mice, G-1, the agonist for the GPER1 increased lordosis to the same extent as EB and EB-induced lordosis was partially blocked by G-15, the antagonist to the GPER1, suggesting that GPER1, a mER is sufficient for lordosis (60).

How does nongenomic signalling contribute to this behaviour? In the arcuate nucleus (ARH), a microcircuit is responsible for the activation of lordosis behaviour in the mPOA (61). E2 administration to the ARH rapidly activated PKCθ (62), leading to the release of neuropeptide Y and the subsequent activation of β-endorphin afferents into the mPOA (63). In the mPOA, this led to the internalization of the μ-opioid receptor (MOR) after 30 minutes of E2-BSA administration in the ARH; this activates and internalizes MOR while transiently inhibiting lordosis until progesterone signalling relieves this inhibition. This non-genomic signalling in the ARH was borne out by the fact that administration of a PKC inhibitor (BIS) in the ARH 30 mins prior to E2-BSA administration reduced MOR internalization in the mPOA (62). In addition, in the ARH, 22% of mGluR1a positive neurons were ERα-positive suggesting that this interaction of ERα with mGluR1a mediated by caveolin at the plasma membrane is important in initiating the rapid non-genomic signalling cascade. Indeed, CAV1 siRNA or an antagonist to mGluR1a in the ARH not only reduced MOR internalization in the mPOA but also reduced lordosis (64,65). Interestingly, both tamoxifen and ICI 182,780, similar to G-1, reduced μ-opioid receptor (MOR) internalization in the mPOA and facilitated female reproductive behavior in the rat. This effect was blocked by the GPER1 antagonist, G-15, suggesting that tamoxifen and ICI 182, 780 drive this behaviour in a GPER1-dependent manner (66).

One molecular pathway initiated by non-genomic signalling in the ARH is the increase in spine density, particularly of mushroom shaped spines by E2 treatment over 48 hours in female rats since this was correlated with increased lordosis. Consistent with this, cytochalsin B, an inhibitor that depolymerises actin, inhibited lordosis when injected into the ARH. Though the 48 hour time-scale is indicative of genomic signalling, the first phase of this process is the generation of filamentous spines, a process promoted by the activation of PKC and LIMK by

the ERα-mGluR1a complex at the cell membrane. LIMK is a kinase that phosphorylates and inactivates the actin depolymerization factor, cofilin and allows for branching and stabilization of spines. Supporting this, administration of a mGluR1 antagonist into the ARH decreased the levels of phosphorylated cofilin within an hour of infusion (61). However, the appearance and increase of mature, mushroom shaped spines in ARH and VMH neurons of the female rat requires longer time scales – approximately 48 hours after E2 administration, in parallel with the E2 induction of lordosis – and requires genomic transcription, including an increase in CREB-mediated transcription (61,67). Therefore, though rapid non-genomic signalling in the ARH is important for initiating the sequence of events, genomic signalling via ERα-mediated transcriptional upregulation of the progesterone receptor in the VMH is needed for the culmination of this process i.e., for lordosis (68,69) (Figure 2A). Indeed, a female mouse that has a mutation in the ERα DNA binding domain and that cannot bind the ERE was deficient in lordosis behaviour (70) though it retained some elements of receptivity towards males.

Furthermore, a two-pulse priming paradigm in female rats where membrane-impermeant E2-BSA injected into the VMH prior to administration of EB and progesterone could drive lordosis to the same extent as traditional longer-priming EB regimens, suggesting that membrane-initiated rapid non-genomic signalling potentiated lordosis behaviour (71), in a coupled or integrated signalling pathway. This coupled signalling paradigm also potentiated transcription of the progesterone receptor gene (72). Our laboratory has demonstrated that brief addition of E2-BSA for 20 minutes in a neuroblastoma cell line increased transcription from a consensus ERE, by increasing PI3K and ERK activation which in turn phosphorylate the ERα (73) to potentiate transcription (Figure 1). Similarly, in a SK-N-SH neuroblastoma cell line, E2 treatment rapidly increased PKA activation and CREB phosphorylation, both of which are needed for the upregulation of the neurotensin gene (Figure 1); upregulation did not occur when the PKA pathway was blocked or was investigated in the preoptic area of PKA-deficient mice (74,75). It is clear from these studies that ERα plays a dominant role in increasing lordosis in female rodents by initiating both genomic and non-genomic events; hence, αERKO females showed no lordosis while βERKO female mice showed normal levels of lordosis (76,77).

Though spinogenesis in the ARH and VMH is a neuromorphological correlate linked to lordosis and is also due to coupled signalling (20,61,78), changes in spine morphology and density with concomitant facilitation of cognition have been shown due to rapid signalling alone, in the female hippocampus. In the dorsal hippocampus, activation of GPER1/GPR30 with G-1 rapidly increased social recognition, object recognition and dendritic spine density within 40 minutes in female ovariectomized mice (79-82),with the time frame suggesting that this is due to rapid, non-genomic action. In contrast to this, the ERβ agonist, DPN, impaired social recognition and decreased spine density in the stratum lacunosum-moleculare layer of the

hippocampus (83) but increased cognitive performance in the novel object placement test at higher doses. However, in the medial amygdala, infusion of pharmacological agonists to all three ERs, i.e. ERα, ERβ and GPER1 increased social recognition within 40 minutes in ovariectomized female mice (84) demonstrating that all ERs are capable of rapidly facilitating memory in a region-dependent manner. Though the mechanisms that underlie the rapid increase in spine density remain unclear, both protein synthesis and actin polymerization inhibitors, but not DNA transcription inhibitors, decreased social recognition when infused into the dorsal hippocampus prior to testing (85). In addition, post-training infusion of a JNK inhibitor but not an ERK inhibitor into the dorsal hippocampus of female ovariectomized mice abolished GPER-1 mediated object recognition memory, suggesting that GPER1 uses a different MAPK pathway for memory retrieval as opposed to memory consolidation (86). Moreover, in some cases, this could solely be due to an increase in protein translation for e.g. in NG108 neuroblastoma cells, E2 rapidly increased PSD-95 protein via the increased phosphorylation of elongation factor 4E-BP1 in a PKB-dependent fashion without changing the expression level of PSD-95 mRNA itself (87). Similarly, corticosterone application to hippocampal slices from male rats increased both spine density and mushroom shaped spines in an hour, similar to spine density increases that were seen in the CA1 1 hour after exposure of the whole animal to acute stressors. Selective blockers showed that this increase in spine density was via the Rho-Rock pathway that inhibits cofilin via phosphorylation by LIMK (88,89).

4.1. NON-GENOMIC SIGNALLING CONTRIBUTES TO SEX BEHAVIOUR IN THE MALE

Cross and Roselli were the first to demonstrate that E2 can exert rapid actions on male reproductive behavior. Within 35 minutes of E2 stimulation, castrated but sexually experienced male rats showed an increase in chemoinvestigation and mounting and a reduction in the latency to mount (90). This suggests that estrogen non-genomically increases both male sexual motivation and appetitive behaviours towards receptive females. Though administration of estradiol benzoate (EB) in adulthood resulted in reduced mounting frequency in aromatase knockout males, these males still showed sexual interest by licking or sniffing genital area of receptive females, suggesting that these behaviours are not dependent on the level of the ligand but more likely due to signalling by the receptor. Like females, αERKO males showed no sex behaviour while βERKO males showed no differences from wildtype mice in the ejaculation frequency or latency of mounting (91,92). These finding suggest that ERα but not ERβ is critical for the display of male-typical sexual behavior in mice. Male sexual behavior requires ERα expression in the mPOA and VMH since knockdown of ERα in these regions, but not in the meA, using siRNA decreased this behaviour in mice (93). siRNA to ERα in the mPOA but not in the meA of male rats across also decreased sex behaviour (94),

suggesting that estrogen signalling mediated by ERα in the mPOA is critical across species. Furthermore, a DNA binding mutant of the ERα (knock-in mouse into a αERKO background) could not show sex behaviour, suggesting that transcription of ERE-containing genes is important in male sex behaviour, similar to females (95) (Figure 2B).

These data contrast with the quail, where ERβ may play an important role in male sexual behaviour. After inhibition of aromatase in the brain with vorozole, male Japanese quail showed significantly reduced rhythmic cloacal sphincter movements (RCSM) (96,97), an appetitive sexual behaviour in birds; this could be rescued 30 mins after E2-BSA administration. Moreover, administration of DPN (ERβ agonist) or the mGluR1a inhibitor prevented this reduction in the frequency of RCSM, that occurred after aromatase inhibitor vorozole injections, within 30 minutes, while there were no changes in the reduction of RCSM when the ERα agonist PPT was injected. These data suggest that sexual motivation in quail may largely depend on nongenomic signalling by ERβ by locally synthesized estrogens (98) via a mGluR1-mediated membrane-initiated mechanism. This is interesting as studies using rodents suggest that sexual motivation is independent of ERβ activation; a comparative endocrine study would determine the relative contribution of each region in the SBN and ERs in male sexual behavior across species.

Very few studies have examined the rapid action of corticosteroids on sexual behavior in male and female rodents. Restraint stress reduced lordosis behaviour within 1 hour of the stressor in female rats (99); chronic stress however increased female receptivity (100). Surprisingly, deoxycorticosterone increased lordosis in the estrogen-primed OVX female rat within 5 mins of i.v. injection, demonstrating an acute effect of a glucocorticoid on female sex behaviour (101). In contrast, acute administration of corticosterone reduced female preference for male odour within 5 minutes, in mice, in a NMDA-dependent manner (102). However, chronic corticosteroid administration decreased lordosis in both ovariectomized female rats and mice, when it preceded estrogen administration (103). Acute electric foot shock and cold-water immersion stress reduced sexual behavior performance 15 to 30 min after stress concurrently with plasma corticosterone increases, in sexually experienced males (104). However, neither acute nor chronic administration of corticosterone itself could change male rat sexual behaviour, suggesting that the effect of stressors on sex behaviour in males is not correlated to increases in corticosterone (105). These studies suggest that though the effects of glucocorticoids are easily discernable and could be rapid, the direction of effect depends on sex, type of glucocorticoid, duration of administration and context.

4.2. NON-GENOMIC SIGNALLING CONTRIBUTES TO AGGRESSIVE BEHAVIOUR IN THE MALE

Aggression in the rodent model in the lab is generally measured in males in a resident-intruder

paradigm that is repeated across days; most studies use timeframes that make it difficult to determine if this is due to solely non-genomic signalling (106).

Though ERα expression was positively correlated with aggressive behavior in several nuclei of the SBN including the LS, BNST, and AH (107), knockdown studies pinpoint critical areas for this behaviour in rodents to be the VMH. For example, Sano *et al,* (93) found that when ERα was suppressed in the mPOA there was a reduction in sexual behavior, but not aggressive behavior. Additionally, there was a reduction in both aggression and sexual behaviors in males with ERα suppression in the VMH. There were no changes with ERα knockdown in the meA for either behavior. This suggest that ERα expression is required in the mPOA and VMH for normal sexual behavior but only in the VMH for normal aggression (93,108). When ERα was deleted in GABergic, but not glutamatergic neurons in the BNST and mPOA, there was attenuation of aggressive and sex behaviour, as well as an increase in ERβ expression suggesting that ERα regulated ERβ expression (109). However, though adult βERKO male mice did not show any difference in aggression when compared to WT mice (77), ERβ may also be a regulator of aggressive behavior in an age-dependent manner. ERβ knockout male mice showed increased aggressive behaviour and shorter latency periods to attack when compared to wildtype during puberty and young adulthood, though the mechanism is unclear (110). These data suggest that ERβ is a negative regulator of ERα-driven aggressive behaviour (Figure 2B).

The importance of rapid signalling by ERα in VMH neurons has further been elegantly demonstrated by optogenetic activation and inhibition of these neurons in the laboratories of David Anderson and Dayu Lin. For example, optogenetic activation of ventrolateral VMH (vlVMH) neurons expressing ERα elicited attacks from male mice towards male and female conspecifics including towards a glove, while inhibition of these neurons reduces inter-male attack demonstrating that these neurons are in the critical pre-motor module of the neuronal circuitry. Therefore, direct high-intensity photostimulation activated these neurons and uncoupled them from the preceding modules that gather sensory information about the opponent i.e., VNO/AOB and the decision making module i.e. the meA and the BNST to attack intruders that would not normally be the subject of aggression (111). In addition, VMH neurons expressing ERα of the resident male fired and showed increased calcium during both the social appraisal and attack stages in intermale-aggression and ablating these neurons did not decrease conspecific sex recognition (112,113). Interestingly, low intensity photostimulation of these vlVMH neurons that express ERα allowed for mounting both male and female conspecifics, showing that ERα-expressing neurons from the VMH (around 40%) control both male mounting and aggression, suggesting that intensity of activation of overlapping neurons in the VMH can drive different social behaviours (112). Furthermore, in rats, the VMH lies

within a defined hypothalamic attack area (HAA) and electrical and pharmacological stimulation elicited intermale aggression, suggesting that ERα signalling in the vlVMH is conserved across species (114). Optogenetic activation of anterior vlVMH neurons expressing ERα that project primarily to the PAG increased self-defense behaviours by the resident in the face of intruder attack while activation of posterior vlVMH neurons expressing ERα that project primarily to the mPOA increased driving attacks (115). These optogenetic tools demonstrate scalable control of aggressive behaviour that can be modulated in a nuanced manner (Figure 2B).

The laboratories of Trainor and Nelson have explored the effect of photoperiod on aggression using several species of Peromyscus which show parental care and aggression. In short photoperiods, male California mice (*Peromyscus californicus*), beach mice (*Peromyscus polionotus*) and deer mice (*Peromyscus maniculatus*) all show increased aggression as compared to mice in longer photoperiods. In beach and deer mice but not in California mice, this was correlated with an increase in ERα in the lateral septum and a decrease of ERβ in the BNST and a decrease in circulating testosterone (116). In addition, in beach mice, both PPT and DPN could restore aggression in fadrozole-treated mice housed in short photoperiods, suggesting that both ERα and ERβ play a role in this species. In the beach mouse and California mouse, fadrozole-treated mice could show normal aggression within 15 mins of E2 injection when housed in short photoperiods, with the time frame following injection suggesting that non-genomic signalling is sufficient for aggression (117). This was supported by the fact that cycloheximide treatment did not change the rapid E2 facilitation of aggression (118). In California mice, aggression in the short photoperiod but not long photoperiod was correlated with increased p-ERK expression in the meA and BNST reinforcing the idea that E2 facilitation of aggression is via rapid, non-genomic kinase-dependent signalling pathways (37). The molecular mechanisms that underlie the rapid control of aggression by E2 remain unclear.

Treatment with corticosterone increased aggressiveness in male rats within 2 minutes (119); this is similar to the increase in aggressive behaviour shown by acute treatment of adrenocorticotrophic hormone (ACTH) in male mice (120). This is an effect in the CNS since administration of corticosterone into the right lateral ventricle increased aggression (119). A glucocorticoid synthesis inhibitor, metyrapone given acutely decreased aggression in male rats, which was rescued by corticosteroids given 2 minutes before a territorial intrusion by the intruder. However, protein synthesis inhibitors did not decrease aggression, suggesting that this is a rapid, non-genomic effect (119). In contrast, adrenalectomy in male rats resulting in long-term loss of glucocorticoids promoted abnormal attack behaviour and social deficits (121). Supporting this, corticosterone injection rapidly decreased the level of electrical stimulation required by the hypothalamus for attack behaviour (122) in these rats. Chronic

increased levels of glucocorticoids as seen in animals subject to chronic variable stress or social defeat paradigms resulted in decreased aggression in rats, mice and hamsters (123,124) (125). Hence, genomic signalling by glucocorticoids appears to reduce aggression while rapid signalling promotes it in rodents.

5. RELEVANCE OF NON-GENOMIC SIGNALLING

Why does non-genomic signalling initiated by mERs or mGRs exist? In many cases, e.g., lordosis or spinogenesis, it appears to be the initiating step in a sequence of events that culminates in transcription, as part of a coupled signalling pathway. This initiating step could either prime or potentiate the later steps or could be antagonistic. For example, work from our laboratory has shown that in neuroblastoma cells, E2-BSA potentiated transcription from a consensus ERE by phosphorylation of the ERα by activated PKB and ERK (73) (Figure 1). Studies from the Dorsa laboratory have shown that rapid activation of PKA by E2-BSA may potentiate transcription from non-ERE containing promoters such as those with CRE enhancer elements (74) (Figure 1). Hence, one function of non-genomic signalling and the rationale for the existence of the mERs is the ability of mER-mediated action to prime transcription from both ERE and non-ERE containing promoters, thereby increasing the number of genes responsive to estrogen (20). This may occur by the mER, and the intracellular ER being arranged in series in a signalling pathway (Figure 3A). However, mERs' may also antagonize the transcriptional activity of the classical ERs though antagonism of classical ERs (Figure 3A). This has been mostly shown in cancer cells with not many examples in the CNS (126). For example, in MCF-7 cells, GPER1 activation by G-1 decreased proliferation that was increased by ERα activation while in MDA-MB-231 cells, GPER1 activation lowered Akt signaling that was critical to ERβ-mediated ezrin phosphorylation and subsequent cell invasion (127).

These studies suggest that activation of mERs and the consequent rapid non-genomic signalling can act as a gain amplifier or dampener of nuclear genomic transcriptional effects, mediated by a predominant ER in that brain area (Figure 3A). In ovariectomized female rats, only the ERα agonist (PPT) and not the ERβ agonist (DPN) induced sex behaviour when injected on two consecutive days prior to testing; combination of both these agonists reduced sex behaviour suggesting that ERβ can negatively regulate ERα's ability to reduce sex behaviour in females, similar to aggression in males (128). Our laboratory has outlined several scenarios where GPER1 as a mER may act as a "collaborator" in series or in parallel to ERα or ERβ (126) (29)) to increase final output (Figure 3B) which could be an alteration in cell phenotype e.g., spinogenesis or an alteration in behaviour e.g., lordosis. For e.g., though EB increases spines in the VMH, this is not on ERα-positive neurons; it is possible that this could be via GPER1 since G-1 administration to ovariectomized female mice increased

lordosis (Anchan, 2014) and GPER1 is expressed widely in the VMH (129). In addition, though GPER1 could phosphorylate ERα in the male hippocampus suggesting that GPER1 activation is upstream of ERα, this interaction has not been shown in any SBN nuclei (130) and the relevance of this to transcription is not understood (Figure 1). In ovariectomized female rats, activation of both ERα and GPER1 using PPT and G-1 respectively decreased food intake within 1 hour (131). However, blockade of GPER1 using G-15 decreased the PPT effect within 1hr suggesting that activation of GPER1 is necessary for ERα, possibly in series in the same signalling pathway, to exert rapid anorexigenic effects (131). Variants of the ERα are predominantly localized to the membrane and could act as mERs. For example, the variant ERα-36 isoform could influence the full length ERα-66-dependent responses and may also act as collaborators. Inhibition of LPS-induced TNFα expression in MCF7 cells was dependent on the full length ERα66 isoform, the variant ERα- 36 isoform and GPER1. When ERα-36 or the GPER1 was knocked down, the anti-inflammatory effects of estrogen were lost (132). Hence, depending on the relative levels of ERs and crosstalk between receptors, the same ligand i.e., E2 could generate different responses in the same cell and at different time points that reflect different modes of signalling i.e., rapid or slow (20). Supporting this contention, both ERα and ERβ could induce lordosis behaviour rapidly but only ERα does so over longer time scales, demonstrating that different temporal modes of signalling may be predominantly used by different ER isoforms. This idea remains to be tested in detail.

Another intriguing possibility is that some of these isoforms or variants may preferentially bind to environmentally relevant molecules and transduce information about the environment to physiologically relevant behaviours. Phytoestrogens such as genistein can bind both the GPER1 (133) and ERβ, reflected in the higher affinity K_d values i.e., 145 nM for ERα and 8.4 nM for ERβ (134) (Figure 3). Female mice raised on the phytoestrogen-free diet (total isoflavones less than 1.0 ppm) showed substantially less sexual receptivity to novel males at puberty (135). Also, a low phytoestrogen diet in male mice results in a decrease in sociability and reduced *c-fos* induction in the cortical amygdala, the lateral septum, the medial preoptic area, and the bed nucleus of the stria terminalis (136), suggesting lower SBN activation. A recent study shows the effects of genistein may reduce the increased depression and microglia seen with chronic social defeat stress in the hippocampus, suggesting that modulation of neuron-microglia signalling could be a molecular mechanism for regulating estrogen-dependent behaviours (137).

6. FUTURE DIRECTIONS

Though nongenomic signalling is increasingly being studied in the CNS and crosstalk between isoforms is probably ubiquitous, several questions, some of which were highlighted in the

previous section, remain. There is no comprehensive map for colocalisation of these receptor isoforms and despite the evidence that various ERα variants may localize to the membrane and act as mERs, there is little demonstration of their action in the CNS. Another issue is the persistent lack of a reliable ERβ antibody (138) though this could be partially abrogated by using an RFP-ERβ mouse that has been used recently to colocalize both ERα and ERβ in different CNS nuclei (139) (140). Though androgens are the substrate for estrogen production, the non-genomic of androgens in regulating lordosis is underexplored. Androgens (141) including the dihydrotestosterone metabolite, 3α-diol (142) generally decrease sexual receptivity but androgen receptor (AR) antagonists fail to block this effect, suggesting a non-classical AR-independent mechanism (143). In addition, rapid neurotransmitter-type signalling has been proposed for steroids synthesized in the brain (neurosteroids) though the behavioural outputs and signaling pathways that underlie these behaviours, particularly for the glucocorticoids, are poorly understood (144). Combining whole animal behavioural phenotypes with novel tools such as the Brainbow mouse (145) and cell transcriptomics (146) will help to uncover the neurocircuitry in the SBN nuclei, the differential ratio of ERs in these nuclei and the crosstalk between different isoforms.

Legends:

Figure 1: Nongenomic and integrated signaling initiated by membrane ERs: Ligand bound ERs (either ERα or ERβ) at the membrane activate rapidly activate within minutes, many kinase and calcium signaling pathways (shown by the solid black arrows) that are dependent on cell type. In some areas such as in the arcuate nucleus of the hypothalamus, ERα can interact with mGluR1 via the caveolin-1 bridge and activate both PKC and LIMK which are in turn important for spinogenesis. Whether integrated signalling is important for maturation of spines is not known. GPER1, a GPCR that binds estradiol has been shown to activate PKA and induce calcium release as well as activate MAPK pathways via epidermal growth factor receptor (EGFR) activation.
Integrated signalling: Several kinase pathways culminate in the nucleus (shown by the green dotted lines). For example, rapid signalling initiated by estrogens at the plasma membrane can regulate transcription from non-ERE containing promoters such as the *c-fos* gene or those containing the cAMP-response-element (cre). In addition, an integrated signalling pathway wherein rapid kinase activation (ERK/PKB) leads to ERα phosphorylation and potentiation of transcription from a consensus estrogen response element (ERE) has also been shown in neuroblastoma cells. Furthermore, GPER1 has been shown to phosphorylate Eralpha in the male mouse hippocampus but the mechanism is not fully understood (depicted by the green solid arrows).
Abbreviations: MAPK: mitogen activated protein kinase; ERK: extracellular regulated kinase; PI3K: phosphatidylinositol-3-kinase; PKB: protein kinase B; PKA: protein kinase A; PLC: phospholipase C; PKC: protein kinase C; LIMK: LIM kinase; CAV1: caveolin1; mGlurR1: metabotrophic glutamate receptor 1; CREB: cAMP response binding protein.
Black solid arrows: denote rapid signaling pathways from membrane-bound ERs.
Green solid arrows: denote molecular signals that impinge on or are initiated by the intracellular ERα
Green dotted lines: denote signals from kinases into the nucleus.
Green dotted arrows: denote pathways that are not yet known.
Green triangle: 17β-estradiol
Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

Figure 2: Sex and aggression are predominantly driven by ERα in the social behaviour network in both males and females in the mouse. Presumably non-genomic and genomic signalling

are both involved in both behaviours, though the role of non-genomic signalling and the exact pathways are underexplored. In the female (top panel A), ERα expression in the VMH is known to important for sex behaviour (Section 3) while in the male, ERα in the mPOA and VMH are critical for sex behaviour (Section 4.1). Apart from this, ERα in the VMH also drives aggressive behaviour in the male while ERβ in the VMH may be inhibitory for aggressive behaviour (Section 4.2). GPER1 activation is sufficient for lordosis behaviour in female mice (not shown in the figure) but the molecular mechanism is not known.
Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/). The male and female symbol is from Male Female Symbol Vectors by Vecteezy (free to use).

Figure 3: Probable functions of non-genomic signalling: A) Rapid actions mediated by ERs (ERα or ERβ) or GPER1 can potentiate or antagonize slow genomic actions mediated by intracellular ERs (ERα or ERβ) to generate a cell output such as transcription. B) Rapid actions may act independently from slower genomic signalling on the same output. In this case, such parallel signalling may result in an output that is a sum of outputs from these two pathways. Note that the ERα or ERβ or GPER1 can bind more than the predominant endogenous estrogen, 17β-estradiol (shown by the green triangle); ERs including ERβ and GPER1 can bind phytoestrogens and environmental estrogens with relatively high affinity (depicted by the grey doughnut).
Parts of the figure were drawn by using pictures from Servier Medical Art. Servier Medical Art by Servier is licensed under a Creative Commons Attribution 3.0 Unported License (https://creativecommons.org/licenses/by/3.0/).

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