The influence of sensory experience on the glutamatergic synapse.

Daniel D. Cooper[+] and Bruno G. Frenguelli*

School of Life Sciences, University of Warwick, Coventry, UK,

*To whom correspondence should be addressed at:

Professor Bruno G. Frenguelli

School of Life Sciences

University of Warwick

Coventry

UK

CV4 7AL

Tel 02467 150591

Email b.g.frenguelli@warwick.ac.uk

[+]Present address

Bayberry Wellness and Recovery Centres Ltd.

Small Lane

Earlswood

Solihull

UK

B94 5EL

Highlights

Environmental enrichment has been repeatedly shown to improve cognition

This improvement likely resides in enhanced plasticity at glutamatergic synapses

BDNF, the MAPK cascade and MSK1 play pivotal roles in these enhancements

MSK1 underpins the metaplastic enhanced dynamic range of enriched synapses

MSK1 also orchestrates a genomic homeostatic downscaling of plasticity gene expression

MSK1 stabilises the enriched brain and makes it better able to respond to novelty

Abstract

The ability of glutamatergic synaptic strength to change in response to prevailing neuronal activity is believed to underlie the capacity of animals, including humans, to learn from experience. This learning better equips animals to safely navigate challenging and potentially harmful environments, while reinforcing behaviours that are conducive to survival. Early descriptions of the influence of experience on behaviour were provided by Donald Hebb who showed that an enriched environment improved performance of rats in a variety of behavioural tasks, challenging the widely-held view at the time that psychological development and intelligence were largely predetermined through genetic inheritance. Subsequent studies in a variety of species provided detailed cellular and molecular insights into the neurobiological adaptations associated with enrichment and its counterparts, isolation and deprivation. Here we review those experience-dependent changes that occur at the glutamatergic synapse, and which likely underlie the enhanced cognition associated with enrichment. We focus on the importance of signalling initiated by the release of BDNF, and a prime downstream effector, MSK1, in orchestrating the many structural and functional neuronal adaptations associated with enrichment. In particular we discuss the MSK1-dependent expansion of the dynamic range of the glutamatergic synapse, which may allow enhanced information storage or processing, and the establishment of a genomic homeostasis that may both stabilise the enriched brain, and may make it better able to respond to novel experiences.

Keywords: environmental enrichment; MSK1, BDNF, cognitive reserve; LTP; LTD; synaptic plasticity; metaplasticity; homeostatic synaptic scaling; RNA seq; transcriptomics; gene expression

Introduction

The concept of the glutamatergic synapse, its ability to undergo activity-dependent modifications in the strength of communication across it, and the contribution this makes to learning and memory, are now widely taken for granted. However, these concepts, in particular that glutamate is a neurotransmitter, were initially met with scepticism. The seminal review written by Jeff Watkins and Dick Evans in 1981, the 40th anniversary of which this series of Special Issues of *Neuropharmacology* celebrates, firmly established the veracity of glutamate as a neurotransmitter through both their careful appraisal of the literature, and more importantly, through their development and use of antagonists for the excitation produced by exogenous glutamate receptor agonists and fiber tract stimulation (Watkins and Evans, 1981). Equally seminal contributions around this time demonstrated that these glutamatergic synapses could undergo activity-dependent modifications in their strength (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973), that this modification – long-term potentiation (LTP) - required the activation of the NMDA subtype of glutamate receptor (Collingridge et al., 1983), and that antagonism of this particular receptor interfered with the learning of a behavioural task (Morris et al., 1986). The rest, as they say, is history, and neatly summarised elsewhere (Bliss and Collingridge, 2019; Takeuchi et al., 2014).

Long before these studies however, psychologists were grappling with questions regarding the mechanisms through which psychological development occurred, and the extent to which experience influenced this development. Was there just a physiological program that emerged through developmental maturity and from which arose cognitive properties based upon a genetic predisposition, or did early developmental experiences shape the outcome for cognition and intelligence (Hunt, 1979)? Quite remarkably, the Canadian psychologist Donald Hebb in his famous book, The Organisation of Behavior (Hebb, 1949), straddled both the concept of activity-dependent changes in the efficiency of the communication between neurones ("plasticity"), and the importance of experience in shaping cognitive development, which today we might refer to as "metaplasticity" – the process through which the propensity to display plasticity is enhanced (Abraham, 2008). The former has given birth to the ideas and observations surrounding the Hebb synapse and the storing of memories in cell assemblies (Brown, 2020). The latter has spawned its own paradigm on the influence of early experience on adult cognitive outcomes (Miguel et al., 2019), and, moreover,

government-led societal programs to assist children from deprived backgrounds (Brown and Milner, 2003; Hunt, 1979). There is considerable evidence as to the efficacy of these programs (Cattan et al., 2019; ETI, 2018; Sanders et al., 2014), but which is all too often unfortunately ignored for political or ideological expediency. Enrichment strategies are also being deployed in the elderly where benefits are also being seen (Dause and Kirby, 2019), in children with autism spectrum disorders (Woo et al., 2015; Woo and Leon, 2013), post-stroke rehabilitation (McDonald et al., 2018), and are starting to be evaluated in the treatment of substance use disorders (Galaj et al., 2020), including alcohol abuse (Pang et al., 2019).

Of the possible interfaces between sensory experience and psychological outcome, the glutamatergic synapse has received the most attention. This is by virtue of the ability to study fast excitatory synaptic transmission at central synapses, the importance of glutamate receptors in synaptic plasticity, together with the occurrence of glutamatergic synaptic plasticity in regions of the brain implicated in a range of cognitive processes. In this review we consider some of the changes that occur at the glutamatergic synapse in response to environmental enrichment. We discuss the often-cited importance of BDNF in initiating the synaptic changes associated with enrichment, and describe our own work with MSK1, an enzyme downstream of BDNF that regulates gene expression. We propose that MSK1, through the regulation of key plasticity-related proteins such as CREB, Arc/Arg3.1 and Egr1, is necessary for the metaplasticity underlying the expansion of the dynamic range of synapses after enrichment and, as a consequence, the full expression of experience-dependent cognitive enhancement (Privitera et al., 2020). Moreover, in order to avoid runaway changes in synaptic strength, we propose that MSK1, in keeping with its regulation of homeostatic synaptic plasticity (Corrêa et al., 2012), orchestrates a genomic homeostasis characterised by its own down regulation, and that of several plasticity-related proteins (Privitera et al., 2020). MSK1 may thus serve as a key transducer in responding to experience-dependent changes in BDNF, and regulating gene expression to enshrine the neuronal and cognitive adaptations provoked by changes in prevailing synaptic activity.

Environmental Enrichment and the glutamatergic synapse

Donald Hebb's unorthodox but effective means by which to provide lab rats with an enriched environment was to allow them free run of his house with the "enthusiastic assistance" of his two young children (Hebb,

1949). Since not many of us have families, or indeed regulatory authorities, so inclined, subsequent studies have revolved around providing laboratory animals with larger cages, more social contacts, toys, ladders, running wheels, tunnels, see-saws, and platforms on various levels to encourage climbing, and all aimed at promoting more naturalistic and ethologically-relevant behaviour. This variety and lack of standardisation across labs is compounded by the various ages at which animals are exposed to enrichment, their sex and the frequency and durations for which enrichment occurs (Table 1), all of which complicates across-lab comparisons of the effects of enrichment. Moreover, with laboratory animal welfare being increasingly recognised, "standard" housing may contain enrichment items such as nesting materials and cardboard tubes, potentially limiting experimental differentials between housing conditions. Nonetheless, enrichment strategies consistently result in observable differences across a range of assays from neuronal morphology to cognitive performance in wild-type animals and those harbouring genetic modifications or bearing an acquired injury (Table 1).

Early studies on the influences of enrichment on the brain focussed on neurochemical and structural changes, which revealed, among other parameters, quite dramatic changes in brain weight in animals exposed to an enriched or complex environment (Kolb and Whishaw, 1998; Rosenzweig and Bennett, 1996; Rosenzweig and Leiman, 1968). Further studies focussed on synaptic function associated with enrichment, with much emphasis placed on glutamatergic neurones and glutamatergic synapses (Hirase and Shinohara, 2014; Ohline and Abraham, 2019). These studies have largely centred on basal synaptic transmission and LTP, predominately in the hippocampus. The effect of enrichment on glutamatergic signalling within hippocampal area CA1 and the dentate gyrus have recently been reviewed by Ohline and Abraham (2019), who conclude that enrichment is generally observed to facilitate LTP within area CA1, but not the dentate gyrus (Ohline and Abraham, 2019). This may reflect differences in the induction or roles of key plasticity proteins BDNF and Arc/Arg3.1 (Zhang and Bramham, 2020), and so below we consider only area CA1 as we discuss glutamatergic synaptic modifications following enrichment.

Basal glutamatergic synaptic transmission and paired-pulse facilitation

Basal glutamatergic synaptic transmission appears resistant to change following exposure to enrichment (Ohline and Abraham, 2019), generally displaying no difference between enriched and standard housed animals (Table 1). That this occurs despite a general facilitation of LTP following enrichment implies that some form of synaptic homeostasis could have occurred to avoid potential runaway potentiation of synapses. Lack of consistent differences in transmission strength or paired-pulse facilitation (Table 1) following enrichment suggest that enhancements in synaptic plasticity following enrichment are unlikely to be due to changes in basal glutamatergic transmission strength or altered glutamate release probability. Instead, they are likely due to changes in factors controlling the regulation and/or induction of LTP and LTD, at either the genomic or post-translational level.

Long-term potentiation

Studies of LTP modification at the CA3-CA1 synapse following enrichment have used a wide range of enrichment periods, from 2 weeks up to 20 weeks, and have been recorded both *in vitro* in hippocampal brain slices, and *in vivo* (Table 1). LTP facilitation in area CA1 can be observed as early as 2 - 3 weeks after introduction to an enriched environment (Buschler and Manahan-Vaughan, 2012; Buschler and Manahan-Vaughan, 2017; Stein et al., 2016). This enhancement is also observed after longer (4 – 8 week) periods of enrichment (Artola et al., 2006; Cortese et al., 2018; Duffy et al., 2001; Hosseiny et al., 2015; Malik and Chattarji, 2012; Novkovic et al., 2015a; Novkovic et al., 2015b; Zhu et al., 2011), but not always (Foster and Dumas, 2001; Hosseiny et al., 2015; Morelli et al., 2014; Zarif et al., 2018). This enhancement of LTP is still seen at more prolonged periods (8 – 20 weeks) of enrichment (Hullinger et al., 2015; Kumar et al., 2012; Privitera et al., 2020), though not consistently (Bouet et al., 2011; Eckert et al., 2010; van Praag et al., 1999). Exposure to environmental enrichment is also associated with an increase in CA1 dendritic spine density, although this does not always accompany changes in LTP or synaptic transmission (Table 1).

Changes in synaptic efficacy in response to external stimuli are thought to play an important part in learning and memory (Bliss et al., 2018; Takeuchi et al., 2014). Accordingly, examining glutamatergic synaptic efficacy after exposure to an enriched environment provides a valuable cellular neurophysiological substrate against which to compare any enhancements in learning and memory observed after enrichment (Table 1). However,

these comparisons have not always provided consistent results. Enrichment-dependent LTP enhancements have been observed to accompany improvements in cognitive tasks such as learning of the Morris water maze (Privitera et al., 2020; Zhu et al., 2011). However, learning improvements have also observed in animals displaying no LTP enhancement (Bouet et al., 2011; van Praag et al., 1999), and LTP enhancement observed in animals with no learning improvement (Cortese et al., 2018; Kumar et al., 2012). Similarly, environmental enrichment-mediated enhancement of LTP has also been seen alongside improvements in memory, both for novel object memory (Novkovic et al., 2015b) and submerged platform location (Cortese et al., 2018; Kumar et al., 2012; Privitera et al., 2020). This too is not always the case, with LTP enhancement observed accompanying no improvement in novel object/spatial memory (Cortese et al., 2018; Zhu et al., 2011).

Such inconsistencies could revolve around LTP induction protocols. For example, theta-burst stimulation (TBS (Larson et al., 1986)) protocols appear much more robust at eliciting enhanced LTP following enrichment than high frequency stimulation (HFS; tetanus) based protocols (Table 1): 9 of the 11 (82 %) TBS LTP studies listed in Table 1 showed an enhancement of LTP after enrichment, whereas only 11 of the 19 (58 %) HFS LTP studies did so. Perhaps enrichment facilitates LTP through a mechanism preferably recruited during TBS, but not HFS stimulation, and this could underlie the heterogeneity between studies of enrichment-mediated LTP changes? One possible candidate is the MAPK cascade which is believed to be preferentially recruited in response to TBS (Winder et al., 1999), and which, as we will see below, plays a pivotal role in the neuronal response to enrichment. Alternatively, strong LTP induction protocols may obscure a change in the threshold for the induction of LTP: Buschler and Manahan-Vaughan (2012) only observed a difference in the magnitude of LTP when a weaker HFS induction protocol was delivered to CA3-CA1 synapses following 2 weeks of enrichment. These observations suggest that enrichment not only has the capacity to increase the magnitude of plasticity, and hence the weight of synapses, it also influences the propensity or modification threshold ($\phi(c)$ and θ_M, respectively, in the Bienenstock, Cooper & Munro (BCM) relationship (Bienenstock et al., 1982) Figure 1) to exhibit this plasticity. Enrichment can thus be thought of as inducing a form of metaplasticity (Abraham, 2008) that underpins the experience-dependent ability of synapses to facilitate changes in the strength of communication across them.

Differences in housing paradigms may also explain some of the differences observed in LTP facilitation and learning and memory following enrichment. Enrichment generally consists of up to three key components: increased cognitive stimulation, social enrichment and exercise, and the inclusion of these three elements varies between different studies (Table 1). Cognitive stimulation is typically provided by means of a larger home cage and the inclusion of colourful objects along with their rearrangement, which provide sources of novelty and exploration opportunities for animals. One month of increased cognitive stimulation has been seen to be sufficient for the enhancement of LTP magnitude in old (23-24 month) rats when compared with socially housed control animals (Cortese et al., 2018). Two of the experiments included in Table 1 do not contain cognitive stimulation as a part of the enrichment paradigm, but consist of a running wheel-only condition. Of these, one demonstrated an enhancement in LTP (Kumar et al., 2012), and the other no difference (van Praag et al., 1999) but this may be due to the strong (4 x HFS trains) stimulation delivered in this study which may preclude observing more subtle differences in LTP induction (amplitude or threshold; Figure 1). The majority of studies examining LTP in equally sized social groups (no social enrichment) have seen LTP enhancement in the enriched group (Artola et al., 2006; Cortese et al., 2018; Duffy et al., 2001; Hullinger et al., 2015; Kumar et al., 2012; Novkovic et al., 2015a; Novkovic et al., 2015b) but see (Buschler and Manahan-Vaughan, 2012). This indicates that increased social stimulation is not necessary to see an increase in LTP at CA3-CA1 synapses.

Long-term depression

Long-term depression (LTD) on the other hand, when compared to LTP, has not been examined often in enrichment studies (Table 1). Those studies examining NMDA receptor-dependent LTD have generally observed an overall enhancement of LTD following enrichment (Artola et al., 2006; Privitera et al., 2020; Stein et al., 2016), although no change (Buschler and Manahan-Vaughan, 2012), and inhbition (Eckert et al., 2010; Kumar et al., 2012) of LTD following enrichment have also been reported. The implications of enhanced LTD may have particular relevance for cognitive flexibility as disruption of hippocampal CA1 LTD has been shown to impair reversal learning behaviour in spatial tasks (Dong et al., 2013; Mills et al., 2014; Wall et al., 2018). Indeed, environmental enrichment has been seen to enhance reversal learning performance (Cortese et al., 2018; Privitera et al., 2020), which may be explained by the enhancement of LTD observed in one study

(Privitera et al., 2020). Additionally, of the 20 studies in Table 1 examining the effects of enrichment on LTP, only 6 also tested the effect of enrichment on LTD. Future studies would benefit from looking at the effects of enrichment on both LTP and LTD, not only in order to capture a wider range of synaptic modifications that enrichment may modulate, but importantly to determine the range of strengths over which synapses operate after exposure to an enriched environment.

Enrichment has been shown to allow the expression of both LTP and LTD in aged rats in which no such plasticity is observed under standard housing conditions (Stein et al., 2016), while another study revealed that enrichment enhanced both LTP and LTD (Privitera et al., 2020). These observations indicate that enriched synapses can bidirectionally weaken and strengthen further than those of their standard housed counterparts – in short, enrichment induces an expansion of the dynamic range of synapses (Figure 1). While this may reflect a change in the threshold (θ_M) for the induction of synaptic plasticity caused by enrichment (Buschler and Manahan-Vaughan, 2012), the study by Artola et al., (2006) indicates that the floor and ceiling of synaptic transmission may also be further apart since they observed greater enhancements and depressions of synaptic transmission ($\phi(c)$) with subsequent presentations of HFS and LFS, respectively (Artola et al., 2006). Thus, enrichment may allow synapses to be both more responsive to activity in terms of the threshold for the induction of plasticity, and to code more information when those thresholds are crossed in terms of greater synaptic weight differentials.

BDNF-dependent signalling cascades in enrichment-induced synaptic modification.

Brain-derived neurotrophic factor (BDNF) has been repeatedly implicated in the neuronal changes associated with environmental enrichment. Enrichment, including exercise in humans, has been shown to directly induce or facilitate increases in the expression of BDNF (Berg et al., 2021; Cowansage et al., 2010; Falkenberg et al., 1992; Martinez-Diaz et al., 2020; Novkovic et al., 2015b; Rogers et al., 2019; von Bohlen und Halbach and von Bohlen und Halbach, 2018; West et al., 2014). Subsequently, the activity-dependent release of BDNF, which may occur through a variety of mechanisms from both presynaptic terminals and postsynaptic dendritic spines (Brigadski and Leßmann, 2020), initiates increases in the size and density of dendritic spines (von Bohlen und Halbach and von Bohlen und Halbach, 2018) and enhances glutamatergic synaptic

transmission in a manner that parallels increases in spine volume (Harward et al., 2016; Hedrick et al., 2016). More direct evidence for an important role of BDNF in regulating the neuronal response to enrichment has been obtained from mice that are heterozygous for the BDNF gene, since the homozygous deletion of the BDNF gene results in death within the first two postnatal weeks (Conover and Yancopoulos, 1997). Studies in BDNF mutant mice, though complicated by some baseline sex-dependent differences in a range of behavioural tasks, synaptic plasticity and spine density, nonetheless show that BDNF is required for the full neurogenesis, morphological, plasticity and cognitive response to enrichment, including cognitive flexibility when responding to novel challenges (Chourbaji et al., 2008; Chourbaji et al., 2012; Novkovic et al., 2015b; Rossi et al., 2006; Sakata et al., 2013; Zhu et al., 2009), with the effects of BDNF most likely mediated via the TrkB receptor (Andreska et al., 2020; von Bohlen und Halbach and von Bohlen und Halbach, 2018).

Downstream of BDNF/TrkB receptors lie three signalling pathways: PI3K/Akt, PLCγ and MAPK (Figure 2), which possess distinct roles in terms of regulating synaptic plasticity (Johnstone and Mobley, 2020; Minichiello, 2009; Panja and Bramham, 2013) and the formation of dendritic spines (Zagrebelsky et al., 2020). For example, LTP was impaired in hippocampal slices from mutant mice lacking the binding site on the TrkB receptor for PLCγ, which results in the activation of CREB (Minichiello et al., 2002). In contrast, a mutation preventing interaction with Shc that signals through MAPKs did not affect the induction or expression of LTP, at least not within a three hour period post-induction (Korte et al., 2000; Minichiello et al., 2002). More recently, others have confirmed the importance of BDNF/TrkB/PLCγ in LTP (Harward et al., 2016), while a requirement for both the TrkB Shc and PLCγ sites seems to be necessary for the appearance and maturation of dendritic spines induced by BDNF (Cowansage et al., 2010; Zagrebelsky et al., 2020). Moreover, neurotrophins (including BDNF), TrkB receptors, and associated signalling cascades have all been implicated in hippocampal neurogenesis (Numakawa et al., 2018; Vilar and Mira, 2016). These observations point to the importance of the BDNF/TrkB signalling in regulating neuronal responses commonly observed following enrichment.

MSK1 as a downstream effector of BDNF-dependent signalling during enrichment

These observations, however, only get us so far in linking the experience- and activity-dependent release of BDNF with the necessary changes in gene expression required to support the long-term modifications in neuronal structure, synaptic function and cognition associated with enrichment. Some mechanism must exist to couple the activation of cell surface TrkB receptors to the genome. To this end, we have been investigating an enzyme, mitogen- and stress-activated protein kinase 1 (MSK1; Figure 2) in the context of environmental enrichment. MSK1 is downstream of the BDNF/TrkB/MAPK cascade and directly regulates transcription via the phosphorylation of CREB at S133, and histone H3 at S10 (Reyskens and Arthur, 2016). In addition, MSK1 is activated by a variety of stimuli, including following the activation of glutamate receptors (Frenguelli and Corrêa, 2012). Moreover, MSK1 is the primary CREB kinase activated by BDNF, since CREB phosphorylation by BDNF was absent in cultured cortical neurones from MSK1 knockout mice (Arthur et al., 2004), and in CA1 neurones from hippocampal slices prepared from mice harbouring a kinase-dead mutation in MSK1 (Daumas et al., 2017). The interaction with CREB is particularly notable given CREB's prominence as a key plasticity-related protein that regulates the activity-dependent genomic response underpinning LTP and certain forms of learning and memory (Barco et al., 2002; Barco and Marie, 2011; Benito et al., 2011; Bourtchuladze et al., 1994). CREB's role in learning and memory may involve both synapse-specific, and more general cell-wide changes in neuronal responsiveness to stimuli. The former may strengthen the involvement of a particular synapse in a neuronal circuit underpinning a memory, and the latter to increase the number of neurones allocated to that memory (Lisman et al., 2018).

Increases in CREB phosphorylation have been observed in response to enrichment, and this has been associated with the facilitation of both LTP and LTD (Stein et al., 2016). Importantly, the BDNF-dependent induction of Arc/Arg 3.1, another important plasticity-related protein (Nikolaienko et al., 2018), and also upregulated in response to enrichment (Pinaud et al., 2001), requires the kinase activity of MSK1 (Hunter et al., 2017). These data implicate MSK1 as a prime regulator of both the genomic activation and expression of proteins implicated in synaptic plasticity and learning and memory. Moreover, they predicted that MSK1 may play an important role in both of these processes. Indeed, studies in MSK1 knockouts suggested that this might be the case. Knockout of MSK1 or both MSK1/2 isoforms impaired learning and memory in a range of tasks including fear conditioning and the Morris water maze (Chwang et al., 2007), and the Barnes maze and

novel object recognition (Karelina et al., 2012). These tantalising observations, including those of deficits in basal and responsive neurogenesis in the subgranular zone of the dentate gyrus (Choi et al., 2012; Karelina et al., 2012; Karelina et al., 2015), and spine density in area CA1 (Karelina et al., 2012), are tempered by the fact that MSK1 may form part of a structural complex that signals to the nucleus (Gutierrez-Mecinas et al., 2011), which may explain why some 275 genes were differentially regulated under basal conditions in the MSK1 knockout mouse (Choi et al., 2017). Thus, the loss of MSK1 protein may complicate the interpretation of its kinase action. To obviate such concerns we have used a mouse harbouring a targeted point mutation in the N-terminal kinase domain of MSK1. This mutation inactivates the kinase activity of MSK1 such that, while the protein is present, it does not phosphorylate downstream targets such as CREB or histone H3 (Corrêa et al., 2012), and only three genes are differentially regulated between wild-type and the MSK1 kinase dead (MSK1 KD) mouse under basal conditions (Privitera et al., 2020).

Using this MSK1 KD mouse, and in contrast to previous studies, we observed an increase in basal neurogenesis in the dentate gyrus (Olateju et al., 2021), suggesting a negative regulation of neurogenesis by MSK1, and increased spine density in area CA1 (Corrêa et al., 2012; Privitera et al., 2020), potentially indicative of an important role for MSK1 in spine pruning. Indeed, reductions in dendritic BDNF are associated with increased spine density in area CA1 (An et al., 2008) and visual cortex (Kaneko et al., 2012). These data suggest that BDNF-driven activation of MSK1 is required for the appropriate regulation of spine number, and hence the number of synaptic contacts.

However, there were no basal deficits in learning and memory in a variety of water maze tasks, in spontaneous entries on a 4-arm radial maze, or in exploratory or anxiety behaviour (Daumas et al., 2017; Privitera et al., 2020). This lack of an effect on learning and memory was paralleled by no deficit in LTP measured up to three hours after induction (Daumas et al., 2017; Privitera et al., 2020). This was true if either HFS or TBS was applied to the Schaffer pathway in area CA1 of the hippocampus (Daumas et al., 2017; Privitera et al., 2020), the latter of which elicited transcription-dependent LTP (Daumas et al., 2017). The lack of effect on LTP may reflect observations made previously that the TrkB Shc site, which signals to MAPK, downstream of which is MSK1, is not required for LTP (Korte et al., 2000; Minichiello et al., 2002).

However, two peculiar aspects of the MSK1 KD mouse pointed to a potentially longer-term influence of MSK1 on neuronal structure and function: an influence on basal synaptic transmission, and on spine density, both of which are regulated, at least in part, through BDNF and TrkB receptors. We observed that while miniature excitatory postsynaptic currents (mEPSCs) were larger in cultured hippocampal neurones from MSK1 KD mice, and associated with greater cell surface GluA1 AMPA receptor subunit expression (Corrêa et al., 2012; Lalo et al., 2018), both field excitatory postsynaptic potentials (fEPSPs) and, to a lesser extent, mEPSCs were smaller in hippocampal slices in MSK1 KD mutants compared to slices from wild-type mice (Corrêa et al., 2012; Daumas et al., 2017; Lalo et al., 2018; Privitera et al., 2020). This latter observation in hippocampal slices was associated with a greater density of CA1 dendritic spines in MSK1 mutant mice (Corrêa et al., 2012; Privitera et al., 2020), precluding a lack of synaptic contacts as the basis of the deficiency in synaptic transmission. Furthermore there were no appreciable differences in GluA1 or GluA2 AMPA receptor subunit expression (Privitera et al., 2020), or in the probability of transmitter release (Daumas et al., 2017; Privitera et al., 2020). We therfore considered the possibility that MSK1 may play a role in long-term regulation of neuronal activity and morphology. To test this we examined the role of MSK1 in homeostatic synaptic plasticity.

Regulation of homeostatic and experience-dependent plasticity by MSK1

Homeostatic synaptic plasticity is regarded as a mechanism by which to regulate activity-dependent Hebbian synaptic plasticity. While the latter seeks to strengthen or weaken synaptic communication at individual glutamatergic synapses, a mechanism must exist to constrain potentially unrestrained synaptic potentiation and depression yet allowing the relative weighting of synaptic strength across synapses to be maintained. Homeostatic synaptic scaling fulfils that role (Turrigiano, 2017). Although initially described in cultured neurones *in vitro* (Turrigiano et al., 1998), homeostatic synaptic scaling has been observed *in vivo*, not least of which in the down-regulation during sleep of increased synaptic activity and neuronal firing above basal levels caused by visual deprivation (Torrado Pacheco et al., 2021). Early indications of a role for BDNF, protein phosphorylation, gene expression, GluA subunit expression changes, the induction of Arc, which is required for the internalisation of GluA subunits (Chowdhury et al., 2006; Rial Verde et al., 2006), and the many hours over which this scaling of synaptic strength develops (Pozo and Goda, 2010), pointed to a potential role of

MSK1. Typically, such homeostatic synaptic scaling can be observed following prolonged exposure of cultured neurones to either activity deprivation (eg with the voltage-gated Na^+ channel blocker tetrodotoxin; TTX), or activity enhancement (eg with the $GABA_A$ receptor antagonist bicuculline) (Turrigiano et al., 1998). We found that mEPSCs in cultured neurones from MSK1 KD mice were indifferent to TTX or bicuculline, in contrast to neurones from wild-type mice which showed the expected increase and decrease in mEPSC amplitude, respectively (Corrêa et al., 2012). Importantly, the time-dependent down-regulation of Arc seen in TTX-exposed wild-type neuronal cultures, and likely responsible for the increased cell surface AMPAR expression, was not observed in neurons from MSK1 KD mice. That the BDNF/TrkB/MAPK cascade was involved was evidenced by application of BDNF, mimicking activity, which caused down-scaling, and a TrkB antagonist or a MEK1/2 inhibitor, mimicking deprivation, both of which caused up-scaling. Notably, these manipulations of the BDNF/TrkB/MAPK pathway did not affect synaptic transmission in MSK1 KD neurones. Subsequent work has confirmed the importance of the MAPK pathway in Arc induction in synaptic scaling (Bateup et al., 2013).

These observations (summarised in Figure 3) indicated that MSK1 responds to prevailing synaptic activity through a BDNF-driven activation of the MAPK cascade to regulate Arc-dependent cell surface expression of AMPA GluA subunits. Thus, MSK1 underpins BDNF-dependent homeostatic synaptic scaling. To extend our understanding of MSK1's role as a homeostat of neuronal activity under more naturalistic conditions, we tested whether MSK1 played a role in the neuronal response to enriched environments. The rationale for this investigation arose because environmental enrichment has been shown to influence synaptic function (Table 1), and has been associated with elevations in BDNF, the activation of the MAPK cascade, CREB phosphorylation and Arc induction, all of which involve MSK1.

Evidence that MSK1 may play a role in the neuronal response to environmental enrichment came from observations that while enrichment enhanced the amplitude of mEPSCs in wild-type CA1 and neocortical neurones from mice raised in an enriched environment from birth, no increase in mEPSC amplitude was observed in MSK1 KD mutant mice (Corrêa et al., 2012; Lalo et al., 2018). Surprisingly, this enrichment-driven enhancement of mEPSCs in wild-type mice did not translate into strengthening of population-level synaptic transmission as measured with fEPSPs. Indeed, if anything, enrichment caused a small (non-significant)

decrease in the wild-type fEPSP across the entire input stimulus range (Privitera et al., 2020). This may be due to a reduction in the probability of electrically-evoked transmitter release, since paired-pulse facilitation, which is inversely proportional to the probability of neurotransmitter release (Jackman and Regehr, 2017), was increased exclusively in enriched wild-type mice (Privitera et al., 2020), suggesting a further MSK1-dependent homeostatic adaptation to enrichment.

In wild-type mice after enrichment, and consistent with the reports of other groups (Cortese et al., 2018; Hullinger et al., 2015; Kumar et al., 2012; Malik and Chattarji, 2012; Novkovic et al., 2015a; Novkovic et al., 2015b), we observed (Figure 4A) an enhancement in the magnitude of TBS CA1 LTP over that seen in standard-housed mice (Privitera et al., 2020). In addition, we also observed an enhancement in low frequency stimulation-induced LTD in area CA1 (Figure 4B). In contrast, there was no such enhancements in LTP or LTD in slices taken from enriched MSK1 KD mice (Figure 4A, B). The selective enhancement of LTP in wild-type mice compared to MSK1 KD mice has been replicated at neocortical synapses in young (6-12 week) and old (9-15 month) mice after enrichment (Lalo et al., 2020). From these observations it may be possible to conclude that the enhancement of both LTP and LTD after enrichment reflects an MSK1-dependent bidirectional expansion of the dynamic range of synapses, such that enriched synapses can weaken further and strength more. This is true when both the sign-free magnitude in the change in synaptic plasticity is considered (Figure 4C) and when the sign of change is taken into account (Figure 4D, *cf* Figure 1). This MSK1-dependent influence on synaptic plasticity represents a form of metaplasticity (Abraham, 2008) whereby synapses are primed, in this case by enrichment, and through the actions of MSK1, to behave in a manner that differs from that which would occur in the absence of the enriched experience. We estimated that this metaplastic synaptic priming increased the dynamic range of synaptic transmission by ~28%, thereby allowing the enriched synapse to code more information. The mechanism by which this expansion occurs is presently unclear, but could either reflect enhanced trafficking of AMPA receptors at the synapse (Diering and Huganir, 2018), post-translational modifications of individual AMPA GluA subunits resulting in reduced or enhanced conductance (Benke and Traynelis, 2019), or indeed in the addition of synaptic structural modules containing AMPA receptors and associated proteins (Liu et al., 2017). In either case, it is clear that MSK1 is required for this enhancement of the dynamic range of synaptic transmission after enrichment.

Parallel behavioural studies revealed tangible benefits in terms of cognitive flexibility and the persistence of memory of this experience- and MSK1-dependent enhancement of the synaptic dynamic range (Privitera et al., 2020).

Activity-, experience-and MSK1-dependent gene expression changes, and the induction of genomic homeostasis

Differential gene expression likely underlies the beneficial effect of enrichment on synaptic plasticity and learning and memory. The expression of Egr1 (zif268) and Arc/Arg3.1 have previously been seen to be upregulated following enrichment (7 – 10 days) (Koh et al., 2005; Pinaud et al., 2001). Both Egr1 and Arc/Arg3.1 have also been observed to be upregulated following LTP induction in area CA1 (Chen et al., 2017; Yilmaz-Rastoder et al., 2011), and their importance stems from their roles in synaptic plasticity and learning and memory (Duclot and Kabbaj, 2017; Minatohara et al., 2015; Wall et al., 2018; Zhang and Bramham, 2020). Arc/Arg3.1 is transcribed in response to neuronal activity and translocated to dendritic spines where it is translated into active Arc/Arg3.1 protein, also in an activity-dependent manner (Steward et al., 2014). Potentially, Arc/Arg3.1 can then mediate differential AMPAR endocytosis at synapses, enhancing the contrast between potentiated and non-potentiated synapses (Okuno et al., 2012; Shepherd et al., 2006; Zhang and Bramham, 2020), yet preventing runaway synaptic enhancement; in essence mediating cell-wide homeostatic plasticity (Turrigiano, 2012). The transcription factor Egr1 instead is believed to contribute to plasticity by regulating the expression of synaptic plasticity genes that promote the stability of synapses (Duclot and Kabbaj, 2017).

The molecular basis for some of the changes at the glutamatergic synapse that are induced by enrichment can also be interrogated through microarray- or sequencing-based approaches. By comparing total gene expression in the enriched hippocampus with that of standard housed counterparts, differences in gene expression due to exposure to enrichment can be appreciated, and perhaps linked to the permissive, metaplastic, effects of enrichment on synaptic plasticity and learning and memory. Previous RNAseq studies have examined the effects of different enrichment exposure periods on gene expression changes within the hippocampus: in the dentate gyrus of 4 month old CD1 mice, 1 month of enrichment (complex environment

and social enrichment, without a running wheel) resulted in differential gene expression enriched for functions including “extracellular matrix organisation”, “collagen metabolic process” and “growth factor binding” (Gregoire et al., 2018). Alternatively, the same mice kept under a running wheel only condition displayed differential gene expression functionally enriched for the terms “glutamate receptor signalling pathway”, “ionotropic glutamate receptor signalling pathway”, “regulation of cellular response to growth factor stimulus” and “Ca^{2+} ion transmembrane transport” (Gregoire et al., 2018). In wild-type mice (C57BL/6N background) gene expression changes at 12 months of an enriched environment, a much longer enrichment period, resulted in an upregulation of BDNF expression and functional enrichment of differentially expressed genes for “learning or memory”, “neuron development”, “response to external stimulus” and “axonogenesis” (Wassouf et al., 2018). Exposure to enrichment therefore seems to induce gene expression changes related to the extracellular environment, growth factor signalling and glutamate receptor signalling.

A more comprehensive way to study the interplay between gene expression changes, synaptic plasticity and behavioural changes following enrichment, is to study each under the same experimental conditions rather than in isolation. We adopted a Next Generation Sequencing approach to examine hippocampal transcriptomic changes after prolonged (~3 months) of enrichment from birth in cohorts of wild-type and mutant mice that underwent electrophysiological, behavioural and spine density analysis. This late interrogation, at a time when adaptation to the enriched environment would likely have occurred, of course runs the risk that early changes in gene expression may dissipate, potentially leaving few and small differences between the enriched and standard housed brain. Alternatively, such an approach may allow the modifications of the genome to be observed that are either necessary to support the enriched brain in terms of the often reported increases in spine density, neuronal architecture, vascularisation and metabolism (Kolb and Whishaw, 1998), or to leave the brain in a metaplastic state that is primed to better respond to future experience. We found that there was evidence of both (Privitera et al., 2020).

Support for structural changes among the 475 genes differentially regulated by enrichment in wild-type mice (Supplemental Table 10 from Privitera et al., (2020), GEO accession number: GSE149210) was evidenced by the large number of the 139 enriched GO terms (at $p < 0.01$) that related to the structure and organisation

of the extracellular matrix, organelle assembly and function – including for the cilium and associated axoneme, solute transport, and regulation of vasculature (Figure 5 & Supplementary Table 1; analysis as in Privitera et al., (2020)).

Pathways identified as being differentially regulated by enrichment display overlap with genes upregulated following LTP induction in hippocampal pyramidal neurons, including “MAPK signalling pathway”, “extracellular matrix-receptor interaction”, “focal adhesion”, “regulation of actin cytoskeleton”, “cell adhesion genes” and cytoskeletal genes” (Chen et al., 2017). As mentioned above, functional enrichment of differentially expressed genes following enrichment include terms such as “extracellular matrix organisation” and “growth factor binding” (Gregoire et al., 2018), “cell adhesion”, “extracellular matrix organization” and “cytoskeleton organization” (Figure 5). Gene expression studies therefore indicate enrichment influences the expression of genes also related to the induction of LTP, particularly “extracellular matrix” and “cell adhesion” genes. This could be one way in which enrichment acts to enhance LTP, by regulating baseline expression of these pathways to facilitate LTP induction. We have also previously observed that enrichment-dependent modulation of extracellular matrix pathway genes was deficient in MSK1 KD mice, in which there was no enhancement of LTP (Privitera et al., 2020).

While there were no dramatic changes in GluR subunit gene expression (Figure 6), save for an enrichment-induced downregulation of Gria2 (coding for GluA2), which was not reflected at the protein level (Privitera et al., 2020), some form of genomic adaptation to enrichment had clearly occurred. This was suggested by the unexpected down-regulation of elements of the GO term “regulation of synaptic plasticity” (Figure 6 and Supplementary Figure 1), the MAPK pathway (Figure 7A), and a number of plasticity-related genes (Figures 6, 7) notably MSK1 (but not MSK2; Figure 7A, B, C), Egr1, Arc/Arg3.1 (Figure 7A), CaMK2α (but not CaMK2β; Figure 6). Of these, the down regulation of Egr1 and Arc/Arg3.1, which were confirmed at the protein level (Figure 7D, E), occurred in an MSK1-dependent manner, and indeed there was a general down-regulation of the MAPK signalling cascade specifically in wild-type mice, a cascade that was highly represented in the GO terms observed after enrichment in both wild-type and MSK1 mutant mice (Privitera et al., 2020).

These observations strongly imply that, as might be expected, the physical development of the brain through enrichment requires the continued production of the necessary infrastructure for its maintenance. Moreover, they suggest that there are no dramatic changes in the gene expression of glutamate receptors, in keeping with the subtle effects on synaptic transmission after prolonged enrichment (Ohline and Abraham, 2019; Privitera et al., 2020). The system, however, does seem to have undergone an unanticipated downregulation of key plasticity-related genes typically associated with increases following the induction of synaptic plasticity or in response to learning. Since several of these genes are under the control of MSK1, MSK1 may represent a mechanism that is recruited by enrichment and experience to downregulate these genes to potentially both avoid runaway Hebbian synaptic potentiation and depression, and stabilise the neuronal networks underlying enhanced cognition. An important corollary of this genomic down regulation is that the signal to noise ratio of newly-induced genes is increased upon induction, and potentially, this may lead to greater cellular consequences upon translation. How this downregulation might come about may stem from the epigenetic control of gene expression that MSK1 provides (Adewumi et al., 2019; Kumar et al., 2017). The down-regulation of MSK1 may result in the loss of permissive epigenetic marks on DNA, such as phosphorylation of S10 on histone H3, which will have consequences on other permissive histone modifications such as H3 lysine 14 (K14) acetylation. Indeed, Chandramohan et al., observed the loss of S10 phosphorylation and K14 acetylation in the dentate gyrus of MSK1/2 double knockouts in response to forced swimming (Chandramohan et al., 2008), and Chwang and colleagues reported the absence of both S10 phosphorylation K14 acetylation in MSK1 knockout mice in response to fear conditioning (Chwang et al., 2007). The down regulation of MSK1 could therefore impart upon the genome a history of recent synaptic- and experience-dependent activity through the absence of permissive epigenetic marks that subsequently limits the expression of plasticity related genes, until as such time as they are needed in response to novel experiences (Figure 8). MSK1 therefore might be considered as orchestrating a homeostatic genomic priming that increases the dynamic range of gene expression within neurones, in addition to its requirement for enhancing the dynamic range of synapses. Indeed, MSK1 may potentially be important in either creating, or accessing, the cognitive reserve brought about by an enriched environment (Gelfo et al., 2018).

Concluding remarks

The study of the influence of enriched and complex environments on the mammalian brain has come a long way since the seminal observations reported by Donald Hebb on the improvements of cognition in rats given free run of his home (Hebb, 1947, 1949). Under more rigorous, but no less varied laboratory conditions, Hebb's behavioural observations have been replicated many times over, and at least some of the putative cellular and molecular substrates have been identified. Not least of these are metaplastic adaptations that occur at the glutamatergic synapse. These adaptations depend upon MSK1 and imbue glutamatergic transmission with even greater capacity to adjust the strength of communication that occurs across it, and to therefore encode more information, and potentially make it more responsive to subsequent activity. These changes are associated with a necessary homeostatic mechanism to constrain runaway Hebbian synaptic potentiation and depression, and seems to also involve MSK1 in an experience-dependent down regulation of a number of genes that are commonly associated with synaptic plasticity and learning and memory. Through this homeostatic mechanism MSK1 may thereby stabilise the enriched brain, yet make it more agile in the face of novel experience.

Acknowledgements: This work was supported by a project Grant from the BBSRC (BB/L00139X/1 to BGF), grants from the WPH Charitable Trust and the Sylvia Waddilove Foundation (BGF), BBSRC Doctoral support (BB/M01116X/1 to DDC.). DDC is currently an employee of Bayberry Wellness and Recovery Centres Ltd., who provided time and resources for the writing of this review. We thank Professor J. Simon Arthur, School of Life Sciences, University of Dundee, for the provision of the MSK1 KD mice, and Dr Daniel Hebenstreit, School of Life Sciences, University of Warwick, for discussions on the implications of gene expression changes. The Graphical Abstract and Figures 1, 2, 3 and 8 were created in Biorender.

Conflicts of Interest: BGF is the Editor-in-Chief of *Neuropharmacology*

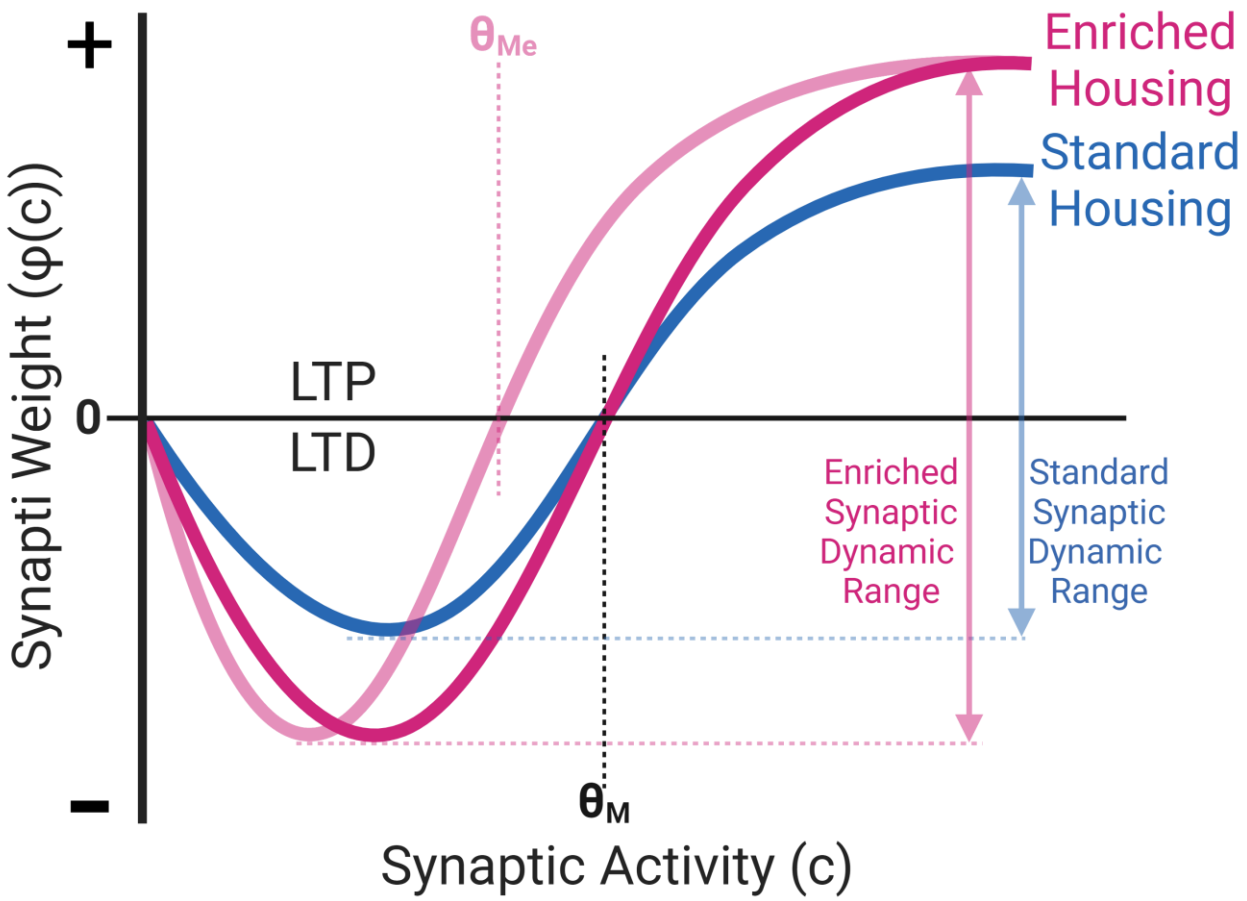


Figure 1. The Bienenstock, Cooper and Munro (BCM) relationship for activity-dependent synaptic modification. The sign (+, long-term potentiation, LTP; - long-term depression, LTD) and weight (ϕ(c)) of synaptic transmission varies as a function of synaptic activity (c) around a modification threshold value (θ_M). The dynamic range of synaptic strength is that represented by the difference between the lowest and highest synaptic weights possible under a given set of experimental conditions. Standard housing conditions (blue curve) engenders its own synaptic dynamic range suited to the cognitive demands upon the animal. Enrichment (deep red curve) enhances the synaptic dynamic range imbuing the animal with greater cognitive ability. In addition to changes in absolute synaptic weights, the modification threshold (θ_M) at which the sign of the synaptic modification occurs may also change in response to enrichment (pale red curve; θ_{Me}). After Bienenstock et al., (1982).

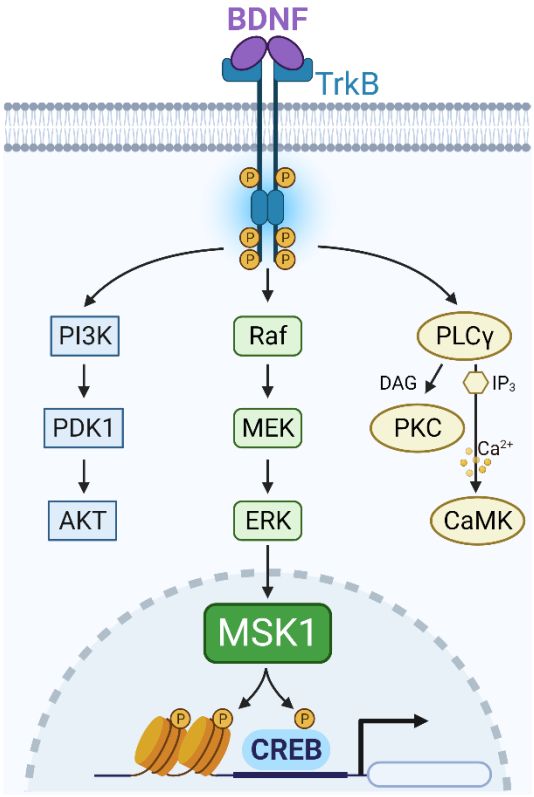


Figure 2. BDNF/TrkB and the MAPK/MSK1 cascade. The activation of the TrkB receptor by BDNF activates a number of intracellular signalling pathways. Of these, the MAPK cascade, characterised by the MEK1/2 activation of ERK1/2 results in the activation of MSK1. MSK1 regulates transcription via the phosphorylation of histone H3 and CREB.

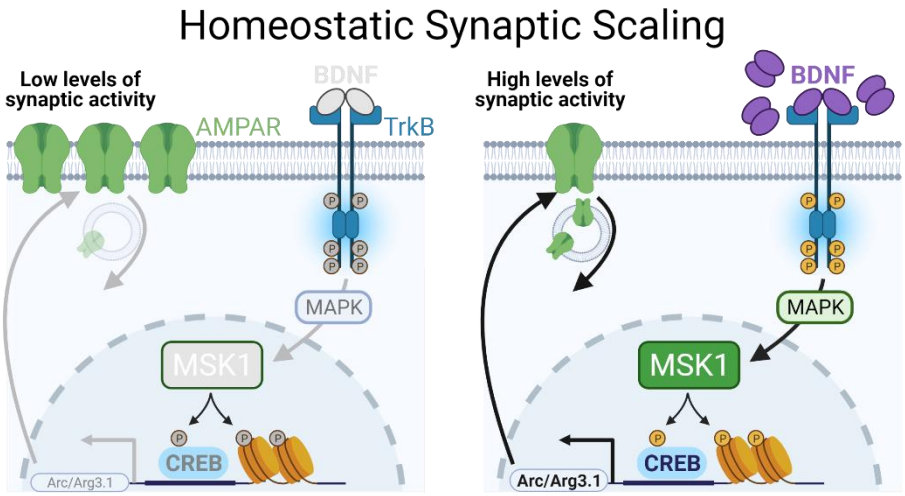


Figure 3. MSK1 regulates homeostatic synaptic scaling in vitro. Reduced release of BDNF during synaptic activity deprivation (left; eg in the presence of the voltage-gated Na^+ channel blocker TTX) results in reduced activation of the MAPK/MSK1 cascade and reduced expression of Arc/Arg3.1. This leads to reduced endocytosis of cell surface AMPA receptors (in green) and an increase in the amplitude of miniature excitatory postsynaptic currents. In contrast, high levels of synaptic activity (right; eg in the presence of the $GABA_A$ receptor antagonist bicuculline) promotes BDNF release and activation of the MAPK/MSK1 pathway leading to increased Arc/Arg3.1 expression and the endocytosis of AMPA receptors. This leads to a corresponding homeostatic decrease in synaptic strength.

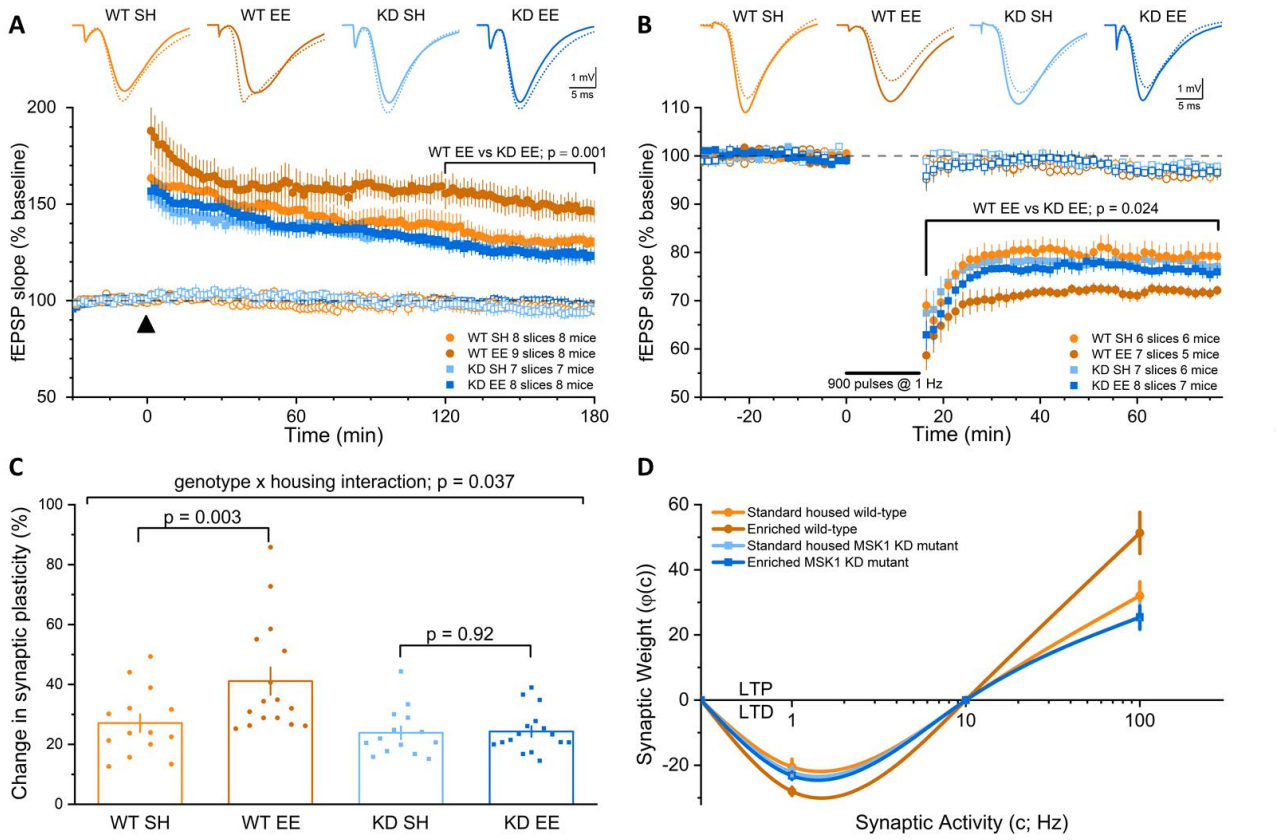


Figure 4. Environmental enrichment enhances the dynamic range of glutamatergic synapses in an MSK1-dependent manner. LTP (**A**) and LTD (**B**) are enhanced in wild-type mice after enrichment. In contrast, mice lacking the kinase activity of MSK1 (MSK1 KD) do not show this enhancement, and the level of synaptic change in response to LTP and LTD are the same as standard-housed mice. Inset are representative fEPSPs from the two genotypes and two housing conditions. **C**) Quantification (mean ± SEM; with individual experimental data points displayed) of the sign-free changes from a baseline value of 100 % in each of the experiments depicted in **A and B**. No change occurred in MSK1 KD mice, whereas enriched mice showed enhanced plasticity compared to their standard-housed counterparts. WT SH: wild-type standard housed; WT EE: wild-type environmentally enriched; KD SH: MSK1 KD mutant mice standard housed; KD EE: MSK1 KD mutant mice environmentally enriched. **D**) Mapping of the mean (± SEM) synaptic potentiation in **A** and synaptic depression in **B** at 100 and 1 Hz, respectively, onto the BCM synaptic modification rule (Figure 1). The data has been fit with a spline curve and an assumption is made that the nominal modification threshold (θ_M; 10 Hz) has not changed. An enhancement of the synaptic dynamic range (ϕ(c)) is observed exclusively in the enriched wild-type mice. Modified from Privitera et al., (2020).

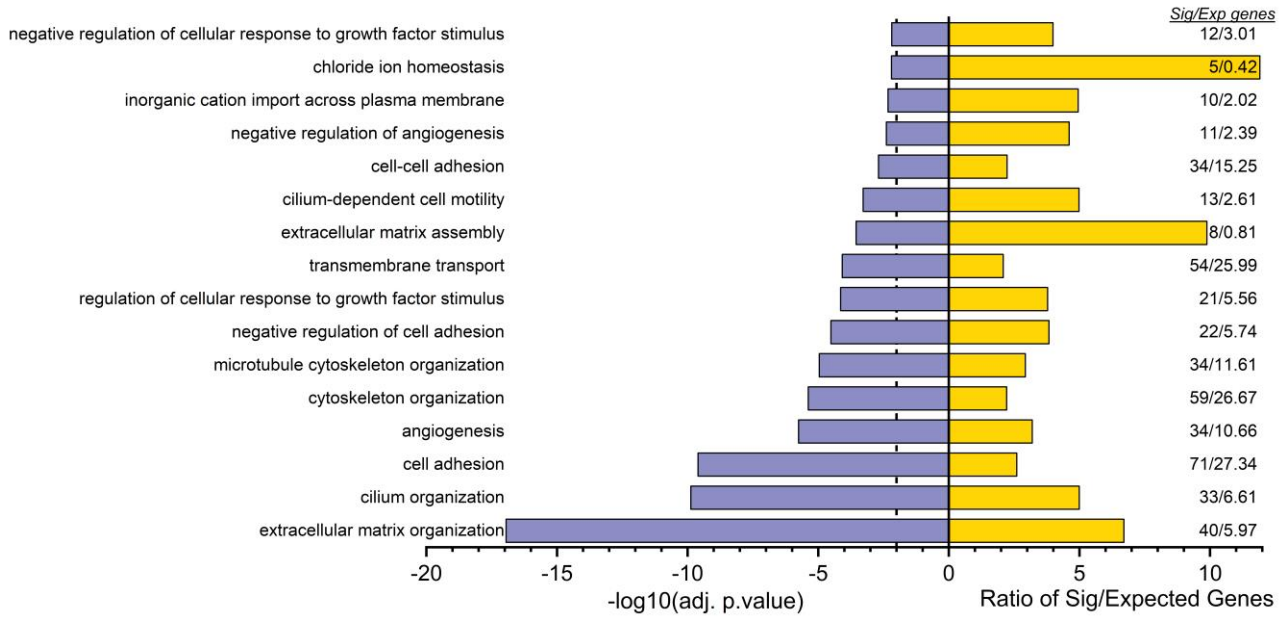


Figure 5. Long-term enrichment recruits GO terms relevant to the development and support of the enriched wild-type brain. Sixteen representative GO terms (from 139) that were significantly different (Benjamini–Hochberg corrected $p < 0.01$; lilac bars) from standard-housed wild-type mice reflecting the emphasis on the extracellular matrix, the cilium, vascular development and solute transport. Yellow bars indicate fold-enrichment of significant genes contributing to each term to the number expected in each category. The actual values for the number of significant and expected genes for each category are given. The broken vertical line at -2 indicates $p = 0.01$. Supplementary Table 1 lists all 139 $p < 0.01$ GO terms plus an additional 151 GO terms where $p < 0.05$ (290 GO terms in total). Analysis and data from Privitera et al., (2020) and GEO accession number: GSE149210.

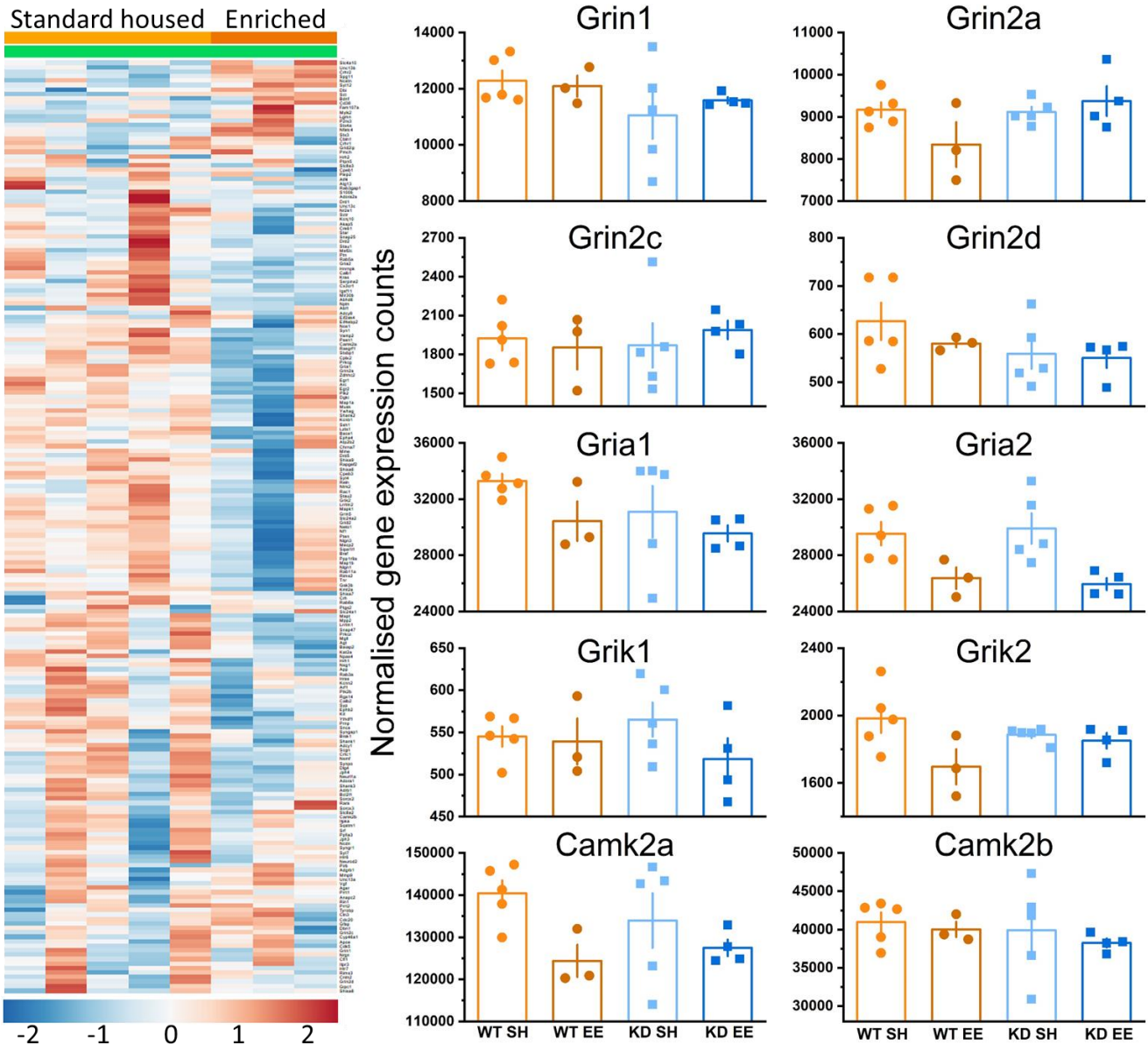


Figure 6. General cooling of hippocampal plasticity-related genes after enrichment in wild-type mice. Left panel shows heat map for the genes in the GO term “regulation of synaptic plasticity” (GO:0048167) and all child terms (GO:0031637, GO:0031914, GO:0031915, GO:0031916, GO:0031917, GO:0031918, GO:0048168, GO:0048169, GO:0048170, GO:0048171, GO:0048172, GO:0048173, GO:0048174, GO:0051913, GO:0051914, GO:0051915, GO:0060291, GO:0060292, GO:0150092, GO:1900271, GO:1900272, GO:1900273, GO:1900452, GO:1900453, GO:1900454, GO:1905512, GO:1905513, GO:1905514, GO:1990926) in the hippocampi of five standard housed wild-type mice and three enriched wild-type mice. Note the predominance of cooler colours in the enriched mice. Left panels show the gene expression counts

for key GluR subunit genes and CaMKII between wild-type (WT) and MSK1 KD (KD) mice raised from birth under standard housed (SH) and environmentally enriched (EE) conditions. Of the GluR subunits genes, only Gria2 showed an enrichment-dependent decrease that was independent of genotype. Similarly, of the CaMKII isoforms, only CaMKIIα showed an enrichment-dependent, MSK1-independent decrease. These data, taken from the data sets generated in Privitera et al., (2020) and GEO accession number: GSE149210, indicate that prolonged enrichment causes a down-regulation of plasticity-related genes. Full, high resolution heat map available in Supplementary Figure 1.

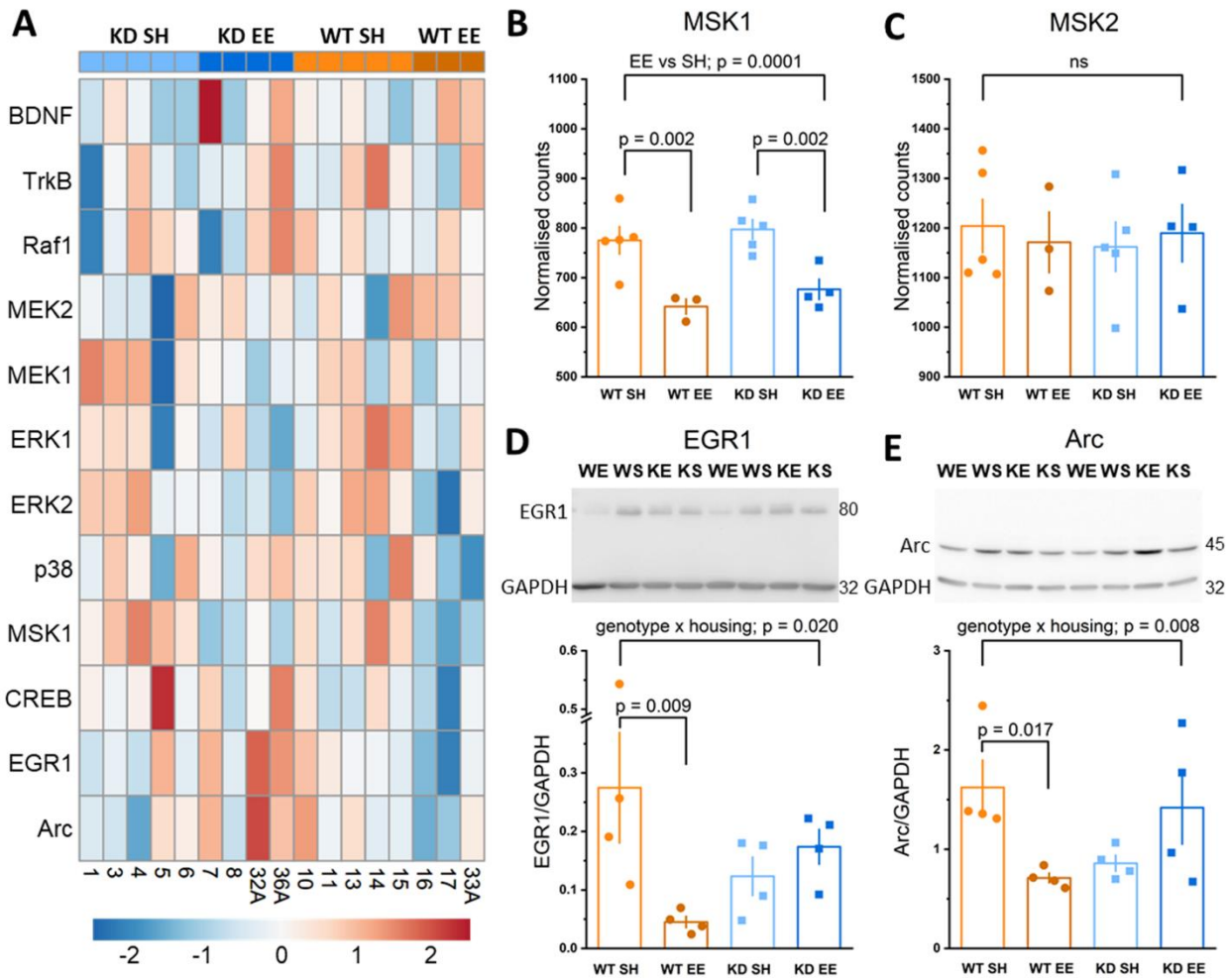


Figure 7. MSK1 coordinates a homeostatic downregulation of plasticity-related genes and proteins in response to prolonged enrichment. **A**. Heatmap of curated genes in the BDNF/MAPK/MSK1 pathway showing downregulation in enriched wild-type mice. **B**. Selective down-regulation of MSK1 gene expression after enrichment, but **C** no effect on MSK2. **D**, **E.** Enrichment- and MSK1-dependent decreases in EGR1 and Arc/Arg3.1 protein expression, respectively. Bar graphs shows quantification of western blot data from four independent hippocampi of contemporaneous mice from the four groups used in the RNA seq analysis. Inset are shown one of the two western blots for each protein used in the analysis. Abbreviations: WT SH, WS: wild-type standard housed; WT EE, WE: wild-type enriched; KD SH, KS: MSK1 KD standard housed; KD EE, KE: MSK1 KD enriched. Figure from Privitera at al., (2020). Gene expression data from GEO accession number: GSE149210.

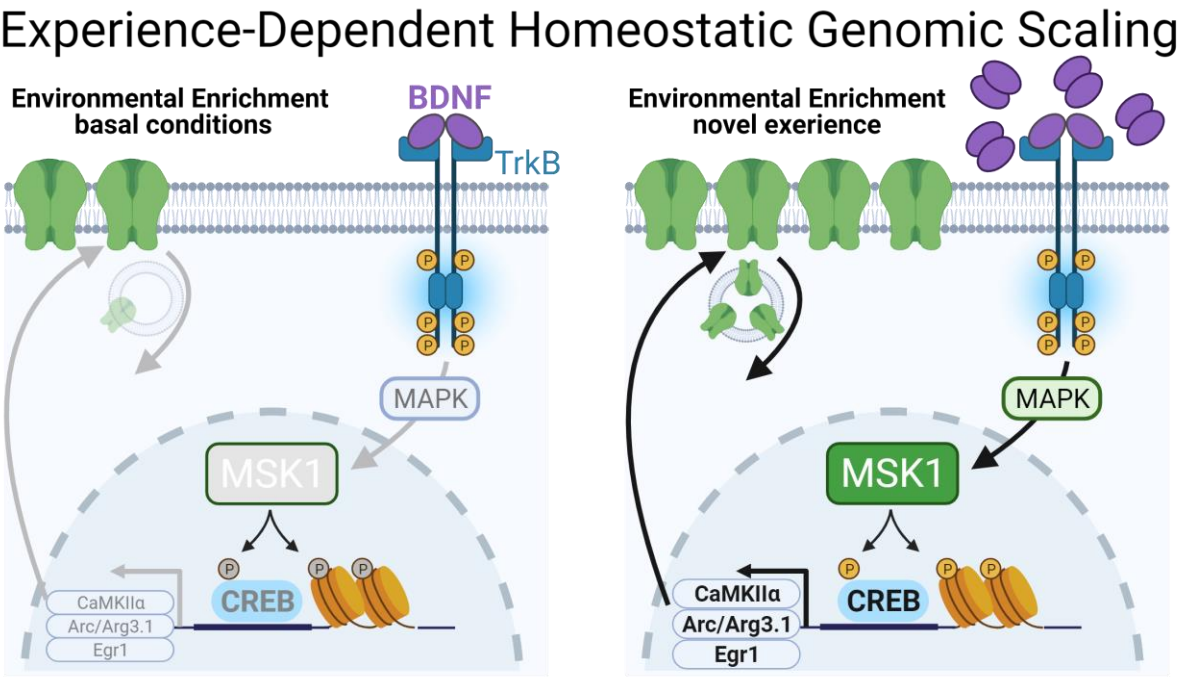


Figure 8. MSK1 as a genomic homeostat. Continuous and prolonged environmental enrichment (left panel) results in the downregulation of key plasticity-related genes, including those for members of the MAPK pathway, MSK1, CaMKIIα, Arc/Arg3.1 and Egr1. This potentially occurs against a background of normal basal synaptic transmission and BDNF signalling via other TrkB-associated pathways. Loss of otherwise gene expression-permissive and MSK1-dependent epigenetic marks (eg phosphorylation of histone H3) may serve as a genomic memory of the enriched experience. Against this backdrop of reduced plasticity-related gene expression, novel experiences (right panel) may provoke an enhanced transcriptional response. This may be functionally expressed as greater bidirectional AMPA receptor trafficking, leading to an expansion of the dynamic range of synapses, and enhanced cognitive function.

Study environmental enrichment parameters							SC-CA1 electrophysiological properties				Other outcomes of interest		
Study	**EE duration (wks)**	**Species / sex**	**Age post EE (wks)**	**Complex Environ-ment?**	**Wheel?**	**Social enrichment?**	**LTP**	**LTD**	**Synaptic input-output**	**Paired-pulse ratio**	**CA1 apical dendrite properties**	**MWM spatial acquisition, retention and reversal performance**	**Other learning or memory tests**
Buschler and Manahan-Vaughan, 2012	2	M / ♂	10 - 11	✓ cont.	✓	✕ 1 EE; 1 IH	**N.D.** <120 m; **N.D.** >24 hr [HFS, *in vivo*]	-	**N.D.**	-	-	-	-
Buschler and Manahan-Vaughan, 2012	2	M / ♂	10 - 11	✓ cont.	✓	✓ 5-6 EE; 1 IH	↑ 30 m; **N.D.** >24 hr [HFS, *in vivo*]	**N.D.** <120 m [LFS, in vivo]	**N.D.**	-	-	-	-
Buschler and Manahan-Vaughan, 2017	2	M / ♂	12 - 16	✓ cont.	✓	✓ 5-6 EE; 1 IH	↑ <120 m; **N.D.** >120 m [HFS; *in vivo*]	-	-	-	-	-	-
Buschler and Manahan-Vaughan, 2012	2 + 2 (6 wks gap)	M / ♂	18 - 19	✓ cont.	✓	✓ 5-6 EE; 1 IH	**N.D.** 30 m**;** ↑ >24 hr [HFS, *in vivo*]	-	-	-	-	-	-
Buschler and Manahan-Vaughan, 2017	2	M / ♂	40 - 56	✓ cont.	✓	✓ 5-6 EE; 1 IH	↑ <120 m; **N.D.** <120 m [HFS, *in vivo*]	-	-	-	-	-	-
Stein et al., 2016	3	R / ♂	87	✓ 3hrs / day	✕	✓ 3hrs / day; 3 EE; 1 IH	↑ 60 m [HFS, *in vitro*]	↑ 60 m [LFS, *in vitro*]	-	-	-		
Novkovic et al., 2015b	3 - 4	M / ♂	6 - 7	✓ cont.	✓	✕ 5-8 EE; 5-6 SH	↑ 180 m [HFS + TBS, *in vitro*]	-	**N.D.**	-	-	-	↑ NOR retention (24 hr) , **N.D.** (1 wk)
Novkovic et al., 2015a	3 - 4	M / ♂	6 - 7	✓ cont.	✕	✕ 5-8 EE; 5-8 SH	↑ 180 m [TBS, *in vitro*]	-	**N.D.**	-	-	-	-
Foster and Dumas, 2001	3 - 4	R / ♂+♀	9 - 10	✓ 1 - 6 hrs / day	✕	✓ 2 - 3 EE; 1 IH	**N.D.** 30 m [HFS, *in vitro*]	-	↑	**N.D.**	-	-	-

Morelli et al., 2014	4	M / ♂+♀	8	✓ cont.	✓	**?** 10-12 EE; N.S. SH	**N.D.** 60 m [HFS, *in vitro*]	-	-	**N.D.**	↑ length	-	-
Hosseiny et al., 2015	4	M / ♀	8	✓ cont.	✓	✓ 12 EE; 5 SH	↓ 40 - 60 m [HFS, *in vitro*]	-	-	**N.D.**	↑ spine density	-	-
Zarif et al., 2018	4	M / ♀	8	✓ cont.	✓	✓ 12-15 EE; 5-6 SH	↓ 40 m [HFS, *in vitro*]	-	-	-	↑ spine density	-	↑ acquisition + retention (9 day delay, BM); **N.D.** retention (5 day delay, BM)
Hosseiny et al., 2015	4	M / ♀	12	✓ cont.	✓	✓ 12 EE; 5 SH	↑ 40 - 60 m [HFS, *in vitro*]	-	-	**N.D.**	↑ spine density	-	-
Cortese et al., 2018	4	R / ♂	92 - 96	✓ cont.	×	× 6 EE; 6 SH	↑ <120 m [TBS, *in vitro*]	-	-	-	-	**N.D.** acquisition; **N.D.** retention (24 hr); **N.D.** reversal	**N.D.** NOR retention (24 hr); ↑ performance in RAWM
Zhu et al., 2011	4	R / ♂	N.S.	✓ cont.	✓	✓ 8 EE; N.S. SH	↑ 60 m [HFS, *in vitro*]	-	**N.D.**	**N.D.**	-	↑ acquisition; **N.D.** retention (24 hr)	-
Malik and Chatterji, 2012	4 - 5	R / ♂	7 - 9	✓ 4 hrs / day	×	✓ 12-13 EE; 2-3 SH	↑ 30 m [TBS, *in vitro*]	-	-	**N.D.**	↑ spine density	-	-
Artola et al., 2006	5 (then SH 3-5)	R / ♂	12	✓ cont.	×	× 5 EE; 5 SH	↑ 30m [HFS, *in vitro*]	↑ 30m [LFS, *in vitro*]	-	↓	-	-	-
Hosseiny et al., 2015	6	M / ♀	10	✓ cont.	✓	✓ 12 EE; 5 SH	**N.D.** 40 - 60 m [HFS, *in vitro*]	-	-	**N.D.**	↑ spine density	-	-
Duffy et al., 2001	8	M / ♀	12	✓ cont.	✓	× 7 EE; 7 SH	↑ 60 m [HFS, *in vitro*]	-	**N.D.**	-	-	-	-

Hosseiny et al., 2015	8	M / ♀	12	✓ cont.	✓	✓ 12 EE; 5 SH	↑ 40 - 60 m [HFS, *in vitro*]	-	-	**N.D.**	↑ spine density	-	-
Van Praag et al., 1999a	8 - 16	M / ♀	20 - 28	✕	✓	**N.S.**	**N.D.** 45m [HFS, *in vitro*]	-	-	**N.D.**	-	↑ acquisition	-
Kumar et al., 2012	10 - 12	R / ♂	90 - 100	✕	✓	✕ 1 EE; 1 IH	↑ 30 m [TBS, *in vitro*]	↓ 30m [LFS, *in vitro*]	-	-	-	↓ acquisition; **N.D.** retention (24 hr)	↑ NOR retention (24 hr)
Kumar et al., 2012	10 - 12	R / ♂	91 - 100	✓ 3hrs / day	✕	✓ 2 EE; 1 IH	↑ 30 m [TBS, *in vitro*]	↓ 30m [LFS, *in vitro*]	-	-	-	**N.D.** acquisition; ↑ retention (24 hr)	-
Bouet et al., 2011	12	M / ♀	24	✓ cont.	✓	✓ 14 EE; 7 SH	↓ 15 m; **N.D.** 60 m [TBS, *in vitro*]	-	**N.D.**	-	-	↑ acquisition; N.D. retention (30 m)	-
Bouet et al., 2011	12	M / ♀	80	✓ cont.	✓	✓ 14 EE; 7 SH	**N.D.** 60 m [TBS, *in vitro*]	-	↑	-	-	**N.D.** acquisition; **N.D.** retention (30 m)	-
Privitera et al., 2020	12 - 16	M / ♂	12 - 16	✓ cont.	✓	✓ 8 EE; 2-4 SH	↑ 120 – 180 m [TBS, *in vitro*]	↑ 60 m [LFS, *in vitro*]	**N.D.**	↑	**N.D.** spine density	↑ acquisition; ↑ reversal (24 hr)	↑ Spontaneous Alternation performance
Eckert et al., 2010	12 - 20	R / ♂	16 - 24	✓ cont.	✕	✓/✕ 4 EE; 1 or 4 SH	**N.D.** 120 m SR; (↑ 120 m SO) [HFS, *in vitro*]	↓ 60m SR; (N.D. 60 m SO) LFS, *in vitro*]	**N.D.** SR; (**N.D.** SO)	**N.D.** SR; (**N.D.** SO)	-	↑ acquisition; **N.D.** retention (24 hr)	-
Hullinger et al., 2015	16	R / ♂	24	✓ cont.	✕	✕ 6 EE; 6 SH	↑ <120 m [TBS, in vitro]	-	**N.D.**	**N.D.**	-	↑ acquisition; ↑ retention	↑ NOR retention (24 hr)
Hullinger et al., 2015	16	R / ♂	24	✓ cont.	✕	✓ **6** EE; 2 SH	↑ <120 m [TBS, in vitro]	-	**N.D.**	**N.D.**		**N.D.** acquisition; ↑ retention	↑ NOR retention (24 hr)

Table 1: Summary of observable electrophysiological and other relevant effects of different enrichment paradigms on hippocampal Schaffer collateral/commissural-CA1 glutamatergic synapses and accompanying morphological and behavioural changes in rodents. Studies are arranged vertically by duration of environmental enrichment. Studies are coloured based on number of EE components, complex environment, exercise wheel, social enrichment relative to control group, included in paradigm used: green (3 components), yellow (2 components), pale red (1 component). **Abbreviations:** EE: environmental enrichment; SH: standard housing; IH: isolated housing; M: mouse; R: rat; ♂male; ♀female; cont.: continuous; N.S.: not specified; N.D.: no significant difference; "-": not tested; LTP: long-term potentiation; LTD: long-term depression; CA1: cornu ammonis area 1; SR: stratum radiatum; SO: stratum oriens; wks: weeks; hr: hour; m: minutes; NOR: novel object recognition; BM: Barnes maze; MWM: Morris water maze; metrics: acquisition (latency), retention (probe trial) and reversal (latency to find new location).

References

Abraham, W. C., 2008. Metaplasticity: tuning synapses and networks for plasticity. Nature Reviews Neuroscience 9, 387-387.

Adewumi, I., López, C., Davie, J. R., 2019. Mitogen and stress- activated protein kinase regulated gene expression in cancer cells. Advances in Biological Regulation 71, 147-155.

An, J. J., Gharami, K., Liao, G. Y., Woo, N. H., Lau, A. G., Vanevski, F., Torre, E. R., Jones, K. R., Feng, Y., Lu, B., Xu, B., 2008. Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. Cell 134, 175-187.

Andreska, T., Lüningschrör, P., Sendtner, M., 2020. Regulation of TrkB cell surface expression—a mechanism for modulation of neuronal responsiveness to brain-derived neurotrophic factor. Cell and Tissue Research 382, 5-14.

Arthur, J. S., Fong, A. L., Dwyer, J. M., Davare, M., Reese, E., Obrietan, K., Impey, S., 2004. Mitogen- and stress-activated protein kinase 1 mediates cAMP response element-binding protein phosphorylation and activation by neurotrophins. J.Neurosci. 24, 4324-4332.

Artola, A., von Frijtag, J. C., Fermont, P. C., Gispen, W. H., Schrama, L. H., Kamal, A., Spruijt, B. M., 2006. Long-lasting modulation of the induction of LTD and LTP in rat hippocampal CA1 by behavioural stress and environmental enrichment. Eur J Neurosci 23, 261-272.

Barco, A., Alarcon, J. M., Kandel, E. R., 2002. Expression of constitutively active CREB protein facilitates the late phase of long-term potentiation by enhancing synaptic capture. Cell 108, 689-703.

Barco, A., Marie, H., 2011. Genetic approaches to investigate the role of CREB in neuronal plasticity and memory. Mol Neurobiol 44, 330-349.

Bateup, H. S., Denefrio, C. L., Johnson, C. A., Saulnier, J. L., Sabatini, B. L., 2013. Temporal dynamics of a homeostatic pathway controlling neural network activity. Front Mol Neurosci 6, 28.

Benito, E., Valor, L. M., Jimenez-Minchan, M., Huber, W., Barco, A., 2011. cAMP response element-binding protein is a primary hub of activity-driven neuronal gene expression. J Neurosci 31, 18237-18250.

Benke, T., Traynelis, S. F., 2019. AMPA-Type Glutamate Receptor Conductance Changes and Plasticity: Still a Lot of Noise. Neurochemical Research 44, 539-548.

Berg, J., Cooper, J., Salonikas, C., Seyedsadjadi, N., Grant, R., 2021. Acute caffeine intake in humans reduces post exercise performance in learning and memory. Hum Psychopharmacol.

Bienenstock, E. L., Cooper, L. N., Munro, P. W., 1982. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. The Journal of Neuroscience 2, 32.

Bliss, T., Collingridge, G. L., 2019. Persistent memories of long-term potentiation and the N-methyl-d-aspartate receptor. Brain Neurosci Adv 3, 2398212819848213.

Bliss, T. V., Gardner-Medwin, A. R., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol 232, 357-374.

Bliss, T. V., Lomo, T., 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232, 331-356.

Bliss, T. V. P., Collingridge, G. L., Morris, R. G. M., Reymann, K. G., 2018. Long-term potentiation in the hippocampus: discovery, mechanisms and function. Neuroforum, p. A103.

Bouet, V., Freret, T., Dutar, P., Billard, J. M., Boulouard, M., 2011. Continuous enriched environment improves learning and memory in adult NMRI mice through theta burst-related-LTP independent mechanisms but is not efficient in advanced aged animals. Mech Ageing Dev 132, 240-248.

Bourtchuladze, R., Frenguelli, B., Blendy, J., Cioffi, D., Schutz, G., Silva, A. J., 1994. Deficient long-term memory in mice with a targeted mutation of the cAMP-responsive element-binding protein. Cell 79, 59-68.

Brigadski, T., Leßmann, V., 2020. The physiology of regulated BDNF release. Cell and Tissue Research 382, 15-45.

Brown, R. E., 2020. Donald O. Hebb and the Organization of Behavior: 17 years in the writing. Molecular Brain 13, 55.

Brown, R. E., Milner, P. M., 2003. The legacy of Donald O. Hebb: more than the Hebb synapse. Nat Rev Neurosci 4, 1013-1019.

Buschler, A., Manahan-Vaughan, D., 2012. Brief environmental enrichment elicits metaplasticity of hippocampal synaptic potentiation in vivo. Frontiers in Behavioral Neuroscience 6.

Buschler, A., Manahan-Vaughan, D., 2017. Metabotropic glutamate receptor, mGlu5, mediates enhancements of hippocampal long-term potentiation after environmental enrichment in young and old mice. Neuropharmacology 115, 42-50.

Cattan, S., Conti, G., Farquharson, C., Ginja, R., 2019. The health effects of Sure Start. The Institute for Fiscal Studies.

Chandramohan, Y., Droste, S. K., Arthur, J. S., Reul, J. M., 2008. The forced swimming-induced behavioural immobility response involves histone H3 phospho-acetylation and c-Fos induction in dentate gyrus granule neurons via activation of the N-methyl-D-aspartate/extracellular signal-regulated kinase/mitogen- and stress-activated kinase signalling pathway. Eur J Neurosci 27, 2701-2713.

Chen, P. B., Kawaguchi, R., Blum, C., Achiro, J. M., Coppola, G., O'Dell, T. J., Martin, K. C., 2017. Mapping Gene Expression in Excitatory Neurons during Hippocampal Late-Phase Long-Term Potentiation. Front Mol Neurosci 10, 39.

Choi, Y.-S., Horning, P., Aten, S., Karelina, K., Alzate-Correa, D., Arthur, J. S. C., Hoyt, K. R., Obrietan, K., 2017. Mitogen- and Stress-Activated Protein Kinase 1 Regulates Status Epilepticus-Evoked Cell Death in the Hippocampus. ASN Neuro 9, 1759091417726607.

Choi, Y. S., Karelina, K., zate-Correa, D., Hoyt, K. R., Impey, S., Simon, A. J., Obrietan, K., 2012. Mitogen- and stress-activated kinases regulate progenitor cell proliferation and neuron development in the adult dentate gyrus. J.Neurochem. 123, 676-688.

Chourbaji, S., Brandwein, C., Vogt, M. A., Dormann, C., Hellweg, R., Gass, P., 2008. Nature vs. nurture: can enrichment rescue the behavioural phenotype of BDNF heterozygous mice? Behav Brain Res 192, 254-258.

Chourbaji, S., Hörtnagl, H., Molteni, R., Riva, M. A., Gass, P., Hellweg, R., 2012. The impact of environmental enrichment on sex-specific neurochemical circuitries - effects on brain-derived neurotrophic factor and the serotonergic system. Neuroscience 220, 267-276.

Chowdhury, S., Shepherd, J. D., Okuno, H., Lyford, G., Petralia, R. S., Plath, N., Kuhl, D., Huganir, R. L., Worley, P. F., 2006. Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. Neuron 52, 445-459.

Chwang, W. B., Arthur, J. S., Schumacher, A., Sweatt, J. D., 2007. The nuclear kinase mitogen- and stress-activated protein kinase 1 regulates hippocampal chromatin remodeling in memory formation. J.Neurosci. 27, 12732-12742.

Collingridge, G. L., Kehl, S. J., McLennan, H., 1983. Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. J Physiol 334, 33-46.

Conover, J. C., Yancopoulos, G. D., 1997. Neurotrophin regulation of the developing nervous system: analyses of knockout mice. Rev Neurosci 8, 13-27.

Corrêa, S. A. L., Hunter, C. J., Palygin, O., Wauters, S. C., Martin, K. J., McKenzie, C., McKelvey, K., Morris, R. G. M., Pankratov, Y., Arthur, J. S. C., Frenguelli, B. G., 2012. MSK1 Regulates Homeostatic and Experience-Dependent Synaptic Plasticity. The Journal of Neuroscience 32, 13039-13051.

Cortese, G. P., Olin, A., O'Riordan, K., Hullinger, R., Burger, C., 2018. Environmental enrichment improves hippocampal function in aged rats by enhancing learning and memory, LTP, and mGluR5-Homer1c activity. Neurobiol Aging 63, 1-11.

Cowansage, K. K., Ledoux, J. E., Monfils, M. H., 2010. Brain-derived neurotrophic factor: a dynamic gatekeeper of neural plasticity. Curr.Mol.Pharmacol. 3, 12-29.

Daumas, S., Hunter, C. J., Mistry, R. B., More, L., Privitera, L., Cooper, D. D., Reyskens, K. M., Flynn, H. T., Morris, R. G., Arthur, J. S., Frenguelli, B. G., 2017. The Kinase Function of MSK1 Regulates BDNF Signaling to CREB and Basal Synaptic Transmission, But Is Not Required for Hippocampal Long-Term Potentiation or Spatial Memory. eNeuro 4.

Dause, T. J., Kirby, E. D., 2019. Aging gracefully: social engagement joins exercise and enrichment as a key lifestyle factor in resistance to age-related cognitive decline. Neural Regen Res 14, 39-42.

Diering, G. H., Huganir, R. L., 2018. The AMPA Receptor Code of Synaptic Plasticity. Neuron 100, 314-329.

Dong, Z., Bai, Y., Wu, X., Li, H., Gong, B., Howland, J. G., Huang, Y., He, W., Li, T., Wang, Y. T., 2013. Hippocampal long-term depression mediates spatial reversal learning in the Morris water maze. Neuropharmacology 64, 65-73.

Duclot, F., Kabbaj, M., 2017. The Role of Early Growth Response 1 (EGR1) in Brain Plasticity and Neuropsychiatric Disorders. Front Behav Neurosci 11, 35.

Duffy, S. N., Craddock Kj Fau - Craddock, K. J., Abel T Fau - Abel, T., Nguyen Pv Fau - Nguyen, P. V., 2001. Environmental Enrichment Modifies the PKA-Dependence of Hippocampal LTP and Improves Hippocampus-Dependent Memory.

Eckert, M. J., Bilkey, D. K., Abraham, W. C., 2010. Altered plasticity in hippocampal CA1, but not dentate gyrus, following long-term environmental enrichment. J Neurophysiol 103, 3320-3329.

ETI, 2018. SureStart Evaluation Report. The Education and Training Inspectorate.

Falkenberg, T., Mohammed, A. K., Henriksson, B., Persson, H., Winblad, B., Lindefors, N., 1992. Increased expression of brain-derived neurotrophic factor mRNA in rat hippocampus is associated with improved spatial memory and enriched environment. Neurosci.Lett. 138, 153-156.

Foster, T. C., Dumas, T. C., 2001. Mechanism for increased hippocampal synaptic strength following differential experience. J Neurophysiol 85, 1377-1383.

Frenguelli, B. G., Corrêa, S. A., 2012. Regulation and role of MSK in the mammalian brain. In: Vermeulen, L., Arthur, J. S., (Eds), MSKs. Landes Bioscience.

Galaj, E., Barrera, E. D., Ranaldi, R., 2020. Therapeutic efficacy of environmental enrichment for substance use disorders. Pharmacol Biochem Behav 188, 172829.

Gelfo, F., Mandolesi, L., Serra, L., Sorrentino, G., Caltagirone, C., 2018. The Neuroprotective Effects of Experience on Cognitive Functions: Evidence from Animal Studies on the Neurobiological Bases of Brain Reserve. Neuroscience 370, 218-235.

Gregoire, C. A., Tobin, S., Goldenstein, B. L., Samarut, E., Leclerc, A., Aumont, A., Drapeau, P., Fulton, S., Fernandes, K. J. L., 2018. RNA-Sequencing Reveals Unique Transcriptional Signatures of Running and Running-Independent Environmental Enrichment in the Adult Mouse Dentate Gyrus. Front Mol Neurosci 11, 126.

Gutierrez-Mecinas, M., Trollope, A. F., Collins, A., Morfett, H., Hesketh, S. A., Kersante, F., Reul, J. M., 2011. Long-lasting behavioral responses to stress involve a direct interaction of glucocorticoid receptors with ERK1/2-MSK1-Elk-1 signaling. Proc.Natl.Acad.Sci.U.S.A 108, 13806-13811.

Harward, S. C., Hedrick, N. G., Hall, C. E., Parra-Bueno, P., Milner, T. A., Pan, E., Laviv, T., Hempstead, B. L., Yasuda, R., McNamara, J. O., 2016. Autocrine BDNF–TrkB signalling within a single dendritic spine. Nature 538, 99-103.

Hebb, D. O., 1947. The effects of early experience on problem-solving at maturity. American Psychologist 2, 306-307.

Hebb, D. O., 1949. The Organization of Behaviour. John Wiley, New York.

Hedrick, N. G., Harward, S. C., Hall, C. E., Murakoshi, H., McNamara, J. O., Yasuda, R., 2016. Rho GTPase complementation underlies BDNF-dependent homo- and heterosynaptic plasticity. Nature 538, 104-108.

Hirase, H., Shinohara, Y., 2014. Transformation of cortical and hippocampal neural circuit by environmental enrichment. Neuroscience 280, 282-298.

Hosseiny, S., Pietri, M., Petit-Paitel, A., Zarif, H., Heurteaux, C., Chabry, J., Guyon, A., 2015. Differential neuronal plasticity in mouse hippocampus associated with various periods of enriched environment during postnatal development. Brain Struct Funct 220, 3435-3448.

Hullinger, R., O'Riordan, K., Burger, C., 2015. Environmental enrichment improves learning and memory and long-term potentiation in young adult rats through a mechanism requiring mGluR5 signaling and sustained activation of p70s6k. Neurobiol Learn Mem 125, 126-134.

Hunt, J. M., 1979. Psychological Development: Early Experience. Annual Review of Psychology 30, 103-144.

Hunter, C. J., Remenyi, J., Correa, S. A., Privitera, L., Reyskens, K., Martin, K. J., Toth, R., Frenguelli, B. G., Arthur, J. S. C., 2017. MSK1 regulates transcriptional induction of Arc/Arg3.1 in response to neurotrophins. FEBS Open Bio 7, 821-834.

Jackman, S. L., Regehr, W. G., 2017. The Mechanisms and Functions of Synaptic Facilitation. Neuron 94, 447-464.

Johnstone, A., Mobley, W., 2020. Local TrkB signaling: themes in development and neural plasticity. Cell and Tissue Research 382, 101-111.

Kaneko, M., Xie, Y., An, J. J., Stryker, M. P., Xu, B., 2012. Dendritic BDNF synthesis is required for late-phase spine maturation and recovery of cortical responses following sensory deprivation. J Neurosci 32, 4790-4802.

Karelina, K., Hansen, K. F., Choi, Y. S., DeVries, A. C., Arthur, J. S., Obrietan, K., 2012. MSK1 regulates environmental enrichment-induced hippocampal plasticity and cognitive enhancement. Learn.Mem. 19, 550-560.

Karelina, K., Liu, Y., zate-Correa, D., Wheaton, K. L., Hoyt, K. R., Arthur, J. S., Obrietan, K., 2015. Mitogen and stress-activated kinases 1/2 regulate ischemia-induced hippocampal progenitor cell proliferation and neurogenesis. Neuroscience 285, 292-302.

Koh, S., Chung, H., Xia, H., Mahadevia, A., Song, Y., 2005. Environmental enrichment reverses the impaired exploratory behavior and altered gene expression induced by early-life seizures. J Child Neurol 20, 796-802.

Kolb, B., Whishaw, I. Q., 1998. Brain plasticity and behavior. Annu Rev Psychol 49, 43-64.

Korte, M., Minichiello, L., Klein, R., Bonhoeffer, T., 2000. Shc-binding site in the TrkB receptor is not required for hippocampal long-term potentiation. Neuropharmacology 39, 717-724.

Kumar, A., Rani, A., Tchigranova, O., Lee, W. H., Foster, T. C., 2012. Influence of late-life exposure to environmental enrichment or exercise on hippocampal function and CA1 senescent physiology. Neurobiol Aging 33, 828 e821-817.

Kumar, R., Deivendran, S., Santhoshkumar, T. R., Pillai, M. R., 2017. Signaling coupled epigenomic regulation of gene expression. Oncogene 36, 5917-5926.

Lalo, U., Bogdanov, A., Moss, G. W., Frenguelli, B. G., Pankratov, Y., 2018. Role for astroglia-derived BDNF and MSK1 in homeostatic synaptic plasticity. Neuroglia 1, 14.

Lalo, U., Bogdanov, A., Moss, G. W., Pankratov, Y., 2020. Astroglia-Derived BDNF and MSK-1 Mediate Experience- and Diet-Dependent Synaptic Plasticity. Brain Sci 10.

Larson, J., Wong, D., Lynch, G., 1986. Patterned stimulation at the theta frequency is optimal for the induction of hippocampal long-term potentiation. Brain Res 368, 347-350.

Lisman, J., Cooper, K., Sehgal, M., Silva, A. J., 2018. Memory formation depends on both synapse-specific modifications of synaptic strength and cell-specific increases in excitability. Nat Neurosci 21, 309-314.

Liu, K. K., Hagan, M. F., Lisman, J. E., 2017. Gradation (approx. 10 size states) of synaptic strength by quantal addition of structural modules. Philos Trans R Soc Lond B Biol Sci 372.

Malik, R., Chattarji, S., 2012. Enhanced intrinsic excitability and EPSP-spike coupling accompany enriched environment-induced facilitation of LTP in hippocampal CA1 pyramidal neurons.

Martinez-Diaz, I. C., Escobar-Munoz, M. C., Carrasco, L., 2020. Acute Effects of High-Intensity Interval Training on Brain-Derived Neurotrophic Factor, Cortisol and Working Memory in Physical Education College Students. Int J Environ Res Public Health 17.

McDonald, M. W., Hayward, K. S., Rosbergen, I. C. M., Jeffers, M. S., Corbett, D., 2018. Is Environmental Enrichment Ready for Clinical Application in Human Post-stroke Rehabilitation? Front Behav Neurosci 12, 135.

Miguel, P. M., Pereira, L. O., Silveira, P. P., Meaney, M. J., 2019. Early environmental influences on the development of children's brain structure and function. Dev Med Child Neurol 61, 1127-1133.

Mills, F., Bartlett, T. E., Dissing-Olesen, L., Wisniewska, M. B., Kuznicki, J., Macvicar, B. A., Wang, Y. T., Bamji, S. X., 2014. Cognitive flexibility and long-term depression (LTD) are impaired following beta-catenin stabilization in vivo. Proc Natl Acad Sci U S A 111, 8631-8636.

Minatohara, K., Akiyoshi, M., Okuno, H., 2015. Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. Front Mol Neurosci 8, 78.

Minichiello, L., 2009. TrkB signalling pathways in LTP and learning. Nat.Rev.Neurosci. 10, 850-860.

Minichiello, L., Calella, A. M., Medina, D. L., Bonhoeffer, T., Klein, R., Korte, M., 2002. Mechanism of TrkB-mediated hippocampal long-term potentiation. Neuron 36, 121-137.

Morelli, E., Ghiglieri, V., Pendolino, V., Bagetta, V., Pignataro, A., Fejtova, A., Costa, C., Ammassari-Teule, M., Gundelfinger, E. D., Picconi, B., Calabresi, P., 2014. Environmental enrichment restores CA1 hippocampal LTP and reduces severity of seizures in epileptic mice. Exp Neurol 261, 320-327.

Morris, R. G. M., Anderson, E., Lynch, G. S., Baudry, M., 1986. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature 319, 774-776.

Nikolaienko, O., Patil, S., Eriksen, M. S., Bramham, C. R., 2018. Arc protein: a flexible hub for synaptic plasticity and cognition. Seminars in Cell & Developmental Biology 77, 33-42.

Novkovic, T., Heumann, R., Manahan-Vaughan, D., 2015a. Ras does not contribute to the facilitation of hippocampal synaptic plasticity enabled by environmental enrichment. Neuroscience 309, 214-223.

Novkovic, T., Mittmann, T., Manahan-Vaughan, D., 2015b. BDNF contributes to the facilitation of hippocampal synaptic plasticity and learning enabled by environmental enrichment. Hippocampus 25, 1-15.

Numakawa, T., Odaka, H., Adachi, N., 2018. Actions of Brain-Derived Neurotrophin Factor in the Neurogenesis and Neuronal Function, and Its Involvement in the Pathophysiology of Brain Diseases. Int J Mol Sci 19.

Ohline, S. M., Abraham, W. C., 2019. Environmental enrichment effects on synaptic and cellular physiology of hippocampal neurons. Neuropharmacology 145, 3-12.

Okuno, H., Akashi, K., Ishii, Y., Yagishita-Kyo, N., Suzuki, K., Nonaka, M., Kawashima, T., Fujii, H., Takemoto-Kimura, S., Abe, M., Natsume, R., Chowdhury, S., Sakimura, K., Worley, P. F., Bito, H., 2012. Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKIIβ. Cell 149, 886-898.

Olateju, O. I., More, L., Arthur, J. S. C., Frenguelli, B. G., 2021. Mitogen and Stress-activated Protein Kinase 1 Negatively Regulates Hippocampal Neurogenesis. Neuroscience 452, 228-234.

Pang, T. Y., Hannan, A. J., Lawrence, A. J., 2019. Novel approaches to alcohol rehabilitation: Modification of stress-responsive brain regions through environmental enrichment. Neuropharmacology 145, 25-36.

Panja, D., Bramham, C. R., 2013. BDNF mechanisms in late LTP formation: A synthesis and breakdown. Neuropharmacology.

Pinaud, R., Penner, M. R., Robertson, H. A., Currie, R. W., 2001. Upregulation of the immediate early gene arc in the brains of rats exposed to environmental enrichment: implications for molecular plasticity. Brain Res.Mol.Brain Res. 91, 50-56.

Pozo, K., Goda, Y., 2010. Unraveling mechanisms of homeostatic synaptic plasticity. Neuron 66, 337-351.

Privitera, L., Morè, L., Cooper, D. D., Richardson, P., Tsogka, M., Hebenstreit, D., Arthur, J. S. C., Frenguelli, B. G., 2020. Experience Recruits MSK1 to Expand the Dynamic Range of Synapses and Enhance Cognition. The Journal of Neuroscience 40, 4644.

Reyskens, K. M., Arthur, J. S., 2016. Emerging Roles of the Mitogen and Stress Activated Kinases MSK1 and MSK2. Front Cell Dev Biol 4, 56.

Rial Verde, E. M., Lee-Osbourne, J., Worley, P. F., Malinow, R., Cline, H. T., 2006. Increased expression of the immediate-early gene arc/arg3.1 reduces AMPA receptor-mediated synaptic transmission. Neuron 52, 461-474.

Rogers, J., Renoir, T., Hannan, A. J., 2019. Gene-environment interactions informing therapeutic approaches to cognitive and affective disorders. Neuropharmacology 145, 37-48.

Rosenzweig, M. R., Bennett, E. L., 1996. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav.Brain Res. 78, 57-65.

Rosenzweig, M. R., Leiman, A. L., 1968. Brain functions. Annu.Rev.Psychol. 19, 55-98.

Rossi, C., Angelucci, A., Costantin, L., Braschi, C., Mazzantini, M., Babbini, F., Fabbri, M. E., Tessarollo, L., Maffei, L., Berardi, N., Caleo, M., 2006. Brain-derived neurotrophic factor (BDNF) is required for the enhancement of hippocampal neurogenesis following environmental enrichment. Eur.J.Neurosci. 24, 1850-1856.

Sakata, K., Martinowich, K., Woo, N. H., Schloesser, R. J., Jimenez, D. V., Ji, Y., Shen, L., Lu, B., 2013. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. Proc Natl Acad Sci U S A 110, 15103-15108.

Sanders, M. R., Kirby, J. N., Tellegen, C. L., Day, J. J., 2014. The Triple P-Positive Parenting Program: a systematic review and meta-analysis of a multi-level system of parenting support. Clin Psychol Rev 34, 337-357.

Shepherd, J. D., Rumbaugh, G., Wu, J., Chowdhury, S., Plath, N., Kuhl, D., Huganir, R. L., Worley, P. F., 2006. Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. Neuron 52, 475-484.

Stein, L. R., O'Dell, K. A., Funatsu, M., Zorumski, C. F., Izumi, Y., 2016. Short-term environmental enrichment enhances synaptic plasticity in hippocampal slices from aged rats. Neuroscience 329, 294-305.

Steward, O., Farris, S., Pirbhoy, P. S., Darnell, J., Driesche, S. J., 2014. Localization and local translation of Arc/Arg3.1 mRNA at synapses: some observations and paradoxes. Front Mol Neurosci 7, 101.

Takeuchi, T., Duszkiewicz, A. J., Morris, R. G., 2014. The synaptic plasticity and memory hypothesis: encoding, storage and persistence. Philos Trans R Soc Lond B Biol Sci 369, 20130288.

Torrado Pacheco, A., Bottorff, J., Gao, Y., Turrigiano, G. G., 2021. Sleep Promotes Downward Firing Rate Homeostasis. Neuron 109, 530-544 e536.

Turrigiano, G., 2012. Homeostatic Synaptic Plasticity: Local and Global Mechanisms for Stabilizing Neuronal Function. Cold Spring Harbor Perspectives in Biology 4, a005736.

Turrigiano, G. G., 2017. The dialectic of Hebb and homeostasis. Philos Trans R Soc Lond B Biol Sci 372.

Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C., Nelson, S. B., 1998. Activity-dependent scaling of quantal amplitude in neocortical neurons. Nature 391, 892-896.

van Praag, H., Christie, B. R., Sejnowski, T. J., Gage, F. H., 1999. Running enhances neurogenesis, learning, and long-term potentiation in mice. Proc Natl Acad Sci U S A 96, 13427-13431.

Vilar, M., Mira, H., 2016. Regulation of Neurogenesis by Neurotrophins during Adulthood: Expected and Unexpected Roles. Front Neurosci 10, 26.

von Bohlen und Halbach, O., von Bohlen und Halbach, V., 2018. BDNF effects on dendritic spine morphology and hippocampal function. Cell and Tissue Research 373, 729-741.

Wall, M. J., Collins, D. R., Chery, S. L., Allen, Z. D., Pastuzyn, E. D., George, A. J., Nikolova, V. D., Moy, S. S., Philpot, B. D., Shepherd, J. D., Müller, J., Ehlers, M. D., Mabb, A. M., Corrêa, S. A. L., 2018. The Temporal Dynamics of Arc Expression Regulate Cognitive Flexibility. Neuron 98, 1124-1132.e1127.

Wassouf, Z., Hentrich, T., Samer, S., Rotermund, C., Kahle, P. J., Ehrlich, I., Riess, O., Casadei, N., Schulze-Hentrich, J. M., 2018. Environmental Enrichment Prevents Transcriptional Disturbances Induced by Alpha-Synuclein Overexpression. Front Cell Neurosci 12, 112.

Watkins, J. C., Evans, R. H., 1981. Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 21, 165-204.

West, A. E., Pruunsild, P., Timmusk, T., 2014. Neurotrophins: Transcription and Translation. In: Lewin, G. R., Carter, B. D., (Eds), Neurotrophic Factors. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 67-100.

Winder, D. G., Martin, K. C., Muzzio, I. A., Rohrer, D., Chruscinski, A., Kobilka, B., Kandel, E. R., 1999. ERK plays a regulatory role in induction of LTP by theta frequency stimulation and its modulation by beta-adrenergic receptors. Neuron 24, 715-726.

Woo, C. C., Donnelly, J. H., Steinberg-Epstein, R., Leon, M., 2015. Environmental enrichment as a therapy for autism: A clinical trial replication and extension. Behav Neurosci 129, 412-422.

Woo, C. C., Leon, M., 2013. Environmental enrichment as an effective treatment for autism: a randomized controlled trial. Behav Neurosci 127, 487-497.

Yilmaz-Rastoder, E., Miyamae, T., Braun, A. E., Thiels, E., 2011. LTP- and LTD-inducing stimulations cause opposite changes in arc/arg3.1 mRNA level in hippocampal area CA1 in vivo. Hippocampus 21, 1290-1301.

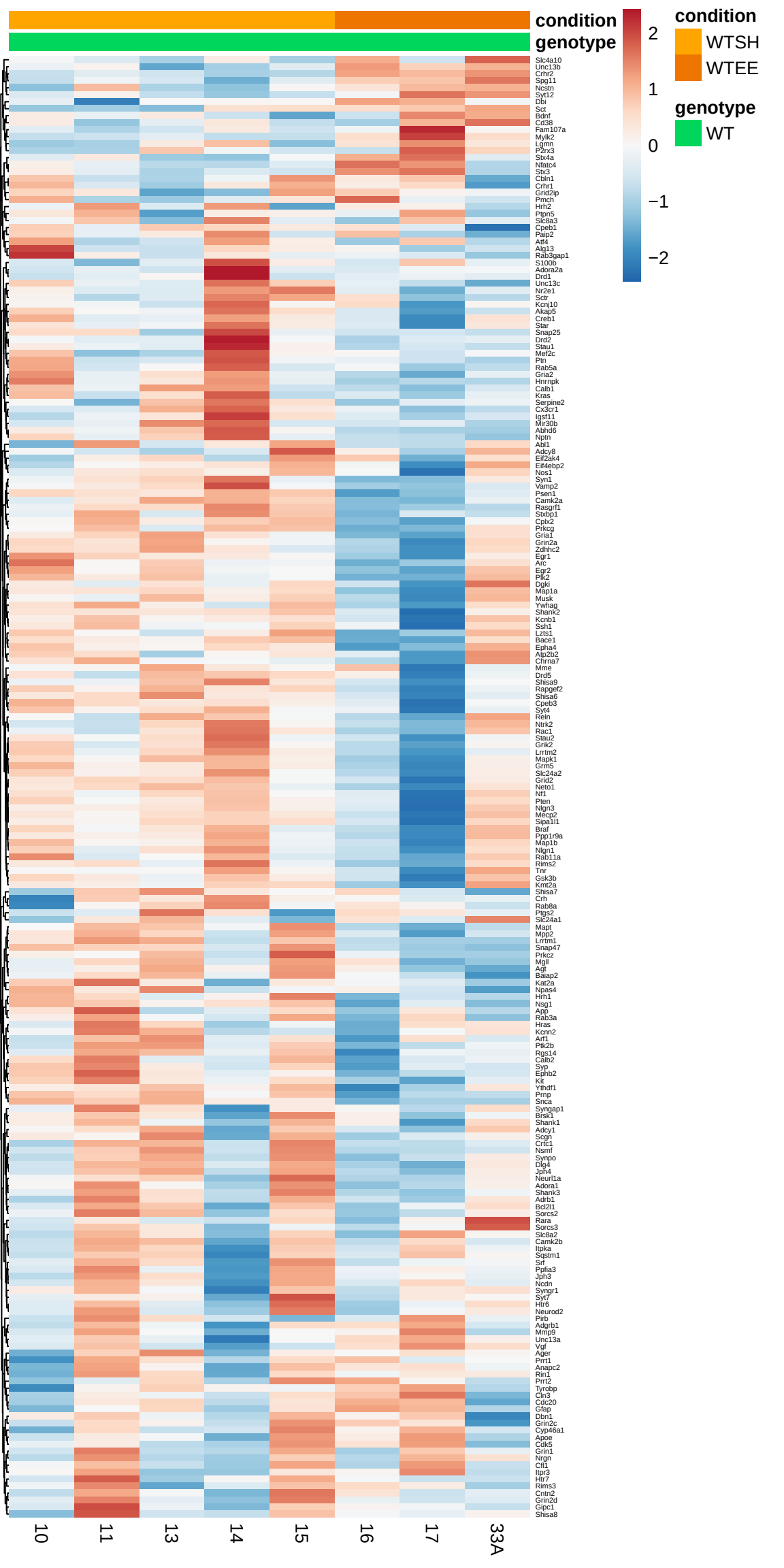
Zagrebelsky, M., Tacke, C., Korte, M., 2020. BDNF signaling during the lifetime of dendritic spines. Cell and Tissue Research 382, 185-199.

Zarif, H., Nicolas, S., Guyot, M., Hosseiny, S., Lazzari, A., Canali, M. M., Cazareth, J., Brau, F., Golzne, V., Dourneau, E., Maillaut, M., Luci, C., Paquet, A., Lebrigand, K., Arguel, M. J., Daoudlarian, D., Heurteaux, C., Glaichenhaus, N., Chabry, J., Guyon, A., Petit-Paitel, A., 2018. CD8(+) T cells are essential for the effects of enriched environment on hippocampus-dependent behavior, hippocampal neurogenesis and synaptic plasticity. Brain Behav Immun 69, 235-254.

Zhang, H., Bramham, C. R., 2020. Arc/Arg3.1 function in long-term synaptic plasticity: Emerging mechanisms and unresolved issues. Eur J Neurosci.

Zhu, H., Zhang, J., Sun, H., Zhang, L., Liu, H., Zeng, X., Yang, Y., Yao, Z., 2011. An enriched environment reverses the synaptic plasticity deficit induced by chronic cerebral hypoperfusion. Neurosci Lett 502, 71-75.

Zhu, S. W., Codita, A., Bogdanovic, N., Hjerling-Leffler, J., Ernfors, P., Winblad, B., Dickins, D. W., Mohammed, A. H., 2009. Influence of environmental manipulation on exploratory behaviour in male BDNF knockout mice. Behav.Brain Res. 197, 339-346.


condition
genotype
2
1
0
−1
−2
condition
WTSH
WTEE
genotype
WT
Slc4a10
Unc13b
Crhr2
Spg11
Ncstn
Syt12
Dbi
Sct
Bdnf
Cd38
Fam107a
Mylk2
Lgmn
P2rx3
Stx4a
Nfatc4
Stx3
Cbln1
Crhr1
Grid2ip
Pmch
Hrh2
Ptpn5
Slc8a3
Cpeb1
Paip2
Atf4
Alg13
Rab3gap1
S100b
Adora2a
Drd1
Unc13c
Nr2e1
Sctr
Kcnj10
Akap5
Creb1
Star
Snap25
Drd2
Stau1
Mef2c
Ptn
Rab5a
Gria2
Hnrnpk
Calb1
Kras
Serpine2
Cx3cr1
Igsf11
Mir30b
Abhd6
Nptn
Abl1
Adcy8
Eif2ak4
Eif4ebp2
Nos1
Syn1
Vamp2
Psen1
Camk2a
Rasgrf1
Stxbp1
Cplx2
Prkcg
Gria1
Grin2a
Zdhhc2
Egr1
Arc
Egr2
Plk2
Dgki
Map1a
Musk
Ywhag
Shank2
Kcnb1
Ssh1
Lzts1
Bace1
Epha4
Atp2b2
Chrna7
Mme
Drd5
Shisa9
Rapgef2
Shisa6
Cpeb3
Syt4
Reln
Ntrk2
Rac1
Stau2
Grik2
Lrrtm2
Mapk1
Grm5
Slc24a2
Grid2
Neto1
Nf1
Pten
Nlgn3
Mecp2
Sipa1l1
Braf
Ppp1r9a
Map1b
Nlgn1
Rab11a
Rims2
Tnr
Gsk3b
Kmt2a
Shisa7
Crh
Rab8a
Ptgs2
Slc24a1
Mapt
Mpp2
Lrrtm1
Snap47
Prkcz
Mgll
Agt
Baiap2
Kat2a
Npas4
Hrh1
Nsg1
App
Rab3a
Hras
Kcnn2
Arf1
Ptk2b
Rgs14
Calb2
Syp
Ephb2
Kit
Ythdf1
Prnp
Snca
Syngap1
Brsk1
Shank1
Adcy1
Scgn
Crtc1
Nsmf
Synpo
Dlg4
Jph4
Neurl1a
Adora1
Shank3
Adrb1
Bcl2l1
Sorcs2
Rara
Sorcs3
Slc8a2
Camk2b
Itpka
Sqstm1
Srf
Ppfia3
Jph3
Ncdn
Syngr1
Syt7
Htr6
Neurod2
Pirb
Adgrb1
Mmp9
Unc13a
Vgf
Ager
Prrt1
Anapc2
Rin1
Prrt2
Tyrobp
Cln3
Cdc20
Gfap
Dbn1
Grin2c
Cyp46a1
Apoe
Cdk5
Grin1
Nrgn
Cfl1
Itpr3
Htr7
Rims3
Cmn2
Grin2d
Glpc1
Shisa8
10
11
13
14
15
16
17
33A

	GO.ID	Term	Annotated	Significant	Expected	Fold Enrichment	Rank in fish.cl	fish.weight	fish.class	Benjamini.fish.class
1	GO:0030198	extracellular matrix organization	295	40	5.97	6.700167504	1	4.10E-06	1.30E-21	1.12E-17
2	GO:0043062	extracellular structure organization	296	40	5.99	6.677796327	2	1	1.40E-21	1.12E-17
3	GO:0016043	cellular component organization	5773	192	116.74	1.644680487	3	1	3.00E-15	1.60E-11
4	GO:0044782	cilium organization	327	33	6.61	4.992435703	4	0.00322	3.30E-14	1.32E-10
5	GO:0072359	circulatory system development	1153	65	23.32	2.787307033	5	0.01356	5.50E-14	1.76E-10
6	GO:0071840	cellular component organization or biogenesis	5956	192	120.44	1.594154766	6	1	8.00E-14	2.13E-10
7	GO:0007155	cell adhesion	1352	71	27.34	2.596927579	7	0.00025	1.10E-13	2.51E-10
8	GO:0022610	biological adhesion	1365	71	27.6	2.572463768	8	1	1.70E-13	3.37E-10
9	GO:0001578	microtubule bundle formation	102	19	2.06	9.223300971	9	0.02618	1.90E-13	3.37E-10
10	GO:0001944	vasculature development	766	50	15.49	3.227888961	10	1	2.90E-13	4.63E-10
11	GO:0035082	axoneme assembly	69	16	1.4	11.42857143	11	2.70E-05	4.30E-13	5.72E-10
12	GO:0006928	movement of cell or subcellular component	2052	91	41.5	2.192771084	12	1	4.30E-13	5.72E-10
13	GO:0001568	blood vessel development	732	48	14.8	3.243243243	13	0.00031	7.70E-13	9.45E-10
14	GO:0060271	cilium assembly	294	29	5.95	4.87394958	14	0.0008	2.20E-12	2.51E-09
15	GO:0030199	collagen fibril organization	56	14	1.13	12.38938053	15	3.00E-10	4.20E-12	4.47E-09
16	GO:0003341	cilium movement	171	22	3.46	6.358381503	16	6.80E-07	5.70E-12	5.68E-09
17	GO:0051179	localization	5906	184	119.43	1.540651428	17	1	1.10E-11	1.03E-08
18	GO:0006820	anion transport	588	40	11.89	3.364171573	18	0.05567	2.30E-11	2.04E-08
19	GO:0040011	locomotion	1819	79	36.78	2.147906471	19	1	6.00E-11	5.04E-08
20	GO:0035239	tube morphogenesis	922	51	18.65	2.73458445	20	0.05501	7.60E-11	5.73E-08
21	GO:0051674	localization of cell	1624	73	32.84	2.222898904	21	1	7.90E-11	5.73E-08
22	GO:0048870	cell motility	1624	73	32.84	2.222898904	22	1	7.90E-11	5.73E-08
23	GO:0035295	tube development	1144	58	23.13	2.507565932	23	1	1.00E-10	6.94E-08
24	GO:0009653	anatomical structure morphogenesis	2759	102	55.79	1.828284639	24	0.36131	6.10E-10	4.06E-07
25	GO:0048646	anatomical structure formation involved in morphogenesis	1168	57	23.62	2.413209145	25	1	6.40E-10	4.08E-07
26	GO:0048514	blood vessel morphogenesis	636	39	12.86	3.032659409	26	1	8.60E-10	5.28E-07
27	GO:0120031	plasma membrane bounded cell projection assembly	487	33	9.85	3.350253807	27	1	1.60E-09	9.45E-07
28	GO:0001525	angiogenesis	527	34	10.66	3.189493433	28	2.60E-05	3.10E-09	1.77E-06
29	GO:0030031	cell projection assembly	502	33	10.15	3.251231527	29	1	3.30E-09	1.82E-06
30	GO:0015849	organic acid transport	332	26	6.71	3.874813711	30	1	4.70E-09	2.50E-06
31	GO:0007010	cytoskeleton organization	1319	59	26.67	2.212223472	31	0.09826	8.00E-09	4.12E-06
32	GO:0009987	cellular process	16467	379	333	1.138138138	32	1	8.40E-09	4.19E-06
33	GO:0007017	microtubule-based process	843	44	17.05	2.580645161	33	1	9.70E-09	4.69E-06
34	GO:0048856	anatomical structure development	5792	170	117.13	1.45137881	34	1	1.60E-08	7.29E-06

35	GO:0015711	organic anion transport	455	30	9.2	3.260869565	35	1	1.60E-08	7.29E-06
36	GO:0009888	tissue development	1917	75	38.77	1.934485427	36	1	2.00E-08	8.86E-06
37	GO:0098656	anion transmembrane transport	218	20	4.41	4.535147392	37	0.28478	2.10E-08	9.06E-06
38	GO:0000226	microtubule cytoskeleton organization	574	34	11.61	2.928509905	38	0.0255	2.60E-08	1.09E-05
39	GO:0048513	animal organ development	3490	115	70.58	1.629356758	39	1	3.00E-08	1.21E-05
40	GO:0048762	mesenchymal cell differentiation	223	20	4.51	4.4345898	40	1	3.10E-08	1.21E-05
41	GO:0032502	developmental process	6246	179	126.31	1.417148286	41	1	3.10E-08	1.21E-05
42	GO:0031589	cell-substrate adhesion	345	25	6.98	3.581661891	42	1	4.30E-08	1.63E-05
43	GO:0060429	epithelium development	1160	52	23.46	2.216538789	43	1	6.40E-08	2.37E-05
44	GO:0016477	cell migration	1471	61	29.75	2.050420168	44	0.77457	6.90E-08	2.50E-05
45	GO:0070925	organelle assembly	780	40	15.77	2.536461636	45	1	7.60E-08	2.69E-05
46	GO:0007162	negative regulation of cell adhesion	284	22	5.74	3.832752613	46	0.57077	8.80E-08	3.05E-05
47	GO:0007167	enzyme linked receptor protein signaling pathway	916	44	18.52	2.375809935	47	1	1.10E-07	3.73E-05
48	GO:0030155	regulation of cell adhesion	713	37	14.42	2.565880721	48	0.13168	1.80E-07	5.98E-05
49	GO:0090287	regulation of cellular response to growth factor stimulus	275	21	5.56	3.776978417	49	1	2.20E-07	7.16E-05
50	GO:0098739	import across plasma membrane	164	16	3.32	4.819277108	50	1	2.40E-07	7.51E-05
51	GO:0022607	cellular component assembly	2538	88	51.32	1.714731099	51	1	2.40E-07	7.51E-05
52	GO:0046942	carboxylic acid transport	328	23	6.63	3.46907994	52	1	2.70E-07	8.27E-05
53	GO:0060485	mesenchyme development	279	21	5.64	3.723404255	53	1	2.80E-07	8.27E-05
54	GO:0055085	transmembrane transport	1285	54	25.99	2.077722201	54	0.00071	2.80E-07	8.27E-05
55	GO:0097435	supramolecular fiber organization	709	36	14.34	2.510460251	55	1	4.50E-07	0.00013054
56	GO:0048731	system development	4738	140	95.81	1.461225342	56	1	4.80E-07	0.00013676
57	GO:0007018	microtubule-based movement	371	24	7.5	3.2	57	0.20429	6.40E-07	0.00017914
58	GO:0006811	ion transport	1574	61	31.83	1.91643104	58	0.59732	7.00E-07	0.00019256
59	GO:0048251	elastic fiber assembly	10	5	0.2	25	59	7.70E-07	7.70E-07	0.00020823
60	GO:0018057	peptidyl-lysine oxidation	5	4	0.1	40	60	8.10E-07	8.10E-07	0.00021539
61	GO:0051270	regulation of cellular component movement	1054	46	21.31	2.158610981	61	0.03774	8.30E-07	0.00021709
62	GO:0009887	animal organ morphogenesis	1094	47	22.12	2.12477396	62	0.43846	9.70E-07	0.00024962
63	GO:0085029	extracellular matrix assembly	40	8	0.81	9.87654321	63	0.18231	1.10E-06	0.00027858
64	GO:0001837	epithelial to mesenchymal transition	142	14	2.87	4.87804878	64	0.08681	1.20E-06	0.00029916
65	GO:1903825	organic acid transmembrane transport	123	13	2.49	5.220883534	65	0.01977	1.30E-06	0.0003191
66	GO:0001539	cilium or flagellum-dependent cell motility	129	13	2.61	4.980842912	66	1	2.20E-06	0.0005239
67	GO:0060285	cilium-dependent cell motility	129	13	2.61	4.980842912	67	0.13048	2.20E-06	0.0005239
68	GO:0071363	cellular response to growth factor stimulus	604	31	12.21	2.538902539	68	1	2.30E-06	0.00053965
69	GO:0090092	regulation of transmembrane receptor protein serine/threor	242	18	4.89	3.680981595	69	1	2.40E-06	0.00054703

70	GO:0042221	response to chemical	4049	121	81.88	1.47777235	70	1	2.40E-06	0.00054703
71	GO:2000145	regulation of cell motility	965	42	19.51	2.152742183	71	1	2.80E-06	0.00062921
72	GO:0120036	plasma membrane bounded cell projection organization	1541	58	31.16	1.861360719	72	1	3.40E-06	0.00075343
73	GO:0070848	response to growth factor	617	31	12.48	2.483974359	73	1	3.60E-06	0.00078682
74	GO:0030030	cell projection organization	1582	59	31.99	1.844326352	74	0.30402	3.70E-06	0.00078711
75	GO:1901343	negative regulation of vasculature development	135	13	2.73	4.761904762	75	1	3.70E-06	0.00078711
76	GO:0040012	regulation of locomotion	1012	43	20.47	2.100635076	76	1	3.80E-06	0.00079775
77	GO:2000026	regulation of multicellular organismal development	2199	75	44.47	1.686530245	77	1	4.20E-06	0.00087027
78	GO:0030154	cell differentiation	4193	123	84.79	1.450642764	78	0.03706	5.00E-06	0.00102276
79	GO:0071702	organic substance transport	2456	81	49.67	1.630763036	79	1	5.80E-06	0.00117138
80	GO:0007275	multicellular organism development	5306	148	107.3	1.379310345	80	0.4692	6.00E-06	0.00119663
81	GO:1905039	carboxylic acid transmembrane transport	122	12	2.47	4.858299595	81	1	7.00E-06	0.00136201
82	GO:0060294	cilium movement involved in cell motility	122	12	2.47	4.858299595	82	0.00015	7.00E-06	0.00136201
83	GO:0051241	negative regulation of multicellular organismal process	1324	51	26.77	1.905117669	83	1	7.50E-06	0.00144172
84	GO:0006810	transport	4320	125	87.36	1.430860806	84	1	8.10E-06	0.00153852
85	GO:0030449	regulation of complement activation	15	5	0.3	16.66666667	85	0.09629	8.40E-06	0.00157673
86	GO:0044085	cellular component biogenesis	2766	88	55.94	1.573114051	86	1	8.70E-06	0.00161405
87	GO:0003006	developmental process involved in reproduction	1051	43	21.25	2.023529412	87	1	9.80E-06	0.00179723
88	GO:0048869	cellular developmental process	4297	124	86.9	1.426927503	88	1	1.00E-05	0.00181307
89	GO:1901342	regulation of vasculature development	351	21	7.1	2.957746479	89	1	1.10E-05	0.00190766
90	GO:0072337	modified amino acid transport	26	6	0.53	11.32075472	90	1	1.10E-05	0.00190766
91	GO:0030334	regulation of cell migration	917	39	18.54	2.103559871	91	0.76695	1.10E-05	0.00190766
92	GO:0015718	monocarboxylic acid transport	172	14	3.48	4.022988506	92	0.00085	1.10E-05	0.00190766
93	GO:0051093	negative regulation of developmental process	1059	43	21.42	2.007469655	93	1	1.20E-05	0.00201537
94	GO:0022603	regulation of anatomical structure morphogenesis	1096	44	22.16	1.985559567	94	1	1.20E-05	0.00201537
95	GO:0098609	cell-cell adhesion	754	34	15.25	2.229508197	95	0.21255	1.20E-05	0.00201537
96	GO:0098657	import into cell	247	17	4.99	3.406813627	96	1	1.30E-05	0.0021383
97	GO:0055114	oxidation-reduction process	823	36	16.64	2.163461538	97	0.11128	1.30E-05	0.0021383
98	GO:0051234	establishment of localization	4455	127	90.09	1.40970141	98	1	1.40E-05	0.00225626
99	GO:0048729	tissue morphogenesis	661	31	13.37	2.318623785	99	1	1.40E-05	0.00225626
100	GO:0006935	chemotaxis	600	29	12.13	2.390766694	100	0.51631	1.50E-05	0.00239325
101	GO:0010717	regulation of epithelial to mesenchymal transition	92	10	1.86	5.376344086	101	0.21413	1.70E-05	0.00265917
102	GO:0070286	axonemal dynein complex assembly	28	6	0.57	10.52631579	102	0.00386	1.70E-05	0.00265917
103	GO:0007178	transmembrane receptor protein serine/threonine kinase sig	363	21	7.34	2.861035422	103	1	1.80E-05	0.00276144
104	GO:0042330	taxis	605	29	12.23	2.371218316	104	1	1.80E-05	0.00276144

105	GO:0050793	regulation of developmental process	2740	86	55.41	1.552066414	105	1	1.90E-05	0.00285986
106	GO:0022414	reproductive process	1520	55	30.74	1.78919974	106	0.09166	1.90E-05	0.00285986
107	GO:0000003	reproduction	1521	55	30.76	1.788036411	107	1	2.00E-05	0.00298224
108	GO:0010812	negative regulation of cell-substrate adhesion	58	8	1.17	6.837606838	108	0.00014	2.10E-05	0.00310236
109	GO:0009636	response to toxic substance	115	11	2.33	4.721030043	109	0.006	2.20E-05	0.00322028
110	GO:0016525	negative regulation of angiogenesis	118	11	2.39	4.60251046	110	0.00183	2.80E-05	0.00406127
111	GO:0040013	negative regulation of locomotion	318	19	6.43	2.954898911	111	0.12981	2.90E-05	0.00416842
112	GO:0075294	positive regulation by symbiont of entry into host	10	4	0.2	20	112	1	3.10E-05	0.00436376
113	GO:0046598	positive regulation of viral entry into host cell	10	4	0.2	20	113	3.10E-05	3.10E-05	0.00436376
114	GO:0051239	regulation of multicellular organismal process	3279	98	66.31	1.477906801	114	1	3.20E-05	0.00436376
115	GO:0044458	motile cilium assembly	31	6	0.63	9.523809524	115	3.20E-05	3.20E-05	0.00436376
116	GO:0060669	embryonic placenta morphogenesis	31	6	0.63	9.523809524	116	0.00386	3.20E-05	0.00436376
117	GO:0060686	negative regulation of prostatic bud formation	4	3	0.08	37.5	117	3.20E-05	3.20E-05	0.00436376
118	GO:0032835	glomerulus development	62	8	1.25	6.4	118	0.16585	3.40E-05	0.0045972
119	GO:0099587	inorganic ion import across plasma membrane	100	10	2.02	4.95049505	119	1	3.50E-05	0.00465354
120	GO:0098659	inorganic cation import across plasma membrane	100	10	2.02	4.95049505	120	1	3.50E-05	0.00465354
121	GO:0051272	positive regulation of cellular component movement	596	28	12.05	2.323651452	121	1	3.60E-05	0.00474694
122	GO:2000181	negative regulation of blood vessel morphogenesis	122	11	2.47	4.453441296	122	1	3.80E-05	0.00496959
123	GO:0002577	regulation of antigen processing and presentation	20	5	0.4	12.5	123	1	4.00E-05	0.00514677
124	GO:0002504	antigen processing and presentation of peptide or polysacch	20	5	0.4	12.5	124	1	4.00E-05	0.00514677
125	GO:0010718	positive regulation of epithelial to mesenchymal transition	47	7	0.95	7.368421053	125	4.10E-05	4.10E-05	0.00523324
126	GO:0060349	bone morphogenesis	102	10	2.06	4.854368932	126	0.11306	4.20E-05	0.00531833
127	GO:0002921	negative regulation of humoral immune response	11	4	0.22	18.18181818	127	1	4.90E-05	0.00615587
128	GO:2000147	positive regulation of cell motility	576	27	11.65	2.317596567	128	1	5.10E-05	0.00630779
129	GO:0055064	chloride ion homeostasis	21	5	0.42	11.9047619	129	0.00088	5.10E-05	0.00630779
130	GO:0090288	negative regulation of cellular response to growth factor stin	149	12	3.01	3.986710963	130	1	5.30E-05	0.00650473
131	GO:0006996	organelle organization	3511	102	71	1.436619718	131	1	6.50E-05	0.0079166
132	GO:0030335	positive regulation of cell migration	554	26	11.2	2.321428571	132	0.02284	6.90E-05	0.00834011
133	GO:0038063	collagen-activated tyrosine kinase receptor signaling pathwa	12	4	0.24	16.66666667	133	7.20E-05	7.20E-05	0.00863729
134	GO:0002009	morphogenesis of an epithelium	558	26	11.28	2.304964539	134	0.28516	7.70E-05	0.00916817
135	GO:0014031	mesenchymal cell development	89	9	1.8	5	135	0.09574	7.90E-05	0.00933663
136	GO:0032963	collagen metabolic process	110	10	2.22	4.504504505	136	0.0308	8.00E-05	0.00936489
137	GO:0040017	positive regulation of locomotion	592	27	11.97	2.255639098	137	1	8.10E-05	0.00936489
138	GO:0001655	urogenital system development	373	20	7.54	2.652519894	138	1	8.10E-05	0.00936489
139	GO:0032879	regulation of localization	2816	85	56.95	1.492537313	139	1	8.70E-05	0.00998622