Exploring the triad of behaviour, genes and neuronal networks: Heritability of instrumental conditioning and the Arc/Arg3.1 gene in hippocampal coding
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Chapter 6. General discussion
In this Discussion, we draw together the results from the previous chapters and relate them to the recent state or research. In chapters 2 and 3, we assessed the heritable background of acquisition and extinction of appetitive operant learning. Seven standard inbred strains and 28 recombinant inbred mouse lines were screened using a sequential learning protocol. The most common laboratory mouse strain, C57BL/6J, performed well in all of the sequential tasks, as did DBA/2J and Balb/cByJ mice. These mouse lines could thus be used as background strains in knock-out studies. We would, however, advice against using 129S1/Sv, which performed poorly on all tasks.

We found that different stages of appetitive operant learning as well as extinction are under genetic control and are phenotypically and genotypically dissociable. The results presented in Chapter 2 suggested that performance in an operant lever press–nose poke task is regulated by an area on chromosome 9, whereas initial magazine checking behaviour is suggested to be regulated by areas on chromosomes 4 and 6. Furthermore, these traits were not correlated when the effect of the intermediate stage (operant nose poke) was taken into account. Similarly, performance and extinction of the lever press–nose poke task were not correlated, as described in Chapter 3.

We suggested that mouse strains NOD/LtJ, BxD-16 and BxD-42 may be used as models for perseverative food-seeking behaviour. We also found that the widely used C57BL/6J strain was expressing perseverative behaviour and failed to show a steadily progressing decline in lever press behaviour in the absence of reward, which is important to take into account when designing behavioural tasks that involve learning tasks that require flexibility. DBA/2J showed normal extinction behaviour and could be used instead. C3H/HeJ expressed the highest flexibility of the tested mouse lines, and could be used in tasks that require flexible changes in behavioural responding.

In chapters 4 and 5, we characterized hippocampal CA1 activity in Arc/Arg3.1 knockout and wildtype mice during a spatial exploration task and sleep, respectively. Basic firing properties of both fast-spiking neurons and pyramidal cells, as well as place field properties, were preserved in Arc/Arg3.1 knockouts. However, we found that local field potential activity was changed in Arc/Arg3.1 knockout mice, which showed attenuated oscillatory activity in beta-2 and gamma ranges during both track running and sleep. Furthermore, in Chapter 4 we showed that spike-
locking to these frequencies as well as to theta was impaired in the awake state of knockout mice, which may explain the previously reported deficits in the Morris water maze.

In addition to altered local field potential and spike–field phase locking activity, we also found that occurrence of hippocampal sharp–wave ripples during sleep (and waking) as well as spiking activity during them was diminished in knockouts, as described in Chapter 5. Furthermore, correlated firing during sleep was decreased in knockouts.

Taking chapter 4 and 5 together, these findings indicate that although the functionality of individual neurons appears intact, coherent population and network activity is altered in Arc/Arg3.1 knockout mice.
6.1 Genetic background of appetitive operant behaviour revisited

Since finishing the research described in Chapter 2 and Chapter 3, a number of interesting quantitative genetics studies have been published. One of the most intriguing recombinant inbred mouse studies assessed 51 BxD strains in an appetitive operant - reversal learning task (Laughlin et al., 2011). Their set of BxD strains as well as task (nose–poke with set performance criterion instead of lever press with set number of sessions) differed somewhat from ours, making direct comparison to our operant - extinction task difficult. Unfortunately, Laughlin et al. also did not test e.g. BxD-27 or BxD-33, which were one of the top performers at both the operant and extinction stages in our studies.

The parental strains DBA/2J and C57Bl/6J performed somewhat similarly in both studies – in line with Laughlin et al., in our operant task, C57Bl/6J was in the midrange and DBA/2J performed better than C57Bl/6J (Chapter 2). The same pattern was observed in the reversal task by Laughlin et al. and also in our extinction task C57Bl/6J was roughly in the midrange and DBA/2J was faster to extinguish responding, although this finding was not significant (Chapter 3). Interestingly, according to Laughlin et al. BxD-16 required the highest number of trials to meet both operant and reversal learning criteria (and thus did not show a dissociation between them, as they did in acquisition vs. extinction of operant behaviour in our experiments), but this strain was among the worst performing (i.e. most perseverative) in our extinction task, whereas it was among the best 30% BxD lines in our operant task.

Consistent with the results presented in Chapter 3, Laughlin et al. reported that acquisition and reversal learning abilities did not covary at the strain level, coming to the same conclusion as we reached for acquisition and extinction, viz. that these traits must be regulated by distinct sets of genes. Because Laughlin et al. screened a far larger number of strains than we did, they were able to identify a genomic region of interest on chromosome 10 with statistically significant linkage to reversal learning, whereas the region of interest we suggest to regulate nose poke–lever press task in Chapter 2 was on chromosome 9. This is in line with our hypothesis that acquisition of operant learning is under distinct genetic control that is separate from control of reversal and extinction learning (Chapter 3).
To conclude, it appears that, although direct comparison between studies is difficult because of different experimental protocols and sets of recombinant-inbred strains, some general principles regarding heritable regulation of operant behaviour are consistent throughout the studies.

As discussed in the Introduction and Chapter 2, many commonly used mouse strains have not been thoroughly characterized, which is a serious caveat in behavioural studies on mice in general. A recent study reported that visual performance, acuity and stereovision in Balb/c mice was significantly worse than that of C57Bl/6 mice (Yeritsyan et al., 2012). However, in our operant task, which took place in a visually rather simple environment with only few distinct cues present, Balb/c performed in fact better than C57Bl/6J, so there was no need to revisit the results in that respect.

These examples, together with comparison to previous behavioural screening studies in Chapters 2 and 3, illustrate the well-known reproducibility issues in behavioural neuroscience. Often the different results can be attributed to different experimental protocols, but it would be important that all the details regarding the experimental procedures would be reported fully.

6.1.1 Compulsive food seeking as a model for addiction

Some researchers have suggested that intermittent intake of sugar would result in "sugar addiction", which would share many aspects with drug addiction (Lenoir et al., 2007; Avena et al., 2008). In fact, rodents work more readily towards sweet rewards rather than cocaine (Lenoir et al., 2007) and prolonged “cafeteria-style” diet, high in sugar and fats, has been shown to result in compulsive food-seeking behaviour that persists even in the face of adverse consequences (Johnson and Kenny, 2010). Withdrawal from high sugar intake also results in physiological and behavioural withdrawal symptoms similar to those observed during opiate discontinuation (Avena et al., 2008).

To some extent, the pathways for food and drug seeking may share the same neuroanatomical pathways (Avena and Hoebel, 2003; Volkow and Wise, 2005) and receptors (Cottone et al., 2009). However, the findings from the rat studies cannot be directly generalized to humans. Indeed, human studies do not provide direct evidence that would support physiological sugar
addiction. For instance, abstinence (fasting) decreases craving of sweet foods instead of increasing it, unlike the addiction model would predict. Interestingly, preference for intensely sweet tastes declines over age, suggesting that there is no development of tolerance. Furthermore, unlike in rats (Avena et al., 2008), in humans, administering opioid antagonists does not cause withdrawal symptoms (Benton, 2010). Furthermore, addiction models may not apply to all human populations with overeating problems (for a critical review, see Ziauddeen et al., 2012), although they may be useful in elucidating the background of binge-eating disorder (for a review, see e.g. Davis and Carter, 2009).

Instead, compulsive seeking of appetitive rewards in rats and mice could provide an interesting model in studying the neuronal patterns that underlie compulsive overeating in humans, which is a widespread public health problem. Some researchers suggest that stress would increase compulsive food-seeking in rats (Nair et al., 2009), which is interesting because stress is a known risk factor for compulsive eating in humans (Troop and Treasure, 1997), although it seems that even in rats, stress-induced relapses are more common in drug as compared to sugar addiction (Kearns et al., 2011). Developing models for compulsive overeating is challenging because there is no consensus of the diagnostic criteria of compulsive eating that is not characterized by bingeing. Possible traits that would indicate compulsive overeating in an animal model could include i) spending an unusual amount of time on food-seeking activity, ii) inability to stop responding to food-related cues, iii) lack of satiety effect and iv) eventual accumulation of weight. The protocol presented in Chapter 3 is able address trait (ii), but by prolonging the daily exposure to the task as well as the duration of the protocol, traits (i), (iii) and (iv) could be addressed as well.

6.1.2 Recent evidence for a link between Arc/Arg3.1 and appetitively motivated operant learning

Prader-Willi syndrome (PWS) is a genetic disorder that involves deletion of multiple genes on chromosome 15. It is characterized by hypotonia and lethargy at birth and infancy, and chronic overeating and cognitive disabilities such as delayed speech, motor skills and lower IQ in later life (Cassidy, 1997). Linking operant and extinction learning with addictive properties of food, a recent study reports that in a genetic mouse model for Prader–Willi syndrome, appetitively motivated learning and reversal learning are enhanced (Relkovic et al., 2012) despite PWS
usually being accompanied with learning disabilities; this mouse model also shows decreased performance in the five-choice serial reaction time task (Relkovic et al., 2010). One explanation might be a different motivational effect of food: there is evidence for increased motivation by food in PWS, and overeating behaviour due to an abnormal lack of satiety response has been described in human patients (Holsen et al., 2009).

PWS provides a potentially interesting linkage between learning disabilities, appetitive motivation and our work on the Arc/Arg3.1 gene to be discussed next. Arc/Arg3.1 mRNA levels are increased in Prader-Willi syndrome (Ingason et al., 2011) and acquisition of a lever-press task has been shown to result in induction of Arc/Arg3.1 expression in rat hippocampal area CA1 and CA3 (Kelly and Deadwyler, 2002), although the causal role of Arc/Arg3.1 in acquisition of operant behaviour is not known.

Caused by deletion in the same area on chromosome 15 as Prader-Willi syndrome, Angelman syndrome is characterized by cognitive disabilities such as impaired speech development and disordered motor function. One of the genes in this chromosomal area is UBE3A, which regulates degradation of Arc/Arg3.1 gene (Greer et al., 2010; see Fig. 1 for a schematic presentation). Similarly to Arc/Arg3.1 KO mice, a recent study reports Angelman syndrome model mice to have impaired hippocampal long-term potentiation and contextual fear memory (Kaphzan et al., 2012). This may appear somewhat contradictory taken the finding in Prader-Willi model mice, which showed enhanced appetitive operant learning, because both Prader-Willi and Angelman syndrome are described to feature increased Arc/Arg3.1 mRNA. However, this might be explained by the enhanced motivational effect of food in the Prader-Will syndrome model mice (Relkovic et al., 2012).

6.2 Arc/Arg3.1 function and new mouse models for brain diseases

Although our study focused on the effects of Arc/Arg3.1 deletion in the hippocampus, system-level changes caused by impaired Arc/Arg3.1 function have been identified in many other areas. In recent years, Arc/Arg3.1 has been subject to wide interest, and new transgenic mouse strains, which express fluorescent proteins under the Arc/Arg3.1 promoter either hetero-
homozygously, enable real-time and longitudinal imaging of Arc/Arg3.1 in the neocortex (Wang et al., 2006; Eguchi and Yamaguchi, 2009).

Some of the most interesting recent papers that involve Arc/Arg3.1–related pathology are those linking it with Alzheimer disease. In the medial frontal cortex of human Alzheimer disease patients, Arc/Arg3.1 protein can be increased to anomalous levels. The finding was reflected in a transgenic mouse model of Alzheimer disease: amyloid plaque load was alleviated in these mice with an additional Arc/Arg3.1 deletion (Wu et al., 2011), and is consistent with previous findings that indicate that both hyper- and hypofunction of Arc/Arg3.1, mediated by impaired ubiquitination, can lead to mental retardation (Greer et al., 2010). Another recent study reported that Arc/Arg3.1-dependent responses to visual stimulation were reduced in neurons near amyloid plaques in the mouse extrastriate visual cortex (Rudinskiy et al., 2012).

![Figure 1. UBE3A tags Arc/Arg3.1 for degradation.](image)

Normally, UBE3A protein attaches a polyubiquitin (PolyUb) tag to mark Arc/Arg3.1 for degradation in proteasomes in order to maintain Arc/Arg3.1 balance in the dendrites. In Angelman syndrome, the UBE3A coding region is deleted, leading to accumulation of Arc/Arg3.1. The end result of this cascade will be an increased endocytosis of AMPARs and thus loss of AMPRs at the postsynaptic surface. From: Tai and Schuman, 2010.

So far, the role of Arc/Arg3.1 in plasticity and memory consolidation has been studied only by disabling its action in the brain. In addition to memory disorders, it will be worthwhile to examine whether constitutive Arc/Arg3.1 knockout mice may serve as a putative mouse model for certain heritable forms of autism, in which low Arc/Arg3.1 mRNA levels have been implicated (Greer et al., 2010). However, most human syndromes with altered Arc/Arg3.1 function (Alzheimer disease, Prader-Willi syndrome and Angelman syndrome) are characterized by increased Arc/Arg3.1 mRNA levels. It would be interesting to construct a mouse model which
would overexpress Arc/Arg3.1 and compare its phenotype to the phenotype of the animal models for the above mentioned syndromes. In addition, assessing how Arc/Arg3.1 overexpression would affect ripple occurrence, LFP spectra and phase locking would provide more detailed information how the differences in these characteristics between Arc/Arg3.1 KO and WT mice arise and whether they can be connected to the cognitive-behavioural deficits observed in human conditions that involve Arc/Arg3.1 dysregulation.

Our results may help in understanding the neuronal background of heritable forms of autism that involve the same chromosomal area as Angelman and Prader-Willi syndrome and result in decreased levels of Arc/Arg3.1 mRNA (Cook et al., 1997). Together, the findings from mouse models and human patients with disorders involving Arc/Arg3.1 dysregulation suggest that a fine-tuned balance of Arc/Arg3.1 expression is required for normal cognitive development.

**6.2.1 Involvement of Arc/Arg3.1 in plasticity and consolidation in different brain areas**

Previously, Arc/Arg3.1 has been shown to be required for experience-dependent homeostatic synaptic scaling (Gao et al., 2010) and experience-dependent plasticity (McCurry et al., 2010) in mouse primary visual cortex (V1). In mice, Arc/Arg3.1 also appears to promote orientation specificity and the reliability of repetitive activation by visual stimuli in area V1 (Wang et al., 2006). Furthermore, the amplitude of the Arc/Arg3.1–dependent response to visual stimuli predicts the reactivation probability in the extrastriate cortex (Rudinskiy et al., 2012; i.e., the chance that the same cell population is activated again following presentation of the same visual stimulus).

If changes in homeostatic synaptic scaling in mouse V1 are present also in the hippocampus, it may be connected with decreased ripple and high frequency oscillatory activity in the Arc/Arg3.1 KO mice: altered homeostatic synaptic scaling and synaptic plasticity may affect functionality of the CA3 network, which is important for generating high-frequency events.

Spatial exploration induces Arc/Arg3.1 expression not only in the rodent hippocampus, but also in the neocortex and dorsal striatum (Vazdarjanova et al., 2002). It is thus not surprising that the role of Arc/Arg3.1 in consolidation of contextual learning is not limited to hippocampus. Rat studies using Arc antisense microinjections also indicate that Arc/Arg3.1 expression in the
nucleus accumbens is required for acquisition, context-induced retrieval and reinstatement of morphine-induced conditioned place preference (Lv et al., 2011). Furthermore, Arc/Arg3.1 in the amygdala has been shown to be essential for consolidation of fear memory (Ploski et al., 2008).

Taken together, these findings seem to make it plausible that Arc/Arg3.1 is involved in the reliability of repetitive activation and consolidation not only in the hippocampus, but to serve such function more generally in cortical and subcortical areas. However, the role of Arc/Arg3.1 in regulating synaptic plasticity and consolidation throughout these areas remains to be confirmed.

6.2.2 Arc and coding of spatial information

Taken that experience-dependent response potentiation in V1 requires Arc/Arg3.1 (Wang et al., 2006; Gao et al., 2010; McCurry et al., 2010), it is somewhat surprising that place fields in whole-body Arc/Arg3.1 mutant mice appeared normal in our study (chapter 4). However, along with visual input, place fields are shaped by multiple environmental stimuli such as auditory and tactile cues as well as idiothetic (self-motion) cues, which may explain that Arc/Arg3.1 KO mice were able to maintain place fields that are indistinguishable from those of WT mice.

Although visual cues have been suggested to be the primary environmental factor to shape place fields, they exist also in the absence of visual cues, even in total darkness (Markus et al., 1994). Furthermore, some recent preliminary data from studies which use a floating ball and virtual environment, thus enabling mismatch between body movement and visual input, suggest that place field formation relies heavily also on other sources of information than visual input (Chen et al., 2013), which might explain why visual cortex plasticity would not be required for formation of place fields. Even though Arc/Arg3.1 is expressed upon exploration and ripples are decreased already during immobile periods on the track (Fig. 2A, Chapter 4), there may be compensatory mechanisms that support spatial learning, because, according to Plath et al. (2006), Arc/Arg3.1 KO mice are not impaired in the early acquisition phase (first day) of the Morris water maze task, or contextual fear conditioning, when tested shortly after acquisition. The deficit in late-phase acquisition in the Morris Water task could thus be explained by memory consolidation deficits alone.
One of the factors that may contribute to the consolidation impairment of Arc/Arg3.1 KO mice in spatial tasks is that the power of oscillatory rhythms in the beta-2 and gamma ranges was significantly decreased (Fig. 3, Chapter 4). In mice, bursts of oscillatory activity in the beta-2 range have been attributed to the novelty of an environment (Berke et al., 2008), and in rats, low and high gamma have been linked with CA1-CA3 and CA1-entorhinal cortex coupling, respectively (Colgin et al., 2009; Colgin and Moser, 2010). Although we could not reproduce the enhancement of beta-2 activity in novel environments, this rhythm may nonetheless regulate late acquisition and consolidation.

Concerning gamma rhythms, the low and high gamma frequency bands have been correlated putatively to memory retrieval and encoding of sensory information, respectively (Colgin and Moser, 2010). Therefore the decline in gamma power in our Arc KO mice may be proposed as a significant factor in the memory disabilities reported for these mice.

One of the most striking findings during track running episodes was reduced coupling of firing and oscillatory activity in the theta, beta and gamma ranges. It could be argued that reduced spike-LFP locking to higher frequency ranges in Arc/Arg3.1 KO mice may be explained by lower power in these ranges. However, this does not explain reduced locking to the theta rhythm, as theta power was not significantly reduced during track running. It has been suggested that plasticity is linked to phase-locking (Cassenaer and Laurent, 2012), which makes it appealing to hypothesize that the impaired theta coupling observed in Arc/Arg3.1 KOs (Fig. 4, Chapter 4) is associated with a long-term plasticity deficit. However, this remains to be tested directly because in our study, no test of long-term memory was included, so a correlation between theta phase locking and long-term memory could not be assessed.

6.2.3. Relationships between Arc, ripples and consolidation

Rat and mouse studies have shown that neuronal activity patterns that reflect previous experiences are often replayed off-line during rest and sleep (Skaggs and McNaughton, 1996; Dragoi and Tonegawa, 2011). During slow wave sleep (SWS), traces of neuronal activity patterns from preceding behavior can be observed in rat hippocampus, neocortex and ventral striatum (Euston et al., 2007; Lansink et al., 2009). These replay events are associated particularly with sharp wave–ripple complexes (SWRs; Kudrimoti et al., 1999). Recordings from the CA1 pyramidal cell layer showed that the spontaneous reactivation of these patterns is reflected by
an increase in pairwise firing-rate correlations during SWS or quiet wakefulness that followed the behavioural experience, whereas firing-rate correlations during REM sleep did not appear to relate to the preceding experience (Kudrimoti et al., 1999).

Furthermore, hippocampal SWRs are associated with replay events in other brain areas, for instance ventral striatum (Pennartz et al., 2004) and medial prefrontal cortex (mPFC; Peyrache et al. 2009). This replay of previously experienced activity is thought to be crucial for memory consolidation (for a review, see O’Neill et al. 2010; Battaglia et al. 2011). Similarly to SWR density, reactivation of neuronal ensemble activity is strongest in sleep sequences following exposure to a novel environment (O’Neill et al., 2008).

Although replay was first observed during SWS (Skaggs & McNaughton 1996; Lee & Wilson 2002), it is possible that it takes place also during REM sleep (Louie & Wilson 2001), but the existence and causal role of REM-sleep replay are less clear than of SWS (Siegel, 2001; Tatsuno et al., 2006). Multiple studies have confirmed replay to occur in the awake state (e.g. Nádasdy et al. 1999; Dragoi & Tonegawa 2011). Awake replay is most pronounced in novel environments (Carr et al., 2011), suggesting a role in processing spatial information.

An interesting form of awake replay is reverse replay of previously experienced sequences, that has so far been observed in the awake state only (Foster & Wilson 2006). It has been suggested to facilitate consolidation of recently experienced events, which may be especially important for reward-driven learning (Diba & Buzsáki 2007) by modulating reactivation in an outcome-dependent manner (Singer and Frank, 2009; Jadhav et al., 2012). Foster & Wilson (2006) and Kalenscher & Pennartz (2008) further hypothesize that reverse replay in combination with decaying transients of dopamine (or other neurotransmitters) can account for reinforcement learning of previous behavioral sequences leading to reward, in a manner that attributes more value to more recent actions, places and cues preceding the reward. Like replay during SWS, replay in the awake state is also considered to facilitate memory consolidation and retrieval (Carr et al., 2011; Jadhav et al., 2012) and it is enhanced by reward during the period when a trial outcome is presented (Singer & Frank 2009).

However, the firing patterns observed during SWR events commonly referred to as replay are not necessarily just replay of recently fired sequences. First of all, Karlsson and Frank (2009)
observed replay of remote memories, viz. to sequences of positions that were not close to the animal’s current location. Secondly, spontaneous generation of novel sequences corresponding to paths that were not previously experienced have been reported. This finding supports the view that replay may subserve integration of representations pertaining to larger environments or episodes, with less dependence on the recency of the experience being replayed (Gupta et al., 2010).

While replay appears to be an interesting candidate process for mediating short-term to long-term memory transfer, direct evidence of its role in memory consolidation is scarce. That is, the main evidence for a causal role stems from ripple-intervention experiments (Girardeau et al., 2009b; Ego-Stengel and Wilson, 2010; Jadhav et al., 2012), but it should be borne in mind that ripples are EEG events, not demonstrating replay per se. To date, there have been no studies that have interfered specifically with replay of specific firing sequences, thus directly confirming its causal importance.

As replay events are assumed to take place predominantly during ripples, it is possible (and even likely) that the decreased occurrence of ripples during both sleep and immobile task periods observed in Arc/Arg3.1 KO mice, as reported in Chapters 4 and 5, is associated with decreased replay. This is further supported by the finding that firing activity during SWRs, occurring in SWS, is decreased in the KOs, as shown in Chapter 5. Taken that reactivation during sleep correlates with learning performance (Dupret et al., 2010), this could be one of the factors that contribute to the spatial learning deficit previously observed in Arc/Arg3.1 KO mice (Plath et al., 2006). A previous study which showed decreased oscillatory activity in the gamma range but intact SWR activity in Connexin-36 deficient mice in vivo (Buhl et al., 2003) would suggest that decreased SWR activity in Arc/Arg3.1 KO mice cannot be explained by decreased high frequency LFP activity alone.

To our knowledge, our studies provide the first evidence of an animal model where a decrease in SWR rate and correlated firing was observed in vivo. However, a follow-up study would be necessary to confirm whether these findings are reflected in the amount of replay present in Arc/Arg3.1 KO mice.
6.2.4. Arc/Arg3.1 may be involved in reversal learning as part of an NMDA receptor-dependent molecular pathway

Arc/Arg3.1 is part of a complex, exquisitely regulated molecular network centered on dendritic spines of pyramidal cells. In the rat hippocampus, localization of Arc mRNA in active synapses has been shown to be dependent on NMDAR-receptor activation (Steward and Worley, 2001). This points to a strong link between synaptically induced calcium influx, NMDAR-dependent synaptic plasticity and Arc expression. Thus, when NMDARs were ablated in the mouse dentate gyrus, the observed impairment in memory recall (Nakazawa et al., 2002) may well have involved also the loss of Arc/Arg3.1 activation.

The observed reversal learning deficit in Arc/Arg3.1 KO mice (Plath et al., 2006) may be linked to impaired long-term potentiation and disrupted function of the NMDA receptor–dependent pathway: VGLUT1-deficient mice show reduced LTP and a specific reversal learning deficit (Balschun et al., 2010) and mice with a local KO of the NMDA receptor subunit NR-1 in dentate gyrus acquired contextual fear conditioning normally, but were impaired in learning less distinct contexts (McHugh et al., 2007). When these findings are juxtaposed to the slower reversal learning of Arc/Arg3.1 KO mice in the face of intact place cell function or place field formation (Chapter 4), we may hypothesize that NMDAR- and Arc-deficient molecular pathways result in impairments of more complex tasks, such as involving fine context discrimination and cognitive flexibility.

Furthermore, a recent mouse study provides more evidence that interfering with the Arc/Arg3.1-dependent cytoskeleton regulation pathway may impair reversal learning. When the Abl-related gene kinase, a protein which regulates the structure of actin cytoskeleton (Wang et al., 2001), was inhibited either genetically or pharmacologically, instrumental reversal learning was found to be impaired, while acquisition was intact (Gourley et al., 2012).

However, there are also indications that Arc/Arg3.1 and NMDAR function can diverge. For instance, NMDA receptor blockade has been reported to lead to stronger synchrony, especially in the gamma band (Hunt et al., 2010; van Wingerden et al., 2012), whereas in our study, synchrony in the gamma band was decreased (Chapter 4). Even though the time scales of the
manipulations differ, this suggests that Arc/Arg3.1 plays a pivotal and distinct role in promoting high-frequency synchronization in the hippocampus.

6.3 Future directions

Causal demonstration of a role of a specific (set of) gene(s) requires manipulation of the expression of the candidate gene e.g. by genetic engineering or RNA interference. However, as the example of initial magazine checking behaviour in Chapter 2 shows, many behavioural traits are polygenic, which can make this approach challenging.

Genome-wide association (GWAS) studies remain a useful tool in sieving candidate chromosomal regions and genes for polygenic traits and disorders for which no animal models exist. For developing more specific mouse models of behavioural-cognitive disorders such as difficulties in associative learning and overtly perseverative behaviour, screening of larger sets of RI strains is necessary in order to increase the resolution to a level at which it is possible to identify single candidate genes. Some promising studies along those lines have already been published, most importantly that of Laughlin et al. (2011). It would also be interesting to test a larger set of RI strains in the task used in Chapter 3 to see whether reversal and extinction learning are regulated by the same chromosomal area.

Because the number of tested strains has a high impact on the resolution of quantitative trait loci mapping (Crusio, 2004), but testing a high number of animals in numerous strains is time-consuming, future studies could be made more effective by first doing a tentative screen with smaller within-strain sample size or chromosome substitution strains to find out in which chromosome or chromosomal area the trait of interest is located, and then proceed to screen only strains that have polymorphisms in the region of interest, if necessary, with a higher number of mice per strain.

Cross-species comparisons can provide a fruitful approach in studying the heritable background of human polygenic behavioural-cognitive disorders. For instance, neurexin 1 gene (NRXN-1) copy number variation in humans has been connected to schizophrenia and autism (Marshall et al., 2008; Walsh et al., 2008; Bucan et al., 2009; Need et al., 2009). Assessment of NRXN-1 – related polymorphisms in BxD mice showed that NRXN-1 is linked to behavioral phenotypes
that are relevant to schizophrenia using, for instance, paired-pulse inhibition. Cross-species co-expression studies revealed that one of the most consistent co-expression covariates of neurexin 1 gene is glycogen kinase 3 beta, which appears to comodulate schizophrenia risk in human populations (Mozhui et al., 2011).

Similarly, cross-species comparison studies could provide interesting information regarding the neuronal and genetic background of compulsive food seeking: combining the information from human patients with high-resolution GWAS studies using appetitive operant and extinction protocols might be an effective way to assess the mechanisms that underlie uncontrolled eating. Furthermore, recognizing genetic risk factors might help in identifying subjects at high risk and intervening before compulsive eating has developed to a level of “food addiction”.

Let us next consider possible future directions for studies on Arc/Arg3.1 function. Although the consolidation deficit caused by loss of Arc/Arg3.1 function has been replicated in multiple tasks and brain areas (Plath et al., 2006; Ploski et al., 2008; Lv et al., 2011), studies disentangling the role of Arc/Arg3.1 in primary consolidation, updating of memories and long-term memory storage would be necessary to clarify which subprocess(es) of memory consolidation are exactly dependent on Arc/Arg3.1.

In Chapters 4 and 5, we report a decrease in ripple rate in area CA1 of Arc/Arg3.1 KO mice during rest. An interesting follow-up study would be to examine whether this is accompanied by a comparable decrease in replay activity. Mouse drives with larger numbers of tetrodes or high-density silicon probes, allowing substantially higher yield of single units, can be feasibly applied in future studies. Repeating the recordings with improved drives and a higher number of animals and cells per session could provide enough data to reliably assess whether the consolidation deficit of Arc/Arg3.1 KO mice can be correlated to a deficit in replay activity.

The mouse strain used in this study had a constitutive, whole-brain knockout of Arc/Arg3.1, which enables assessing the role of impaired Arc/Arg3.1 function in a situation that is comparable to heritable mental retardation forms, thus providing information that is potentially useful in Arc/Arg3.1 related pathogeny, e.g. in Fragile X and Angelman syndrome.

Arc/Arg3.1 is expressed in the brain already during prenatal development (E11.5; Allen Mouse Brain Atlas; 2006), but the exact role of Arc/Arg3.1 during different stages of development has
not been systematically assessed. Inducible Arc/Arg3.1 knockouts have been developed by multiple groups, although no studies have been published in peer-reviewed journals today. According to preliminary studies, behavioural phenotypes of conditional Arc/Arg3.1 knockouts, in which Arc/Arg3.1 function is abolished from P6 onwards, are comparable to the constitutive knockouts (Claudia Mahlke, personal communication), which would suggest that Arc/Arg3.1 plays an elemental role during postnatal development.

Knocking out Arc/Arg3.1 temporarily would allow investigating several interesting questions, such as whether the behavioural and neuronal changes caused by Arc/Arg3.1 deletion can be induced in a reversible manner. For instance, would temporary suppression of Arc/Arg3.1 expression also diminish ripple rate and/or replay and hinder memory consolidation to same extent seen in constitutive knockouts, or are the effects of Arc/Arg3.1 deletion at least partially developmental?

An inducible Arc/Arg3.1 knockout model would thus provide a way to disentangle the role of Arc/Arg3.1 in shaping the development of nervous system, versus its role in learning and plasticity in the mature brain. For instance, it would be interesting to test the role of Arc/Arg3.1 in generating LFP rhythms using an inducible Arc/Arg3.1 knockout model to assess whether there is a critical period during which Arc/Arg3.1 participates in forming pyramidal cell–interneuron connections, or whether its regulatory role is necessary for generating and maintaining the oscillatory rhythms also during adulthood.

A further direction for future research is to examine how regional loss of Arc/Arg3.1 is reflected in firing activity e.g. in the prefrontal cortex or ventral striatum. For instance, would a local deletion of Arc/Arg3.1, restricted to the hippocampus, impair reactivation of neuronal ensembles in these areas? Taken previous findings showing that firing activity in PFC and striatum is dynamically organized by hippocampal theta rhythm (Berke et al., 2004; Jones and Wilson, 2005; DeCoteau et al., 2007; Lansink et al., 2009; Benchenane et al., 2010; van der Meer and Redish, 2011), it would be interesting to assess whether reduced phase locking in the hippocampus in Arc/Arg3.1 KO mice would result in lower theta locking or phase precession in other areas of the brain.
Furthermore, it would be of great interest to explore why loss of Arc/Arg3.1 results in decreased phase-locking, even in the presence of a nearly intact LFP rhythm, as in the case of theta phase locking (Chapter 4). If individual neurons do not lock to a rhythm that is still present in synaptic mass activity, as reflected in LFPs, this could indicate a lack of coordinated spike timing, possibly by a loss of STDP (Cassenaer and Laurent, 2012) or an excessively strong form of phase precession. We found no evidence in favor of the latter possibility, as phase precession was generally modest or not demonstrable at a statistically significant level.

Subregional deletion of Arc/Arg3.1 would also provide a way to dissect the mechanisms behind the decreased ripple rate in Arc/Arg3.1 KO mice. Besides studying models that would disentangle the involvement of hippocampus and neocortex, it would be interesting to investigate whether CA3-specific deletion of Arc/Arg3.1 would be sufficient to reduce ripple generation.

In summary, we have seen how current behavioural, molecular and electrophysiological evidence suggests that Arc/Arg3.1 fulfills a key role in synaptic plasticity and neural substrates for memory consolidation; at the same time further studies appear to be needed to buttress this suggestion, particularly by manipulating molecular Arc-pathways more selectively and expanding recording arrays to scrutinize off-line processing at a larger scale.

References


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