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Estrogen Promotes Learning Related Plasticity by Modifying the Synaptic Cytoskeleton

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Abstract

Estrogen's acute, facilitatory effects on glutamatergic transmission and long-term potentiation (LTP) provide a potential explanation for the steroid's considerable influence on behavior. Recent work has identified mechanisms underlying these synaptic actions. Brief infusion of 17 β -estradiol (E2) into adult male rat hippocampal slices triggers actin polymerization within dendritic spines via a signaling cascade beginning with the GTPase RhoA and ending with inactivation of the filament severing protein cofilin. Blocking this sequence, or actin polymerization itself, eliminates E2's effects on synaptic physiology. Notably, the theta burst stimulation used to induce LTP activates the same signaling pathway as E2 plus events that stabilize the reorganization of the sub-synaptic cytoskeleton. These observations suggest that E2 elicits a partial form of LTP, resulting in an increase of fast EPSP's and a reduction in the threshold for lasting synaptic changes. While E2's effects on the cytoskeleton could be direct, results described here indicate that the hormone activates synaptic TrkB receptors for Brain Derived Neurotrophic Factor, a releasable neurotrophin that stimulates the RhoA to cofilin pathway. It is therefore possible that E2 acts via transactivation of neighboring receptors to modify the composition and structure of excitatory contacts. Finally, there is the question of whether a loss of acute synaptic actions contributes to the memory problems associated with estrogen depletion. Initial tests found that ovariectomy in middle-aged rats disrupts RhoA signaling, actin polymerization, and LTP consolidation. Acute applications of E2 reversed these defects, a result consistent with the idea that disturbances to actin management are one cause of behavioral effects that emerge with reductions in steroid levels.

Keywords

actin; cofilin; pTrkB; hippocampus; LTP; RhoA

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Introduction

A now substantial literature indicates that estrogen, in addition to its gonadal and somatic effects, has a potent influence on memory and cognition (Brinton, 2009; Dumitriu et al., 2010; Srivastava and Penzes, 2011). Given that the hormone produces many of its peripheral actions via transcription, it is reasonable to assume that similar mechanisms occurring in forebrain networks play a central role in the behavioral changes it produces. In accord with this, circulating levels of estrogen affect dendritic spine densities (Gould et al., 1990), as well as neurotransmitter levels (Fink et al., 1996; Lee and Pfaff, 2008), in certain hippocampal and cortical subfields. Concentrations of brain growth factors also vary with the estrus cycle (Scharfman et al., 2003), an observation that provides a possible explanation for the steroid's influence on spines and learning.

However, recent work suggests that very rapid, and therefore presumably non-genomic, synaptic events also contribute to at least some of the behavioral changes triggered by estrogen. Specifically, several laboratories have shown that brief infusions of 17 β -estradiol (E2), at concentrations found in the blood during phases of the estrus cycle, result in an almost immediate enhancement of fast excitatory postsynaptic potentials (EPSPs) in hippocampal slices from male (Foy et al., 1999; Bi et al., 2000; Sharrow et al., 2002; Kim et al., 2006; Foy et al., 2008; Kramár et al., 2009a) and female (ovariectomized; OVX) rats (Smejkalova and Woolley, 2010). Notably, this facilitation involves both the slope and amplitude, but not the waveform, of monosynaptic potentials and reverses quickly upon washout (Fig 1A). These observations point to one or more of the following as potential substrates for the acute actions of E2: i) an increase in numbers of activated axons, ii) enhanced neurotransmitter release, or iii) an expanded pool of post-synaptic AMPA receptors.

Estrogen has a second, acute physiological action that is logically and experimentally related to memory encoding: it markedly facilitates long-term potentiation in male rats (Foy et al., 1999; Kramár et al., 2009a), and OVX rats (Smith and McMahon, 2005 and 2006) and mice (Yun et al., 2007). This involves both a reduced threshold for inducing LTP and an elevated ceiling on the maximum degree of potentiation produced by conventional stimulation paradigms (Fig 1B). Diverse manipulations that promote LTP are reported to improve memory in animal models of intellectual impairment and control subjects (Harvey et al., 2005; Porrino et al., 2005; Lynch et al., 2008 for review). The dramatic influence of E2 on the potentiation effect is thus a likely factor in the widely reported memory enhancing actions of the hormone.

The present review addresses the neurobiological mechanisms whereby E2 facilitates excitatory transmission and LTP. We begin with the question of whether the former effect is due to pre- vs. post-synaptic changes (or both) and then ask if factors in addition to response facilitation are involved in the LTP effects. Subsequent sections review signaling pathways initiated by E2 that potentially account for its electrophysiological actions, and discuss barriers to the development of formal hypotheses. Finally, we consider the clinically important question of how the acute actions of E2 might relate to the memory and cognitive problems found after extended periods in which levels of the hormone are reduced.

1. E2 and fast, excitatory transmission

Presynaptic explanations for the modest increase in EPSPs produced by E2 include an increase in axonal excitability or a change in transmitter release kinetics in slices from OVX rat (Smejkalova and Woolley, 2010). The first of these can be simply tested by measuring the magnitude of the fiber volley, a small potential preceding the evoked potential and composed of axon potentials, produced by different stimulation currents. Experiments of this kind failed to detect any steroid effects on the slope of the curve describing fiber volley amplitude and stimulation current in slices from male rats (Kramár et al., 2009a). Similarly, there were no evident differences from controls in the relationship between fiber volley and EPSP size across different intensities of stimulation in slices from OVX rats and mice (Smith and McMahon, 2005; Yun et al., 2007). Repetitive stimulation, at sites throughout the nervous system, increases transmitter release probability via accumulation of residual calcium in axon terminals. Manipulations that increase release probability reduce this facilitation effect, something that has not been shown to occur with E2 treatments in slices from male rat hippocampus (Kramár et al., 2009a). However, as mentioned above estrogen is recently reported to affect frequency facilitation in a subpopulation of hippocampal pyramidal cells using slices from OVX rats (Smejkalova and Woolley, 2010), results that raise the possibility that gender differences may exist with regard to the pre-synaptic actions of E2.

Several studies have shown that E2 causes the formation of dendritic spines and there is evidence that this can occur in less than an hour (Mukai et al., 2007; Srivastava et al., 2008; Brinton, 2009). Whether the effect develops in the 5-10 minute period over which E2 increases EPSPs remains to be tested. Beyond this, it is not clear if spine growth under the influence of E2 in adults is accompanied by synaptogenesis; the new structures are generally described as being thin (Mukai et al., 2007) and could be filopodia as opposed to innervated spines. Transfer of AMPA receptors to the post-synaptic apposition zone from the perisynaptic membrane is widely held to be the expression mechanism for LTP and, if so, this process is an obvious candidate for the route whereby E2 increases EPSPs. Evidence that the steroid increases surface expression of AMPA receptors accords with this idea (Liu et al., 2008) but the pertinent studies used four hour intervals and did not establish the presence of added receptors at synapses. It is therefore possible that the observed surface expression reflects delayed addition to a reserve pool in spine membranes. In all, current results do not resolve the question of whether the acute effects of E2 in mature neurons are due to proliferation of contacts as opposed to a modification of existing synapses.

The development of dual, immunofluorescence microscopy techniques for measuring the concentration of defined antigens within limited cellular compartments (Rex et al., 2009; Chen et al., 2010; Seese et al., 2012) has made it possible, in principle, to test if E2 increases the number of synapses with high concentrations of AMPA receptors (AMPARs). However, such experiments are challenging due to the relatively modest size of the expected signal since the approximately 15% facilitation of EPSPs elicited by E2 treatment could be accommodated by a similarly small addition of AMPARs. This point raises an issue that has received little attention in the field; namely, that the enhanced EPSPs obtained with E2 treatment could reflect much larger effects at a *subpopulation of the synapses formed by the*

stimulated axons. Pertinent to this, experiments using selective agonists indicate that the facilitatory effects of E2 are mediated by estrogen receptor beta (ER β) rather than estrogen receptor alpha (ER α) (Kramár et al., 2009a; Srivastava et al., 2010), and there is evidence that the ER β receptors are localized to the perisynaptic membrane on dendritic spines (Milner et al., 2005; Waters et al., 2011). However, immunohistochemical methods have not yet determined if the receptors are ubiquitously distributed or instead are restricted to particular types or subsets of excitatory contacts. Were the latter to be the case, then estrogen-driven increases in synaptic AMPARs might readily be detected with the new light microscopy techniques. The absence of experimental tests of the point constitutes a major barrier to the development of a hypothesis regarding E2's positive effects on fast EPSPs.

2. Estrogen and long term potentiation

The most straightforward interpretation of E2-induced facilitation of LTP is that enhanced EPSPs lead to greater net depolarization, and thus greater opening of voltage sensitive NMDA receptors (NMDARs), during the delivery of theta burst stimulation (TBS) used to induce potentiation. Greater than normal LTP occurs when afferent stimulation is delivered in the presence of drugs that prolong the opening time of ligand bound AMPARS (Arai et al., 2004). However, these compounds also prolong the duration of EPSPs, resulting in greatly increased temporal summation and net depolarization during the composite response to a theta burst. As noted, E2 amplifies EPSPs but does not substantially affect their waveforms (Kramár et al., 2009a). In any event, it would be surprising if the *only* LTP-related effect resulting from the complex events presumably resulting from ER β activation would be an increase in the size of synaptic responses.

Another mechanism for E2's influence on LTP would be an augmentation of NMDAR responses to released glutamate (Brinton, 2009), something that would not be evident in single EPSPs where depolarization is too brief to remove the voltage block on the NMDARs. Prior studies found that ovariectomy causes a disruption in NMDAR subunits (Smith and McMahon, 2006) and that estrogen interacts with NMDARs to overcome learning deficits in OVX mice (Lewis et al., 2008). Most recently, it was suggested that estrogen rescues hippocampal plasticity in the OVX model by recruiting NR2B-containing NMDA receptors to the synapse (Snyder et al., 2011). However, we found that E2 has no detectable effects on pharmacologically isolated NMDAR-mediated responses in slices from male rats while producing the expected enhancement of isolated AMPAR responses (Fig 2A, B). The composite post-synaptic depolarization produced by a theta burst is shunted by feedforward GABAergic connections formed by interneurons that are driven by the same fibers that produce glutamate-mediated depolarization in pyramidal neurons. These inhibitory responses are also unaffected by E2 (Kramár et al., 2009a). Interestingly, a more recent study by Huang and Woolley (2012) demonstrated that acute infusions of E2 depress inhibitory postsynaptic potentials in slices from adult OVX rats, but not in male rat slices. These new findings again emphasize the possibility of gender differences in the acute response of hippocampus to estrogen; however, work is needed on the contributions of estrogen depletion, as opposed to male/female differences, before strong conclusions can be reached.

The magnitude of the LTP elicited by TBS is also dependent on the facilitation of burst responses that occurs across a series of theta bursts separated from each other by 200 msec (Fig 2C). This effect is in part regulated by the magnitude of the long after-hyperpolarizations set in motion by each burst response (Arai and Lynch, 1992); suppressing the after-hyperpolarizations leads to a marked enhancement of theta burst responses and LTP (Kramár et al., 2004), and a change of this kind could be a substantial contributor to E2's positive actions on LTP. However, experimental tests in slices from male rats were negative: within train facilitation of theta burst responses was not detectably affected by the hormone (Fig 2D).

Collectively, the above results point to an unexpectedly selective physiological effect of acute exposure to E2, at least in males. Following from this, a legitimate simplifying hypothesis is that the enhanced plasticity produced by the hormone in the male hippocampus is a secondary consequence of its actions on fast EPSPs which themselves, as argued above, are most likely due to movement of AMPARs into the synapse (Liu et al., 2008; Zadran et al., 2009). This model is, however, incomplete because it does not specify a mechanism for the proposed, and as yet unconfirmed, receptor translocation, nor does it address the possibility that the machinery for translocation has parallel effects on the substrates for LTP. In other words, there may be a deeper, cell biological explanation for the acute actions of E2 on both baseline physiology and plasticity. The next section explores this idea.

3. Estrogen and actin signaling pathways

One current line of thinking on LTP substrates posits that they involve an increase of synaptic AMPARs followed by a reorganization of the sub-synaptic actin cytoskeleton that anchors the altered state of the contact (Lynch et al., 2007; Bramham, 2008). There are reasons to think that the expression and consolidation events are related; specifically, assembly of actin networks, and triggering of actomyosin motors, could serve to transfer receptors into the synapse (Wang et al., 2008; Yang et al., 2008; Gu et al., 2010) while independently creating a new spine cytoskeleton that maintains the altered, receptor-rich configuration of the connection. E2, according to these arguments, triggers actin signaling pathways that result in AMPAR translocation *and* facilitates the steps leading to stable LTP.

Tests of the above ideas produced interesting results. TBS under baseline conditions engages two (and likely more) small GTPase initiated cascades that result in the assembly and subsequent elaboration of actin filament networks within spines (Harvey et al., 2008; Rex et al., 2009; Chen et al., 2010; McNair et al., 2010;); multiple lines of evidence indicate that these steps are essential for the production of LTP (Krucker et al., 2000; Kramár et al., 2006; Rex et al., 2009). E2 applied to adult male hippocampal slices similarly triggers actin polymerization in dendritic spines but to a lesser degree than TBS (Fig 3A, B). Critically, blocking E2-induced actin filament assembly with the highly selective toxin latrunculin A completely eliminates the enhancing effect of the steroid on EPSPs (Fig 3C). These results suggest that E2 initiates a weak version of the actin reorganization produced by induction of LTP, from which it follows that the E2-induced increase in synaptic responses *could be due to the same mechanisms used to express the potentiation effect*. This hypothesis has the advantage of explaining the enhancement of LTP, the second of the physiological effects of

E2. That is, partial activation of cytoskeletal remodeling by the hormone could reduce the number of theta bursts needed for triggering the actin filament assembly that underlies the production of stable LTP. Tests of this idea proved positive: while three theta bursts are insufficient to cause the accumulation of F- (filamentous) actin in spine heads, they generate a robust effect of this kind in slices pretreated with E2 (Fig 3D).

The generation of TBS-induced increases in spine F-actin is associated with phosphorylation (inactivation) of cofilin (Chen et al., 2007), a constitutively active protein that severs dynamic actin filaments (Sarmiere and Bamburg, 2004). E2 infused into hippocampal slices from male rats, as in the physiological experiments, increased the concentration of phosphorylated (p)-cofilin as determined by western blots (Fig 3E). This effect was selective because levels of phosphorylated p21-activated kinase (PAK), a second actin signaling protein, were slightly reduced in the same treated slices. A primary pathway regulating cofilin phosphorylation involves the serine kinases RhoA kinase (ROCK) and LIM kinase; the latter directly phosphorylates cofilin (Meng et al., 2003). It is of considerable interest, then, that a selective and reversible inhibitor of ROCK completely blocks E2-induced increases in EPSPs and that the effect reverses upon washout of the inhibitor (Kramár et al., 2009a).

ROCK, as indicated by its name (RhoA kinase), is a primary effector of the small GTPase RhoA. The results with cofilin and the ROCK inhibitor thus strongly suggest that E2 activates RhoA and thereby initiates the ROCK > Lim kinase > cofilin > actin polymerization pathway. GTPase pull down assays confirmed that E2 causes a marked increase in activated, GTP-bound RhoA in adult male hippocampal slices and further showed the effect is selective in that activities of two close relatives of RhoA, Cdc42 and Rac, were not affected by the hormone infusions (Fig 3F) (Kramár et al., 2009a).

Collectively, the above findings indicate that E2 selectively stimulates a prominent actin signaling pathway leading to cytoskeletal reorganization and in this way produces a weak and reversible form of LTP in adult male hippocampal pyramidal cells. As noted, E2 does not engage a parallel Rac/Cdc42 > PAK pathway, that is targeted by TBS and appears to promote elaboration and stabilization of the newly formed actin filaments (Rex et al., 2009) (Fig 4). An intriguing possibility is that the failure of E2 to initiate this second pathway explains why its effects are not more persistent.

It is highly unlikely that the RhoA pathway illustrated in figure 4 constitutes the only actin related cascade set in motion by E2 at hippocampal spines and synapses. Studies on dissociated neurons have shown that estrogen activates the small Ras-family GTPase Rap and its effector Erk1/2 and that these events are associated with translocation of the actin binding protein AF-1 to the synaptic membrane (Srivastava and Penzes, 2011). The Rap-driven responses are critical for the rapid spine growth produced by E2 treatment of cultured cortical neurons (Srivastava and Penzes, 2011). It will be of considerable interest to test if comparable results are obtained in adult hippocampal slices.

4. E2 and signaling cascades: activation or transactivation?

The results reviewed thus far lead to the question of how E2 stimulates RhoA. A direct effect on RhoA at adult synapses is a plausible, but not the only, possibility. McEwan and colleagues (Spencer et al., 2008) have proposed that prolonged treatments with E2 transactivate TrkB receptors for BDNF and in this way promote spine growth. An ‘acute’ version of this hypothesis is intriguing in the present context because BDNF is known to stimulate Rho GTPase signaling (Reichardt, 2006) including the activation of RhoA (Yamauchi et al., 2004). Moreover, Scharfman and colleagues (2003) found increases in excitatory transmission that occur during proestrus are prevented by the kinase inhibitor K252a which blocks Trk signaling. These findings are consistent with a model in which E2 causes rapid TrkB activation that, in turn, engages a RhoA-initiated sequence leading to enhanced EPSPs.

We examined the first part of the above argument using dual, immunofluorescence microscopy to estimate the number of synapses, labeled with antibodies to the synaptic scaffold protein PSD95, associated with activated neurotrophin receptor (Fig. 5). TrkB subunits dimerize and autophosphorylate at multiple tyrosine sites as part of the receptor activation process (Reichardt, 2006; Minichiello, 2009), with the latter effect being detectable at synapses using phospho-specific antisera (Rex et al., 2010). Using adult male hippocampal slices, we found that E2 causes an ~60% increase in the number of field CA1 synapses associated with high levels of activated TrkB (phosphorylated at Y515), as assessed 10 min after the onset fEPSP slope facilitation (Fig. 5C). It thus appears that the steroid either transactivates TrkB (Lee and Chao, 2001; Nagappan et al., 2008; Huang and McNamara, 2010) or facilitates the TrkB response to released BDNF. An alternative explanation is that E2 releases BDNF (Krizsan-Agbas et al., 2003; Scharfman et al., 2003; Sato et al., 2007) which then stimulates TrkB receptors through conventional ligand-binding. Future studies are needed to determine whether there is an absence of acute estrogen effects in a BDNF-knockout mouse.

TrkB is not the only synaptic receptor through which E2 could initiate actin signaling and modifications to transmission (Kramár et al., 2009a). In particular, adhesion receptors belonging to the beta 1 integrin family are synthesized at high levels in hippocampus (Pinkstaff et al., 1998; Pinkstaff et al., 1999), concentrated in excitatory contacts (Mortillo et al., 2012), and have been shown to facilitate synaptic transmission in adult hippocampal slices (Kramár et al., 2003). Recent work indicates that synaptic integrins, and integrin signaling, are activated by afferent activity (Babayan et al., 2012) and that this results in LTP-related actin polymerization (Kramár et al., 2006). Accordingly, there are multiple transactivation routes for rapid, E2-driven synaptic modifications. First, as indicated by studies of neurons and non-neuronal cells, estrogen receptors interact with various GTPase regulatory factors (Srivastava et al., 2010; Oviedo et al., 2011; Srivastava and Penzes, 2011) and therefore could directly stimulate RhoA. Together with other synaptic modulatory receptors (TrkB, integrins), this would give the synapse three independent routes to the head stage of the actin assembly cascade (Fig 6A). Second, E2's interactions with GTPase regulatory factors may be too weak, if acting alone, to meaningfully affect RhoA; its observed effects on the small GTPase would then be solely due to transactivation of TrkB

and possibly the integrins (Fig 6B). Third, E2 could stimulate RhoA to a degree that by itself is insufficient to activate the RhoA-to-cofilin sequence but is adequate to lower the threshold for input from the other modifier receptors; the combination of a modest direct influence and partial transactivation would then result in actin filament assembly (Fig 6C).

Results described here (figure 5), together with earlier work cited above, suggest that transactivation (or indirect stimulation) (model B) does occur but this observation by itself does not rule out the other two scenarios. That is, the consequences of transactivation could be relatively minor compared to a direct action of E2 (model A) on RhoA signaling or full signaling could require both a direct action and transactivation (model C). Some discrimination between these possibilities can be achieved using highly selective agents that depress activation of either TrkB or the integrins. Negative results in such blocking experiments would indicate that the direct stimulation hypothesis is likely to be correct. A positive outcome would argue against that hypothesis but would not distinguish between models B and C. In other words, a direct action of E2 could require augmentation from transactivation to cause actin filament assembly. Detailed quantitative studies of synaptic RhoA activation using immunohistochemical methods have the potential to resolve the issue.

Assuming that experimental work narrows the likely post-synaptic mechanism of action to one of the hypotheses in figure 6, there would still remain the very large question of whether the effects obtained with infusions reflect the effects of circulating estrogen or are also related to the effects of locally produced and released hormone. A series of elegant experiments have established that axon terminals in hippocampus of male and female rats synthesize the steroid and have the capacity to release it (Prange-Kiel et al., 2003; Kretz et al., 2004). While evidence that the physiologically realistic patterns of afferent activity cause release of estrogen is lacking, recent work indicates that blocking local synthesis of estrogen depresses LTP in cultured hippocampal slices (Vierk et al., 2012). Further work along these lines will likely close a critical gap in the development of a unifying hypothesis regarding E2's acute effects on synaptic physiology and plasticity in the male hippocampus.

5. Significance for conditions in which estrogen levels are chronically reduced

It is well established that ovariectomy impairs learning and memory in rats (Gibbs and Johnson, 2008; Hammond et al., 2009; Inagaki et al., 2010; Jacome et al., 2010; Luine et al., 2003; Rhodes and Frye, 2006), monkeys (Rapp et al., 2003) and mice (Rissman et al., 2002; for review see Bodo and Rissman, 2006). Similar findings are reported for humans after surgical ovariectomy (Moller et al., 2010; Sherwin, 1988). Results for women in menopause are less clear but careful studies suggest that measurable declines in learning scores occur in this case as well (Vearncombe et al., 2011; Devi et al., 2005; Wegesin and Stern, 2007). The question thus arises as to whether long term depletion of estrogen negatively affects the LTP-related actin signaling systems that are driven by acute administrations. Initial results suggest that this is likely to be the case.

Baseline synaptic physiology in hippocampal field CA1 of middle aged, OVX rats receiving injections of estradiol benzoate every other day for 2 weeks was not detectably different than that in slices from OVX rats receiving vehicle injections. Consistent with other reports, input/output curves and paired pulse facilitation appeared normal, as did the waveform of synaptic responses in OVX-vehicle animals (Foy et al., 1999; Smith and McMahon, 2005; Kim et al., 2006; Kramár et al., 2009a). Interestingly, this contrasts with a recent report (Smejkalova and Woolley, 2010) showing that acute estrogen enhances paired-pulse facilitation in slices prepared from young OVX rats that had been pre-treated with estradiol benzoate for two days. An intriguing feature of this study is that only a subpopulation of synapses with low initial levels of release (high paired-pulse facilitation ratios) was found to respond to E2. Whether the discrepancies between these results and our findings reflect a differential effect of OVX in young vs. middle-aged rats, or involve changes relating to the duration of estrogen replacement treatments, is an important topic for future research.

In any event, long-term ovariectomy produced a marked impairment in LTP that was fully reversed by chronic estrogen replacement therapy (Fig 7A). Induction and initial expression of the potentiation effect were comparable in the OVX (no replacement) and OVX (replacement) groups, as indicated by the comparable levels of response facilitation at 1-2 minutes post-TBS (Kramár et al., 2009a). It follows from this that the essential defect produced by long term estrogen depletion most likely lies in the above described processes that rapidly consolidate LTP. Consonant with this, TBS failed to trigger actin polymerization within dendritic spines in vehicle-treated OVX rats but was fully effective in animals that had received replacement treatments (Fig 7B). As anticipated from these results, and the data summarized in figure 3, ovariectomy produced a pronounced depression of RhoA activity (Fig 7C). It should be noted that not all studies report a LTP impairment in OVX animals (Barraclough et al., 1998; Foy et al., 2008). The discrepancy could indicate that the defect is partial in that it can be overcome with stimulation that is well above threshold for inducing LTP, as found in recent work on mouse models of human conditions associated with memory problems (Lauterborn et al, 2007). This issue can only be resolved by assessing the degree of stable potentiation produced in OVX slices by different amounts of inducing stimulation (e.g., theta burst trains of different length).

A clinically important question that emerges from the above findings is whether long term depletion of estrogen produces changes in gene expression that depress actin signaling, and therefore would be presumably resistant to acute therapies, or instead simply removes a normally present agent (E2) that maintains actin signaling in a state that responds to patterned afferent activity. The latter argument predicts that acute infusions of the steroid will enhance baseline synaptic responses and fully restore the substrates of LTP in long term OVX rats. Experimental work has confirmed this prediction. In particular, acute administration of E2 to hippocampal slices from OVX rats rescues TBS-induced increases in spine F-actin as well as LTP (Kramár et al., 2009a). These findings are consistent with a recent report by Smith and colleagues (2010) showing that acute administration of E2 rescues LTP in slices from 9 and 15 mo post-OVX rats.

These last results strongly point to the unexpected conclusion that the extended loss of circulating estrogen does not substantially affect the baseline physiological or plasticity

characteristics of hippocampal synapses. There has been little work on the extent to which acute E2 rescues learning in long term, middle aged OVX animals, but the evident prediction from the neurobiological studies is that such treatments will have short term, positive effects (Fan et al. 2010).

In any event, the seemingly complete restoration of theta-driven actin signaling and LTP in the face of extended estrogen depletion has to be viewed as surprising and worthy of closer examination. A first question concerns how fully the acute treatments restore plasticity after long term OVX. The material reviewed above was largely concerned with events occurring in the first few minutes after single trains of theta stimulation and did not address the later stages of LTP development. There is, for example, the delayed, protein synthesis phase of potentiation generally assumed to begin 30-90 minutes after induction (Fonseca et al., 2004; Fonseca et al., 2006; Bramham, 2008; but see Abbas et al., 2009 and Villars et al, 2012 for a serious challenge to this argument). If present, this 'L-LTP' stage represents a likely target for any genomic changes following the protracted absence of estrogen. Other work has identified a quite different set of late developing LTP effects and, in particular, a novel, integrin dependent stage of consolidation that emerges about one hour after the initial expression of potentiation; behavioral studies confirmed that memory also passes through this delayed phase of stabilization (Babayan et al., 2012). Related to this, LTP is now known to exhibit a 'spaced trials' effect such that a second and third episode of theta burst stimulation can more than double the magnitude of potentiation if separated by about one hour (Kramár et al., 2012a). Studies involving these secondary characteristics of LTP are needed before strong statements can be made about the degree to which acute E2 fully rescues learning-related plasticity in OVX rats.

6. Summary and Discussion

The material reviewed here indicates that brief infusions of E2 at low, physiologically relevant concentrations initiate some, but not all, of the cytoskeletal machinery used in the production of LTP. E2 was surprisingly selective in its action on the male hippocampus, as it did not measurably change inhibitory transmission, NMDAR-mediated responses, or any of the large number of physiological events triggered by brief periods of high frequency afferent stimulation (Kramár et al., 2009a). However, a broader array of effects has been described for OVX rats (Smejkalova and Woolley, 2010; Huang and Woolley, 2012), a set of observations that raises the possibility of gender differences in the response of the hippocampus to acute estrogen. Estrogen applied for longer periods than those reviewed here induces the formation of thin spines in male rats (Mukai et al., 2007) and cultured slices (Srivastava et al., 2010). These effects are logically related to the actin polymerization that appears to underlie the short latency increases in EPSPs, as spine growth is dependent on the formation of dynamic actin filaments. Possible sex differences and the physiological significance of the spines that develop after long infusions are important topics for future research.

Insights into how E2 acts on the sub-synaptic cytoskeleton have also emerged. The steroid sets in motion a signaling cascade that begins with RhoA and ends with inactivation (phosphorylation) of the actin severing protein cofilin (Kramár et al., 2009a); this last step is

a well established mechanism for allowing actin monomers to assemble into filaments (Gungabissoon and Bamburg, 2003; Sarmiere and Bamburg, 2004). Selectivity is also found at this cell biological level since E2 does not engage pathways that are likely related to filament branching and stabilization. The present review also considered the possibility that E2's links to RhoA involve transactivation (or indirect stimulation) of 'modifier' receptors that potentially stimulate the small GTPase. New results reported here confirm an earlier suggestion that the hormone produces rapid activation of synaptic TrkB receptors for BDNF, a neurotrophin known to activate RhoA (Yamauchi et al., 2004), increase phosphorylation of cofilin, and promote both actin polymerization and LTP (Rex et al., 2007). Questions now arise about i) the importance of the TrkB response to the observed augmentation of EPSPs and LTP, and ii) the likelihood that E2 transactivates other synaptic receptors known to regulate the cytoskeleton (DeMali et al., 2003; Wiesner et al., 2005; Kramár et al., 2006). The essential issue concerns the extent to which synapses use multiple, independent routes as opposed to a more complex, interactive system to produce structural modifications.

Superimposed on these uncertainties about post-synaptic processing are critical questions about the release of locally generated estrogen from axon terminals and its role in vivo. Work using an aromatase inhibitor has provided evidence that endogenous E2 is required for LTP (Grassi et al., 2011) but didn't establish if this reflects constitutive actions vs. release during theta bursts. Thus, it remains to be seen whether brain-derived estrogen serves a constitutive role to maintain normal synaptic transmission and/or whether it is released only following activity-dependent activation. Resolving this point will be difficult given technical problems associated with measuring the small quantities of estrogen that would need to be detected. Nonetheless, any attempt to develop a detailed hypothesis of how E2 acutely affects transmission and plasticity will have to incorporate statements about the relative contributions of peripheral and central sources of the steroid.

The E2 results provide a striking illustration of the manner in which circulating factors can modulate synaptic plasticity in forebrain structures critical to learning. Such effects may be more general than is widely appreciated. For example, multiple studies have shown that interleukins, agents released as part of the inflammatory response, profoundly disrupt LTP stabilization (Imamura et al., 2011; Liu et al., 2012; Lynch, 1998). There is also substantial evidence that glucocorticoids have both positive and negative effects on LTP and learning (Chen et al., 2012; Spyрка et al., 2011; de Quervain et al., 2009). Testosterone has similarly been linked to plasticity and memory encoding (Ball et al., 2004; Bolhuis and Gahr, 2006). In all, the striking influence of E2 on LTP needs to be considered in the broader context of peripheral regulation of central plasticity. Indeed, the intriguing combination of effects potentially arising from local and peripheral sources may not be unique to estrogen since a similar issue arises with stress hormones (Maras and Baram, 2012).

While there is almost no information on how the acute effects reviewed above relate to, or interact with, the genomic actions of estrogen as found in the periphery (Carroll et al., 2006), there are reasons to suspect that links exist (Pechenino and Frick, 2009; Aenlle and Foster, 2010). The LTP related actin signaling initiated by E2 is reported to engage translational activity by dendritic ribosomes via mechanisms that also stimulate transcription (Smart et al., 2003). There is thus the possibility that the acute events are directly involved in the

presumed long term influence of estrogen on neuronal growth and viability. It has to be noted, however, that we have almost no hippocampal data on estrogen binding to the ER α receptors that mediate many of the hormone's actions on transcription in peripheral tissues. Possibly, then, E2's acute and delayed effects could occur in parallel. Insight into this critical issue will require an analysis of gene activation by estrogen under conditions in which its acute effects are well characterized. It should then be possible to ask if the minimal conditions for the rapid changes in physiology are sufficient to trigger genomic responses.

Finally, the discrete yet potent effects of E2 on actin signaling and LTP point to the possible utility of the hormone as a therapeutic for conditions in which cognitive impairments may involve defects in activity-driven cytoskeletal reorganization. We have identified several such cases using rodent models of neuropsychiatric conditions (Baudry et al. 2012; Lauterborn et al., 2007; Chen et al., 2010) including, as described above, instances in which estrogen levels have been reduced in middle-aged animals (Kramár et al., 2012b). The latter studies found that TBS-induced actin filament assembly and LTP do not occur after long term ovariectomy in older rats and that these pronounced impairments can be fully reversed by a brief infusion of E2. How broadly estrogen might be used to treat 'spine dysfunction' disorders is a topic that merits detailed examination.

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References

- Abbas AK, Dozmorov M, Li R, Huang FS, Hellberg F, Danielson J, Tian Y, Ekstrom J, Sandberg M, Wigstrom H. Persistent LTP without triggered protein synthesis. *Neurosci Res.* 2009; 63:59–65. [PubMed: 19013486]
- Aenlle KK, Foster TC. Aging alters the expression of genes for neuroprotection and synaptic function following acute estradiol treatment. *Hippocampus.* 2010; 20:1047–1060. [PubMed: 19790252]
- Arai A, Lynch G. Factors regulating the magnitude of long-term potentiation induced by theta pattern stimulation. *Brain Res.* 1992; 598:173–184. [PubMed: 1486479]
- Arai AC, Xia YF, Suzuki E. Modulation of AMPA receptor kinetics differentially influences synaptic plasticity in the hippocampus. *Neuroscience.* 2004; 123:1011–1024. [PubMed: 14751292]
- Babayan A, Kramár EA, Barrett R, Jafari M, Jakob J, Chen L, Rex CS, Lauterborn JC, Wood M, Gall CM, Lynch G. Integrin Dynamics Produce a Delayed Stage of LTP and Memory Consolidation. *J Neurosci.* 2012; 32:12854–12861. [PubMed: 22973009]
- Barracough DJ, Ingram CD, Brown MW. Chronic treatment with oestradiol does not alter in vitro LTP in subfield CA1 of the female rat hippocampus. *Neuropharmacology.* 1999; 38:65–71. [PubMed: 10193899]
- Ball GF, Auger CJ, Bernard DJ, Charlier TD, Sartor JJ, Ritters LV, Balthazart J. Seasonal plasticity in the song control system: multiple brain sites of steroid hormone action and the importance of variation in song behavior. *Ann N Y Acad Sci.* 2004; 1016:586–610. [PubMed: 15313796]
- Baudry M, Kramár E, Xu X, Zadran H, Moreno S, Lynch G, Gall C, Bi X. Ampakines promote spine actin polymerization, long-term potentiation, and learning in a mouse model of Angelman syndrome. *Neurobiol Dis.* 2012; 47:210–215. [PubMed: 22525571]
- Bi R, Broutman G, Foy MR, Thompson RF, Baudry M. The tyrosine kinase and mitogen-activated protein kinase pathways mediate multiple effects of estrogen in hippocampus. *Proceedings of the National Academy of Sciences of the United States of America.* 2000; 97:3602–3607. [PubMed: 10725383]

- Bodo C, Rissman EF. New roles for estrogen receptor beta in behavior and neuroendocrinology. *Front Neuroendocrinol.* 2006; 27:217–232. [PubMed: 16603234]
- Bolhuis JJ, Gahr M. Neural mechanisms of birdsong memory. *Nat Rev Neurosci.* 2006; 7:347–357. [PubMed: 16760915]
- Bramham CR. Local protein synthesis, actin dynamics, and LTP consolidation. *Curr Opin Neurobiol.* 2008; 18:524–531. [PubMed: 18834940]
- Brinton RD. Estrogen-induced plasticity from cells to circuits: predictions for cognitive function. *Trends Pharmacol Sci.* 2009; 30:212–222. [PubMed: 19299024]
- Carroll JS, Meyer CA, Song J, Li W, Geistlinger TR, Eeckhoutte J, Brodsky AS, Keeton EK, Fertuck KC, Hall GF, Wang Q, Bekiranov S, Sementchenko V, Fox EA, Silver PA, Gingeras TR, Liu XS, Brown M. Genome-wide analysis of estrogen receptor binding sites. *Nat Genet.* 2006; 38:1289–1297. [PubMed: 17013392]
- Chen LY, Rex CS, Babayan AH, Kramár EA, Lynch G, Gall CM, Lauterborn JC. Physiological activation of synaptic Rac>PAK (p-21 activated kinase) signaling is defective in a mouse model of fragile X syndrome. *J Neurosci.* 2010; 30:10977–10984. [PubMed: 20720104]
- Chen LY, Rex CS, Casale MS, Gall CM, Lynch G. Changes in synaptic morphology accompany actin signaling during LTP. *J Neurosci.* 2007; 27:5363–5372. [PubMed: 17507558]
- Chen Y, Kramár EA, Chen LY, Babayan AH, Andres AL, Gall CM, Lynch G, Baram TZ. Impairment of synaptic plasticity by the stress mediator CRH involves selective destruction of thin dendritic spines via RhoA signaling. *Mol Psychiatry.* 2012 ePub, ahead of print.
- de Quervain DJ, Aerni A, Schelling G, Roozendaal B. Glucocorticoids and the regulation of memory in health and disease. *Front Neuroendocrinol.* 2009; 30:358–370. [PubMed: 19341764]
- DeMali KA, Wennerberg K, Burridge K. Integrin signaling to the actin cytoskeleton. *Curr Opin Cell Biol.* 2003; 15:572–582. [PubMed: 14519392]
- Devi G, Hahn K, Massimi S, Zhivotovskaya E. Prevalence of memory loss complaints and other symptoms associated with the menopause transition: a community survey. *Gend Med.* 2005; 2:255–264. [PubMed: 16464737]
- Dumitriu D, Rapp PR, McEwen BS, Morrison JH. Estrogen and the aging brain: an elixir for the weary cortical network. *Ann N Y Acad Sci.* 2010; 1204:104–112. [PubMed: 20738280]
- Fan L, Zhao Z, Orr PT, Chambers CH, Lewis MC, Frick KM. Estradiol-induced object memory consolidation in middle-aged female mice requires dorsal hippocampal extracellular signal-regulated kinase and phosphatidylinositol 3-kinase activation. *J Neurosci.* 2010; 30:4390–4400. [PubMed: 20335475]
- Fink G, Sumner BE, Rosie R, Grace O, Quinn JP. Estrogen control of central neurotransmission: effect on mood, mental state, and memory. *Cell Mol Neurobiol.* 1996; 16:325–344. [PubMed: 8818400]
- Fonseca R, Nagerl UV, Bonhoeffer T. Neuronal activity determines the protein synthesis dependence of long-term potentiation. *Nat Neurosci.* 2006; 9:478–480. [PubMed: 16531998]
- Fonseca R, Nagerl UV, Morris RG, Bonhoeffer T. Competing for memory: hippocampal LTP under regimes of reduced protein synthesis. *Neuron.* 2004; 44:1011–1020. [PubMed: 15603743]
- Foy MR, Akopian G, Thompson RF. Progesterone regulation of synaptic transmission and plasticity in rodent hippocampus. *Learn Mem.* 2008; 15:820–822. [PubMed: 18984562]
- Foy MR, Xu J, Xie X, Brinton RD, Thompson RF, Berger TW. 17beta-estradiol enhances NMDA receptor-mediated EPSPs and long-term potentiation. *J Neurophysiol.* 1999; 81:925–929. [PubMed: 10036289]
- Gibbs RB, Johnson DA. Sex-specific effects of gonadectomy and hormone treatment on acquisition of a 12-arm radial maze task by Sprague Dawley rats. *Endocrinology.* 2008; 149:3176–3183. [PubMed: 18292188]
- Gould E, Woolley CS, Frankfurt M, McEwen BS. Gonadal steroids regulate dendritic spine density in hippocampal pyramidal cells in adulthood. *J Neurosci.* 1990; 10:1286–1291. [PubMed: 2329377]
- Grassi S, Tozzi A, Costa C, Tantucci M, Colcelli E, Scarduzio M, Calabresi P, Pettorossi VE. Neural 17beta-estradiol facilitates long-term potentiation in the hippocampal CA1 region. *Neuroscience.* 2011; 192:67–73. [PubMed: 21749911]

- Gu J, Lee CW, Fan Y, Komlos D, Tang X, Sun C, Yu K, Hartzell HC, Chen G, Bamburg JR, Zheng JQ. ADF/cofilin-mediated actin dynamics regulate AMPA receptor trafficking during synaptic plasticity. *Nat Neurosci.* 2010; 13:1208–1215. [PubMed: 20835250]
- Gungabissoon RA, Bamburg JR. Regulation of growth cone actin dynamics by ADF/cofilin. *J Histochem Cytochem.* 2003; 51:411–420. [PubMed: 12642619]
- Hammond R, Mauk R, Ninaci D, Nelson D, Gibbs RB. Chronic treatment with estrogen receptor agonists restores acquisition of a spatial learning task in young ovariectomized rats. *Horm Behav.* 2009; 56:309–314. [PubMed: 19560466]
- Harvey J, Shanley LJ, O'Malley D, Irving AJ. Leptin: a potential cognitive enhancer? *Biochem Soc Trans.* 2005; 33:1029–1032. [PubMed: 16246038]
- Harvey CD, Yasuda R, Zhong H, Svoboda K. The spread of Ras activity triggered by activation of a single dendritic spine. *Science.* 2008; 321:136–140. [PubMed: 18556515]
- Huang YZ, McNamara JO. Mutual regulation of Src family kinases and the neurotrophin receptor TrkB. *J Biol Chem.* 2010; 285:8207–8217. [PubMed: 20064930]
- Huang GZ, Woolley CS. Estradiol acutely suppresses inhibition in the hippocampus through a sex-specific endocannabinoid and mGluR-dependent mechanism. *Neuron.* 2012; 74:801–808. [PubMed: 22681685]
- Imamura Y, Wang H, Matsumoto N, Muroya T, Shimazaki J, Ogura H, Shimazu T. Interleukin-1 β causes long-term potentiation deficiency in a mouse model of septic encephalopathy. *Neuroscience.* 2011; 187:63–69. [PubMed: 21571042]
- Inagaki T, Gautreaux C, Luine V. Acute estrogen treatment facilitates recognition memory consolidation and alters monoamine levels in memory-related brain areas. *Horm Behav.* 2010; 58:415–426. [PubMed: 20553724]
- Jacome LF, Gautreaux C, Inagaki T, Mohan G, Alves S, Lubbers LS, Luine V. Estradiol and ER β agonists enhance recognition memory, and DPN, an ER β agonist, alters brain monoamines. *Neurobiol Learn Mem.* 2010; 94:488–498. [PubMed: 20828630]
- Kim MT, Soussou W, Gholmieh G, Ahuja A, Tanguay A, Berger TW, Brinton RD. 17 β -Estradiol potentiates field excitatory postsynaptic potentials within each subfield of the hippocampus with greatest potentiation of the associational/commissural afferents of CA3. *Neuroscience.* 2006; 141:391–406. [PubMed: 16725270]
- Kramár EA, Babayan AH, Gavin CF, Cox CD, Jafari M, Gall CM, Rumbaugh G, Lynch G. Synaptic evidence for the efficacy of spaced learning. *Proc Natl Acad Sci USA.* 2012a; 109:5121–5126. [PubMed: 22411798]
- Kramár EA, Bernard JA, Gall CM, Lynch G. Integrins modulate fast excitatory transmission at hippocampal synapses. *J Bio Chem.* 2003; 278:10722–10730. [PubMed: 12524441]
- Kramár EA, Chen LY, Brandon NJ, Rex CS, Liu F, Gall CM, Lynch G. Cytoskeletal changes underlie estrogen's acute effects on synaptic transmission and plasticity. *J Neurosci.* 2009a; 29:12982–12993. [PubMed: 19828812]
- Kramár EA, Chen LY, Lauterborn JC, Simmons DA, Gall CM, Lynch G. BDNF upregulation rescues synaptic plasticity in middle-aged ovariectomized rats. *Neurobiol Aging.* 2012b; 33:708–719. [PubMed: 20674095]
- Kramár EA, Chen LY, Rex CS, Gall CM, Lynch G. Estrogen's Place in the Family of Synaptic Modulators. *Mol Cell Pharmacol.* 2009b; 1:258–262. [PubMed: 20419049]
- Kramár EA, Lin B, Lin CY, Arai AC, Gall CM, Lynch G. A novel mechanism for the facilitation of theta-induced long-term potentiation by brain-derived neurotrophic factor. *J Neurosci.* 2004; 24:5151–5161. [PubMed: 15175384]
- Kramár EA, Lin B, Rex CS, Gall CM, Lynch G. Integrin-driven actin polymerization consolidates long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America.* 2006; 103:5579–5584. [PubMed: 16567651]
- Kretz O, Fester L, Wehrenberg U, Zhou L, Brauckmann S, Zhao S, Prange-Kiel J, Naumann T, Jarry H, Frotscher M, Rune GM. Hippocampal synapses depend on hippocampal estrogen synthesis. *J Neurosci.* 2004; 24:5913–5921. [PubMed: 15229239]

- Krizsan-Agbas D, Pedchenko T, Hasan W, Smith PG. Oestrogen regulates sympathetic neurite outgrowth by modulating brain derived neurotrophic factor synthesis and release by the rodent uterus. *Eur J Neurosci.* 2003; 18:2760–2768. [PubMed: 14656325]
- Krucker T, Siggins GR, Halpain S. Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proceedings of the National Academy of Sciences of the United States of America.* 2000; 97:6856–6861. [PubMed: 10823894]
- Lauterborn JC, Rex CS, Kramár E, Chen LY, Pandeyarajan V, Lynch G, Gall CM. Brain-derived neurotrophic factor rescues synaptic plasticity in a mouse model of fragile X syndrome. *J Neurosci.* 2007; 27:10685–10694. [PubMed: 17913902]
- Lee AW, Pfaff DW. Hormone effects on specific and global brain functions. *J Physiol Sci.* 2008; 58:213–220. [PubMed: 18505601]
- Lee FS, Chao MV. Activation of Trk neurotrophin receptors in the absence of neurotrophins. *Proceedings of the National Academy of Sciences of the United States of America.* 2001; 98:3555–3560. [PubMed: 11248116]
- Lewis MC, Kerr KM, Orr PT, Frick KM. Estradiol-induced enhancement of object memory consolidation involves NMDA receptor and protein kinase A in the dorsal hippocampus of female C57BL/6 mice. 2008
- Liu F, Day M, Muniz LC, Bitran D, Arias R, Revilla-Sanchez R, Grauer S, Zhang G, Kelley C, Pulito V, Sung A, Mervis RF, Navarra R, Hirst WD, Reinhart PH, Marquis KL, Moss SJ, Pangalos MN, Brandon NJ. Activation of estrogen receptor-beta regulates hippocampal synaptic plasticity and improves memory. *Nat Neurosci.* 2008; 11:334–343. [PubMed: 18297067]
- Liu X, Wu Z, Hayashi Y, Nakanishi H. Age-dependent neuroinflammatory responses and deficits in long-term potentiation in the hippocampus during systemic inflammation. *Neuroscience.* 2012; 216:133–142. [PubMed: 22554776]
- Luine VN, Jacome LF, Maclusky NJ. Rapid enhancement of visual and place memory by estrogens in rats. *Endocrinology.* 2003; 144:2836–2844. [PubMed: 12810538]
- Lynch G, Rex CS, Chen LY, Gall CM. The substrates of memory: defects, treatments, and enhancement. *Eur J Pharmacol.* 2008; 585:2–13. [PubMed: 18374328]
- Lynch G, Rex CS, Gall CM. LTP consolidation: substrates, explanatory power, and functional significance. *Neuropharmacology.* 2007; 52:12–23. [PubMed: 16949110]
- Lynch MA. Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? *Prog Neurobiol.* 1998; 56:571–589. [PubMed: 9775404]
- Maras PM, Baram TZ. Sculpting the hippocampus from within: stress, spines, and CRH. *Trends Neurosci.* 2012; 35:315–324. [PubMed: 22386641]
- Meng Y, Zhang Y, Tregoubov V, Falls DL, Jia Z. Regulation of spine morphology and synaptic function by LIMK and the actin cytoskeleton. *Rev Neurosci.* 2003; 14:233–240. [PubMed: 14513866]
- Milner TA, Ayoola K, Drake CT, Herrick SP, Tabori NE, McEwen BS, Warrior S, Alves SE. Ultrastructural localization of estrogen receptor beta immunoreactivity in the rat hippocampal formation. *J Comp Neurol.* 2005; 491:81–95. [PubMed: 16127691]
- Minichiello L. TrkB signalling pathways in LTP and learning. *Nat Rev Neurosci.* 2009; 10:850–860. [PubMed: 19927149]
- Moller MC, Bartfai AB, Radestad AF. Effects of testosterone and estrogen replacement on memory function. *Menopause.* 2010; 17:983–989. [PubMed: 20555288]
- Mortillo S, Elste A, Ge Y, Patil SB, Hsiao K, Huntley GW, Davis RL, Benson DL. Compensatory redistribution of neuroligins and N-cadherin following deletion of synaptic beta1-integrin. *J Comp Neurol.* 2012; 520:2041–2052. [PubMed: 22488504]
- Mukai H, Tsurugizawa T, Murakami G, Kominami S, Ishii H, Ogiue-Ikeda M, Takata N, Tanabe N, Furukawa A, Hojo Y, Ooishi Y, Morrison JH, Janssen WG, Rose JA, Chambon P, Kato S, Izumi S, Yamazaki T, Kimoto T, Kawato S. Rapid modulation of long-term depression and spinogenesis via synaptic estrogen receptors in hippocampal principal neurons. *J Neurochem.* 2007; 100:950–967. [PubMed: 17266735]
- Nagappan G, Woo NH, Lu B. A “zinc” link between TrkB transactivation and synaptic plasticity. *Neuron.* 2008; 57:477–479. [PubMed: 18304477]

- Oviedo PJ, Sobrino A, Laguna-Fernandez A, Novella S, Tarin JJ, Garcia-Perez MA, Sanchis J, Cano A, Hermenegildo C. Estradiol induces endothelial cell migration and proliferation through estrogen receptor-enhanced RhoA/ROCK pathway. *Mol Cell Endocrinol.* 2011; 335:96–103. [PubMed: 20615453]
- Pechenino AS, Frick KM. The effects of acute 17 beta-estradiol treatment on gene expression in the young female mouse hippocampus. *Neurobiol Learn Mem.* 2009; 91:315–322. [PubMed: 18938255]
- Pinkstaff JK, Detterich J, Lynch G, Gall C. Integrin subunit gene expression is regionally differentiated in adult brain. *J Neurosci.* 1999; 19:1541–1556. [PubMed: 10024342]
- Pinkstaff JK, Lynch G, Gall CM. Localization and seizure-regulation of integrin beta 1 mRNA in adult rat brain. *Brain Res Mol Brain Res.* 1998; 55:265–276. [PubMed: 9582434]
- Porrino LJ, Daunais JB, Rogers GA, Hampson RE, Deadwyler SA. Facilitation of task performance and removal of the effects of sleep deprivation by an ampakine (CX717) in nonhuman primates. *PLoS Biol.* 2005; 3:e299. [PubMed: 16104830]
- Prange-Kiel J, Wehrenberg U, Jarry H, Rune GM. Para/autocrine regulation of estrogen receptors in hippocampal neurons. *Hippocampus.* 2003; 13:226–234. [PubMed: 12699330]
- Rapp PR, Morrison JH, Roberts JA. Cyclic estrogen replacement improves cognitive function in aged ovariectomized rhesus monkeys. *J Neurosci.* 2003; 23:5708–5714. [PubMed: 12843274]
- Reichardt LF. Neurotrophin-regulated signalling pathways. *Philos Trans R Soc Lond B Biol Sci.* 2006; 361:1545–1564. [PubMed: 16939974]
- Rex CS, Chen LY, Sharma A, Liu J, Babayan AH, Gall CM, Lynch G. Different Rho GTPase-dependent signaling pathways initiate sequential steps in the consolidation of long-term potentiation. *J Cell Biol.* 2009; 186:85–97. [PubMed: 19596849]
- Rex CS, Gavin CF, Rubio MD, Kramár EA, Chen LY, Jia Y, Haganir RL, Muzyczka N, Gall CM, Miller CA, Lynch G, Rumbaugh G. Myosin IIb regulates actin dynamics during synaptic plasticity and memory formation. *Neuron.* 2010; 67:603–617. [PubMed: 20797537]
- Rex CS, Lin CY, Kramár EA, Chen LY, Gall CM, Lynch G. Brain-derived neurotrophic factor promotes long-term potentiation-related cytoskeletal changes in adult hippocampus. *J Neurosci.* 2007; 27:3017–3029. [PubMed: 17360925]
- Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA. Disruption of estrogen receptor beta gene impairs spatial learning in female mice. *Proc Natl Acad Sci USA.* 2002; 99:3996–4001. [PubMed: 11891272]
- Rhodes ME, Frye CA. ERbeta-selective SERMs produce mnemonic-enhancing effects in the inhibitory avoidance and water maze tasks. *Neurobiol Learn Mem.* 2006; 85:183–191. [PubMed: 16326119]
- Sarmiere PD, Bamberg JR. Regulation of the neuronal actin cytoskeleton by ADF/cofilin. *J Neurobiol.* 2004; 58:103–117. [PubMed: 14598374]
- Sato K, Akaishi T, Matsuki N, Ohno Y, Nakazawa K. Beta-Estradiol induces synaptogenesis in the hippocampus by enhancing brain-derived neurotrophic factor release from dentate gyrus granule cells. *Brain Res.* 2007; 1150:108–120. [PubMed: 17433270]
- Scharfman HE, Mercurio TC, Goodman JH, Wilson MA, MacLusky NJ. Hippocampal excitability increases during the estrous cycle in the rat: a potential role for brain-derived neurotrophic factor. *J Neurosci.* 2003; 23:11641–11652. [PubMed: 14684866]
- Seese RR, Babayan AH, Katz AM, Cox CD, Lauterborn JC, Lynch G, Gall CM. LTP induction translocates cortactin at distant synapses in wild-type but not Fmr1 knock-out mice. *J Neurosci.* 2012; 32:7403–7413. [PubMed: 22623686]
- Sharrow KM, Kumar A, Foster TC. Calcineurin as a potential contributor in estradiol regulation of hippocampal synaptic function. *Neuroscience.* 2002; 113:89–97. [PubMed: 12123687]
- Sherwin BB. Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology.* 1988; 13:345–357. [PubMed: 3067252]
- Smart FM, Edelman GM, Vanderklish PW. BDNF induces translocation of initiation factor 4E to mRNA granules: evidence for a role of synaptic microfilaments and integrins. *Proc Natl Acad Sci U S A.* 2003; 100:14403–14408. [PubMed: 14623964]

- Smejkalova T, Woolley CS. Estradiol acutely potentiates hippocampal excitatory synaptic transmission through a presynaptic mechanism. *J Neurosci.* 2010; 30:16137–16148. [PubMed: 21123560]
- Smith CC, McMahon LL. Estrogen-induced increase in the magnitude of long-term potentiation occurs only when the ratio of NMDA transmission to AMPA transmission is increased. *J Neurosci.* 2005; 25:7780–7791. [PubMed: 16120779]
- Smith CC, McMahon LL. Estradiol-induced increase in the magnitude of long-term potentiation is prevented by blocking NR2B-containing receptors. *J Neurosci.* 2006; 26:8517–8522. [PubMed: 16914677]
- Smith CC, Vedder LC, Nelson AR, Bredemann TM, McMahon LL. Duration of estrogen deprivation, not chronological age, prevents estrogen's ability to enhance hippocampal synaptic physiology. *Proc Natl Acad Sci USA.* 2010; 107:19543–19548. [PubMed: 20974957]
- Snyder MA, Cooke BM, Woolley CS. Estradiol potentiation of NR2B-dependent EPSCs is not due to changes in NR2B protein expression or phosphorylation. *Hippocampus.* 2011; 21:398–408. [PubMed: 20082293]
- Spencer JL, Waters EM, Romeo RD, Wood GE, Milner TA, McEwen BS. Uncovering the mechanisms of estrogen effects on hippocampal function. *Front Neuroendocrinol.* 2008; 29:219–237. [PubMed: 18078984]
- Spyrka J, Danielewicz J, Hess G. Brief neck restraint stress enhances long-term potentiation and suppresses long-term depression in the dentate gyrus of the mouse. *Brain Res Bull.* 2011; 85:363–367. [PubMed: 21624439]
- Srivastava DP, Penzes P. Rapid estradiol modulation of neuronal connectivity and its implications for disease. *Frontiers in endocrinology.* 2011; 2:77. [PubMed: 22654827]
- Srivastava DP, Woolfrey KM, Jones KA, Shum CY, Lash LL, Swanson GT, Penzes P. Rapid enhancement of two-step wiring plasticity by estrogen and NMDA receptor activity. *Proceedings of the National Academy of Sciences of the United States of America.* 2008; 105:14650–14655. [PubMed: 18801922]
- Srivastava DP, Woolfrey KM, Liu F, Brandon NJ, Penzes P. Estrogen receptor β activity modulates synaptic signaling and structure. *J Neurosci.* 2010; 30:13454–13460. [PubMed: 20926671]
- Vearncombe KJ, Rolfe M, Andrew B, Pachana NA, Wright M, Beadle G. Cognitive effects of chemotherapy-induced menopause in breast cancer. *Clin Neuropsychol.* 2011; 25:1295–1313. [PubMed: 22047059]
- Vierk R, Glassmeier G, Zhou L, Brandt N, Fester L, Dudzinski D, Wilkars W, Bender RA, Lewerenz M, Gloger S, Graser L, Schwarz J, Rune GM. Aromatase inhibition abolishes LTP generation in female but not in male mice. *J Neurosci.* 2012; 32:8116–8126. [PubMed: 22699893]
- Wang Z, Edwards JG, Riley N, Provance DW Jr, Karcher R, Li XD, Davison IG, Ikebe M, Mercer JA, Kauer JA, Ehlers MD. Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell.* 2008; 135:535–548. [PubMed: 18984164]
- Waters EM, Yildirim M, Janssen WG, Lou WY, McEwen BS, Morrison JH, Milner TA. Estrogen and aging affect the synaptic distribution of estrogen receptor beta-immunoreactivity in the CA1 region of female rat hippocampus. *Brain Res.* 2011; 1379:86–97. [PubMed: 20875808]
- Wegesin DJ, Stern Y. Effects of hormone replacement therapy and aging on cognition: evidence for executive dysfunction. *Aging Neuropsychol Cogn.* 2007; 14:301–328.
- Wiesner S, Legate KR, Fassler R. Integrin-actin interactions. *Cell Mol Life Sci.* 2005; 62:1081–1099. [PubMed: 15761669]
- Yamauchi J, Chan JR, Shooter EM. Neurotrophins regulate Schwann cell migration by activating divergent signaling pathways dependent on Rho GTPases. *Proceedings of the National Academy of Sciences of the United States of America.* 2004; 101:8774–8779. [PubMed: 15161978]
- Yang Y, Wang XB, Frerking M, Zhou Q. Delivery of AMPA receptors to perisynaptic sites precedes the full expression of long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America.* 2008; 105:11388–11393. [PubMed: 18682558]
- Yun SH, Park KA, Kwon S, Woolley CS, Sullivan PM, Pasternak JF, Trommer BL. Estradiol enhances long term potentiation in hippocampal slices from aged apoE4-TR mice. *Hippocampus.* 2007; 17:1153–1157. [PubMed: 17696167]

- Villers A, Godaux E, Ris L. Long-lasting LTP requires neither repeated trains for its induction nor protein synthesis for its development. *PLoS One*. 2012; 7:e40823. [PubMed: 22792408]
- Zadran S, Qin Q, Bi X, Zadran H, Kim Y, Foy MR, Thompson R, Baudry M. 17-Beta-estradiol increases neuronal excitability through MAP kinase-induced calpain activation. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:21936–21941. [PubMed: 19995977]

Abbreviations

17β-estradiol	E2
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
APV	(2 <i>R</i>)-amino-5-phosphonopentanoate
BDNF	brain-derived neurotrophic factor
DMSO	dimethyl sulfoxide
EB	estradiol benzoate
EPSP	excitatory postsynaptic potential
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
fEPSP	field excitatory postsynaptic potential
GABA	γ -Aminobutyric acid
GEFs	guanine exchange factors
GTP	guanosine-5'-triphosphate
LTP	long term potentiation
LFS	low-frequency stimulation
NMDAR	<i>N</i> -methyl -D-aspartate receptor
OVX	ovariectomized
PAK	p21-activated kinase
PPT	4,4',4''-[4-propyl-(1 <i>H</i>)-pyrazole-1,3,5-triyl]trisphenol
pTrkB	phosphorylated TrkB
PTX	picrotoxin
ROCK	RhoA kinase
str	stratum
TBS	theta burst stimulation
TrkB	tropomyosin-related kinase B

Highlights

Brief infusions of estrogen enhance synaptic transmission and LTP via ER β stimulation

Estrogen promotes actin polymerization by activating RhoA-GTPase signaling

Acute estrogen treatment rapidly increases postsynaptic phospho-TrkB

Possible routes by which estrogen influences RhoA signaling are discussed

Long term ovariectomy disrupts actin remodeling and LTP, and reduces activated RhoA

Figure 1. E2 facilitates baseline synaptic transmission and LTP through estrogen receptor beta
 A) Infusions of 17 β -estradiol (E2) reversibly increases the field excitatory postsynaptic potential (fEPSP) slope and amplitude in acute hippocampal slices prepared from adult male rats. Inset: Traces collected during baseline recordings and at the end of E2 infusion (calibration: 1mV, 5ms). B) Hippocampal slices were continuously perfused with vehicle (0.01% DMSO), 100nM of the estrogen receptor α agonist PPT, or 1nM E2. Two or three theta bursts did not markedly increase the fEPSP slope in slices treated with vehicle or PPT, but did produce stable potentiation in slices treated with E2. Five and ten theta bursts elicited similar levels of LTP in slices infused with vehicle or PPT, but induced much greater potentiation in the presence of E2. The upper dashed line denotes maximum LTP during control (aCSF) conditions. Modified from Kramár et al., 2009a.

Figure 2. E2 enhances AMPAR-mediated components of the fEPSP

In adult hippocampal slices stimulation was applied to the Schaffer-commissural projection and fEPSPs were recorded from CA1b stratum radiatum. A) The AMPAR-mediated component of the fEPSP was isolated by continuous infusion of the NMDAR antagonist, APV (100 μ M) and the GABA_A-receptor antagonist, picrotoxin (PTX, 100 μ M). Under these conditions application of 1 nM E2 rapidly increased the amplitude of the AMPAR-mediated response in a manner comparable to that obtained in otherwise untreated slices (see Fig 1); elimination of the fEPSP by infusion of the AMPAR antagonist DNQX (20 μ M) verified that responses were indeed AMPAR-mediated. B) Representative traces illustrate the effect of E2 on isolated AMPAR-mediated fEPSPs (slices stimulated in the presence of PTX and APV). C) Traces show the composite fEPSP response to the first and fourth bursts in a theta train and the typical increase in the size of later burst responses. D) Graph shows the percent increase in burst response area for bursts 2-10, expressed as a percent of the first burst response, for slices bathed in aCSF plus vehicle (0.01% DMSO), 100 nM PPT, or 1 nM E2; as shown, no significant differences in within train-facilitation were found between groups. Modified from Kramár et al., 2009a.

Figure 3. E2 promotes actin polymerization in CA1 dendritic spines by activating the RhoAGTPase signaling pathway

A, B) Fluorescent-tagged phalloidin was applied to adult rat hippocampal slices which were then processed for localization of phalloidin-labeled F-actin using epifluorescence microscopy (Kramár et al., 2006, Rex et al., 2007). A) Images show phalloidin labeling in CA1 stratum (str.) radiatum (scale bar, 5 μm). The two left panels show the typical increase in labeled spine-like profiles (white puncta, arrow) in slices treated for 20 min with 1 nM E2 as compared to vehicle (Veh). The two right panels show that latrunculin A (500nM) blocks the E2-induced increase in spine F-actin but does not influence phalloidin labeling when applied alone. B) Bar graph shows the mean ($\pm$ SEM) number of phalloidin-labeled spines in the CA1b sample field was greater in E2-treated slices as compared to vehicle controls (* $p < 0.001$), and that latrunculin blocks E2-induced increases in phalloidin labeling. C) Hippocampal slices received stimulation of Schaffercommissural afferents to field CA1; plot shows fEPSPs recorded from CA1b str. radiatum collected over time. Pretreatment with latrunculin blocked both E2-induced increases in synaptic transmission in the control pathway and TBS-induced LTP in the experimental pathway. Following latrunculin washout, the fEPSP response to E2 was restored. D) Bar graph shows quantification of phalloidin-labeled spines in CA1 str radiatum of slices that received low frequency stimulation (LFS, 0.03Hz) or 3 theta bursts, with vehicle (Veh) or 1 nM E2 in the tissue

bath; in these experiments rhodaminephalloidin was applied 60 min after E2 washout. Three bursts increased numbers of phalloidin-labeled (F-actin enriched) spines in the presence of E2, but not vehicle, and the increase was sustained through the 60 min E2 washout (* $p < 0.001$ vs E2 without). E) Western blot analysis showed that 20 min infusion of 1nM E2 increased phosphorylated (p) cofilin (Ser 3) (* $p = 0.04$) without changing total cofilin levels. F) Pull-down assays show that E2 treatment of adult hippocampal slices significantly increased levels of “Active” (GTP-bound) RhoA without altering activated Rac or Cdc42 (* $p < 0.05$) or “Total” GTPase levels. Activated RhoA returned to control levels after a 60 min E2 washout. Modified from Kramár et al., 2009a.

Figure 4. E2 selectively activates RhoA-to-actin signaling in adult hippocampal slices Estrogen receptor beta (ERβ), BDNF's receptor TrkB, and β1-family integrins have been shown to facilitate, or be required for, LTP and the effects of synaptic activity on the actin cytoskeleton (see Lynch et al., 2008, Kramár et al., 2009b for review). All three receptors activate Rho-GTPases via various guanine exchange factors (GEFs). We found that, in adult hippocampal slices, E2 activates RhoA which, through its effector ROCK, triggers phosphorylation, and thus inactivation, of cofilin. This last event persists for at least 15 min (left, light gray bar) and allows actin monomers to assemble into filaments. The β1 integrins and TrkB also activate Rac and Cdc42, and their effector PAK, and thereby coordinate activities of a number of actin binding proteins (e.g., cortactin, ARP2/3) that promote actin filament branching and stabilization (lower gray bar; length of this bar denotes progressive stabilization over time). The actin networks resulting from the above events contribute to a number of activity-dependent changes in the synaptic zone including activation and translocation to the postsynaptic density of CaMKII, a kinase that phosphorylates the AMPAR GluA1 Ser831 site (black dots). This phosphorylation increases ligand-gated conductance and thus EPSP size.

Figure 5. The E2-induced increase in fEPSP slope is associated with increased postsynaptic TrkB phosphorylation

A) Hippocampal slices infused with 1nM E2 were collected at 5, 10, and 30 min following the onset of increased fEPSP responses and processed for deconvolution immunofluorescence microscopy as described (Rex et al., 2009). B) Deconvolved immunofluorescence images show punctate immunolabeling for phosphorylated (p) TrkB^{Y515} (red) and PSD95 (green), and a merged image of the two: scattered double-labeled puncta appear yellow. Scale bar: 5μm. C) Graph shows counts of double-labeled, pTrkB^{Y515} / PSD95+ synapses in the CA1b sample field of slices receiving low frequency stimulation (LFS, 0.03Hz) alone (white bar) or LFS plus 1 nM E2 (grey bars), the latter values were normalized to paired LFS-controls. E2 increased the number of postsynaptic elements immunoreactive for pTrkB^{Y515} after 10 min following the onset of increased field responses (numbers in each bar denote slices/group; **p=0.004 vs LFS).

Figure 6. Signaling pathways that could mediate E2's effects on the spine cytoskeleton

There is evidence that estrogen's ERβ, TrkB and the β1 family integrins all activate signaling through RhoA to facilitate activity-induced remodeling of the dendritic spine actin cytoskeleton. The diagrams above illustrate three different schemes through which E2/ERβ may facilitate actin polymerization. A) The three receptor families may act independently with each influencing guanine exchange factors (GEFs) to activate RhoA signaling leading to actin filament assembly. B) E2, acting through ERβ, may transactivate neighboring TrkB, and possibly also β1 integrins; the latter receptors would then signal through GEFs to activate RhoA. C) Both direct and transactivation processes may be present with weak activation of RhoA via downstream ERβ signaling (dotted line) being reinforced by signaling from transactivated TrkB and β1 integrins

Figure 7. E2 may rescue synaptic plasticity in middle aged ovariectomized (OVX) rats through effects on spine actin polymerization and RhoA activity

A) A conventional ten burst theta train (TBS) produced stable LTP in hippocampal slices from 9-10 month old ovariectomized (OVX) rats treated with estradiol benzoate (EB) (10ug/ml injection every other day for 2 weeks) but not from OVX rats given vehicle. Insets show representative traces collected during baseline (solid line) and 60 min post-TBS (dotted line). Calibration: 1mV, 5ms. B) Bar graph shows quantification of densely phalloidin+ puncta within the CA1b field of afferent stimulation in slices from OVX rats receiving low-frequency stimulation (0.03Hz, LFS) or TBS with or without EB pretreatment: TBS increased the number of densely labeled, F-actin enriched, spines in slices from EB-treated OVX rats relative to slices from vehicle-treated OVX rats (* $p<0.0001$). C) Representative western blots and bar graph show pull down assay quantification of active and total RhoA in hippocampal slices from EB- and vehicle-treated OVX rats: as shown, levels of active, GTP-bound RhoA were significantly elevated by EB treatment (* $p=0.03$) whereas total RhoA levels were comparable between groups. Modified from Kramár et al., 2009a.