Familiarity-Related Neuronal Responses Under Normal Conditions and in an Animal Model of Mental Illness

A thesis submitted to Cardiff University for the degree of Doctor of Philosophy

By

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Summary

Recognition memory is one of the most basic types of memory. One type of recognition memory is visual familiarity, which is the sense that a visual stimulus has been encountered before. This type of memory is affected in different mental illnesses, like schizophrenia and autism. The perirhinal cortex, which is a part of the medial temporal lobe has long been believed to be crucial for recognition. This work had 2 main lines of research. The first part, described in chapters Chapter 3 and Chapter 4 , set out to investigate whether activity at either the population level or at the single-unit level in the mouse perirhinal cortex was correlated with visual familiarity. In these chapters, the recordings were made also in the visual cortex and the hippocampus to put the perirhinal response in the context of the activity both upstream and downstream from it, respectively. Visual stimuli evoked responses in the perirhinal cortex, no familiarity-related modulation could be detected at both levels of analysis. That was true even when the visual cortex demonstrated familiarity-related differences in response. The second part of the work, described in chapters Chapter 5 and Chapter 6 examined the mice with haplo-insufficiency of the CYFIP1 gene as a possible new model for mental illness. First, the animal's validity as a model of mental-illness was tested using auditory-evoked potential, one of the most ubiquitous phenomena that appears with mental illness in people and animal models. Then, recognition memory and the neuronal activity in the perirhinal and the visual cortex was tested, to see if they demonstrate other symptoms relating to schizophrenia. In the second line of work, the CYFIP1 mice demonstrated an auditory-evoked potential profile more like that observed in fragile-X syndrome, rather than schizophrenia and did not present any changes in both recognition memory or the electrical activity in the visual and the perirhinal cortex. This work showed that the perirhinal cortex does not show any familiarity-related activity unlike the current assumption in the literature. It also showed that the CYFIP1 mice might be a possible model for autism and Fragile-X syndrome.

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List of abbreviations

AEP – auditory evoked potentials

AMTL - anterior medial temporal lobe

ASD – autism spectrum disorder

ASSR - auditory steady-state response

BS – Burst spiking

DMS – delayed matching-to-sample

DNMS – delayed non-matching-to-sample task

EC – entorhinal cortex

ERP - Event-related potential

FS – fast spiking

FXS - fragile-x syndrome

HPC – hippocampus

ISI – inter stimulus interval

LEC– lateral entorhinal cortex

LM - Lateral medial field

LS – Late Spiking

LTD – long term depression

LTP – long term potentiation

MCPG - (*S*)-α-methyl-4-carboxyphenylglycine

MEC – medial entorhinal cortex

MMN - mismatch negativity

mPFC - medial prefrontal cortex

MTL – medial temporal lobe

N1 – first negative peak

NOR - Novel object recognition

NR – non-responsive

P1 - first positive peak

PCA - principle component analysis

PCP – phenylcyclidine

PHC – Parahippocampal cortex in primates/ postrhinal cortex in rodents

PPI – prepulse inhibition

PRH – perirhinal cortex

PSTH - peri-stimulus histogram

ROC – Receiver operating characteristic

RS – Regular spiking

SOE - spontaneous object exploration

SS – Single Spiking

SZ – schizophrenia

T/C – Test to Control

TEv – ventral temporal association area

VE – visually excited

VEP – Visually evoked potential

VI – visually inhibited

WT – wild-type

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Chapter 1 General Introduction

1.1 Recognition Memory

1.1.1 Recognition memory in humans: Familiarity and Recollection

Animals have two types of memory, one is episodic memory, which is memory for events and the other is procedural memory, which is a memory for learned actions. Recognition memory is one of the most fundamental types of episodic memory. This type of memory is the one that signals whether an event was encountered in the past or not. Such a form of memory is vital for survival, for example recognising a predator or a familiar food source. Recognition memory is believed to comprise of two distinct and dissociable processes: familiarity and recollection[1,2]. According to this distinction, familiarity is a fast process that only signals a previous encounter with an object, but is devoid of any contextual information such as where or when the item was encountered. In contrast, recollection is a slower process that evokes the contextual details surrounding this object. Both behavioural and electrophysiological experiments in humans support this notion[3–8].

An indication for the existence of two distinct processes can be seen in a timed recognition test. In this type of test, when asked whether a word was previously encountered or about the modality in which it was presented, reaching the same level of discrimination takes longer when making a modality judgment than when only making novelty judgement[3]. The same is true when asked to make judgement about a word's location on a screen[4]. This are taken as evidence to the existence of a quick familiarity process underlying old/new judgements, and a slower recollection process underlying the extraction of additional contextual information.

Another behavioural indication for two distinct processes comes from their receiver operating characteristic curves (ROC). Using the remember/know paradigm[5,6], where participants are asked to say if an item is only familiar or they recollect it, together with confidence ratings enables to generate ROC curves for the two processes. This reveals two completely distinct processes. Familiarity judgements, indicated by the 'know' response, have a standard ROC curve showing an underlying normal distribution. However, 'remember' judgements,

indicating recollection, show the same percentage of hits for any number of false alarms, indicating a threshold process, where the process is either reached or not, regardless of any decision criterion[9].

Some evidence for the distinctiveness of the two processes comes from human event related potential (ERP) studies. An indication by a human subject of 'knowing' an item was seen in a previous trial, elicits a positive going voltage over frontal areas 325-600 ms after presentation, and a late voltage negativity 600-1000, after word presentation. When the person indicates fully remembering the item, however, a late voltage positivity can be observed 600-1000 ms after stimulus presentation[10]. A similar late positivity as seen after a remember response is seen when participants make source judgement of whether a word was presented in a female or male voice[7]; and when they make judgements about the modality of the word presented[8]. Also, the frontal early positive response remained when subjects make a correct novelty judgement with a wrong contextual judgement, despite the decrease in the late response[7,8], indicating the activation of an early familiarity response, that might later activate a recollection response that enables the retrieval of further contextual details.

In summary, experiments suggest that recognition memory consists of 2 processes, familiarity and recollection. Behavioural and EEG studies support this idea and show that the process of recognition starts with the fast, earlier stage of familiarity and is then followed by recollection, which is a slower complementary process. In the next section recollection and familiarity in animals will be discussed.

1.1.2 Recognition memory in animals: Familiarity and Recollection

Recognition can be observed in animals too. In rodents in can be observed using the novel object exploration task[11]. In this task, the rodent's natural tendency to explore novel features of the environment is exploited. When presented with a familiar object and a novel object, in a simultaneous test, rodents typically spend more time exploring the novel object over the familiar. The strongest aspect of this test is that it does not involve reward, thus allowing to examine the studied behaviour directly. The same task can be used to measure different aspects of recognition memory, such as recognition of an object's spatial position, object-context association, and even memory for the temporal order of object presentation[12]. Thus, it can be helpful in differentiating between different types and processes of recognition memory.

As discussed above, recognition memory is made up of two processes in humans. But, do these processes exist or, indeed, can they be demonstrated in animals too? Some studies seem to suggest they can. One study[13] using olfaction in rats tried to answer this question using the ROC procedure described above. Rats were trained to give a rewarded 'new' or ''old' reaction by either sniffing a cup with the new odour or an empty cup at the other end of an arena. To obtain an ROC curve, different biases were introduced by manipulating the height of the 'odour' cup and the reward for the 'old' response. Analysing the resulting ROC curves showed that rats used both familiarity and recollection processes. That was shown by the rats having both a threshold process ROC consistent with recollection, and a symmetrical ROC consistent with a familiarity process. Furthermore, lesioning the hippocampus (HPC) impaired only the recollection process, while sparing familiarity, providing further anatomical support for separate recognition memory processes.

Other studies using lesions have also provided more evidence to the existence of different recognition memory processes in the rat. In most studies the lesion to the HPC is in the fornix region, the main output path of the HPC[14]. In an E-maze, where two objects are hidden from view, rats show a preference to go explore an arm where they had previously encountered an object to which they had not been habituated[15], thus showing a form of recollection by remembering that this arm is associated with a more novel object. Fornix lesioned animals do not show this

preference, thus showing lack of recollection. However, after a primary exploration of the cage, thus knowing where the objects are, they explore the novel object more, showing normal familiarity[15]. Also, rats were presented with an item in two different locations in two different contexts, such that each context was associated with the item being either to the left or the right. When tested in one of the contexts fornix lesioned rats did not show any preference in exploration of the item in the contextually novel location, which indicates an impairment in recognition. However, they still explored an item in a novel location more, showing preference to a novel location, when faced with just one context, thus showing a separate familiarity process that was not impaired by the lesion[16].

1.2 Structures of the MTL

A region of the brain repeatedly implicated in recognition memory is the medial temporal lobe (MTL)[17–19]. Much of the subsequent interest in the MTL was prompted by the study of patient HM[20]. After undergoing an MTL excision to stop epilepsy, HM, showed severe memory impairments, that included, among others, problems in recognition memory[21].

The main components of the medial temporal lobe are the parahippocampal region that includes the parahippocampal cortex (the postrhinal cortex in rodents; PHC), the perirhinal cortex (PRH) and the entorhinal cortex; and the HPC. The PRH and PHC components of the MTL system are connected in a segregated manner[22,23] (**Figure 1.1**). While the PHC is mainly interconnected with the medial part of the entorhinal cortex, the PRH is mainly connected to its lateral part. The same separation seems to follow in the HPC. Thus, the PHC is more strongly interconnected mainly with the dorsal HPC and subiculum, while the PRH is more strongly interconnected with the ventral HPC[22,24]. Functional connectivity studies in humans corroborate these findings in rats. Thus, the PRH is more strongly connected to the anterior HPC, while the PHC is more strongly connected to its posterior parts[25,26], the equivalent of the ventral-dorsal distinction in the rat[27].

Studies in both humans and animals suggest that the PRH is involved in memory for specific objects, while the PHC is involved in memory for the context and ‘source’ of the memory. Indeed, studies in humans have shown the PHC’s role in ‘source memory’, that is, the ability to remember the context in which an item,

usually a word, was encountered[28,29]. For example, the PHC is distinctly more active when a subject remembered the colour of a memorised picture, than if he just recognised the picture or forgot it[28].

In line with its role underlying the memory for the source, or the environment in which an item was encountered, the PHC plays a part in spatial navigation and perception. Thus, when subject were required to virtually navigate to the location of previously seen 3D object in an fMRI scanner, stronger BOLD signal in the PHC gyrus was associated with remembering the target location[30]. Also, in patients undergoing surgery for epilepsy, most neurons responding to a specific landmark in a virtual environment, regardless of its spatial location, were located in the PHC[31]. In line with the human data, c-fos expression levels increase in rats that are exposed to novel spatial configurations[32]. Also, rats[33] and monkeys[34] that undergo lesion to the PHC show deficits in spatial-configuration-dependent tasks.

The functional difference and the segregation in connectivity of the PHC and PRH is suggestive of two distinct systems underlying different aspects of recognition memory, namely familiarity and recollection[18,27,35]. Yet, for episodic memory, that is the memory of both an item and its context, to occur these two systems need to be functionally connected. The connectivity pattern in which these two areas converge in the HPC (**Figure 1.1**), would suggest that it functions as the hub that connects these two distinct system, which together, theory suggests, give rise to episodic memory[36] and memory-guided behaviour in general[37].

These theories suggest that item representations first reach the PRH and the lateral entorhinal cortex (LEC). This information can then support familiarity. In parallel, another flow of information sends contextual representations to the PHC and the medial entorhinal cortex (MEC). Then, the information is combined in the HPC, where these two pathways converge giving rise to later recollection[36,37]. Then the HPC feeds the information forward through its main output path of the fornix[14] (**Figure 1.1**).

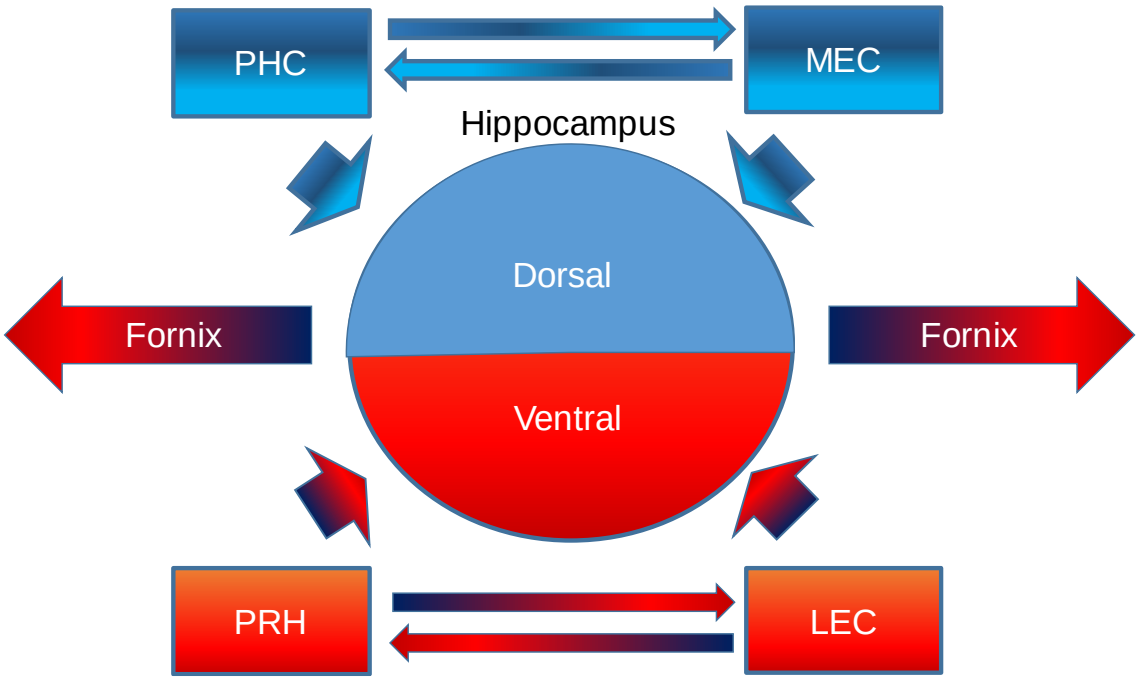

Figure 1.1. The connectivity of the MTL system. *The figure shows the connectivity pattern within the MTL system. There is a clear segregation between the interconnected PHC-MEC system that connects mainly to the dorsal HPC, and the PRH-LEC system that connects mainly to the ventral HPC. PHC: parahippocampal gyrus/postrhinal cortex (in rodents); PRH: perirhinal cortex; MEC: medial entorhinal cortex; LEC: lateral entorhinal cortex. MTL – medial temporal lobe. The dorsal and ventral HPC are homologues of the posterior and anterior HPC in primates, respectively.*

This difference in function between these two pathways underlying familiarity and recollection can be examined by studying patients with selective lesions to the different parts of the MTL. However, one should always bear in mind that these results are sometimes hard to interpret due to the large and general extent of the damage. MTL damaged amnesiacs show a deficit in temporal order memory[38], but not in familiarity-based word recognition. Similarly, a patient, SS who had damage to the HPC and the MTL showed deficits in recollection and familiarity, while another patient, PS, with a damage limited to the HPC, showed only decreased recollection performance, but spared familiarity[39]. A study testing a group of hypoxic patients and controlling for the extent of the lesion reported similar results, with patients with limited hippocampal damage having spared familiarity, while a more extensive damage causing damage to both familiarity and recollection[40]. Other studies on hypoxic patients show the same results[41,42]. A rarer case in the literature of a patient with a damage to the anterior temporal lobe, that includes the PRH, highlights the importance of this region in familiarity. The patient, NB, maintained normal recollection, but had an impaired familiarity[43].

Yet, some studies show that even damage limited to the HPC is enough to harm both recall and recognition performance. Patients with temporal lobe lesions presented deficits in recollection, alongside a substantial but smaller deficit in familiarity, judged with a fitting of an ROC curve[44]. This finding can be explained by damage mainly to HPC but including other structures important to familiarity like the entorhinal cortex. Yet, patient VC who had damage specific to his hippocampi, while having intact MTL showed deficits in both free recall, that mainly uses recollection, and in recognition test, which mainly requires familiarity[45].

More evidence comes from a review of fMRI imaging studies[17]. 84% and 58% of the studies looking at recollection showed increased BOLD activity in the HPC and the PHC, respectively, compared to 11% showing increased activity in the PRH. When examining only familiarity the opposite was seen. Then, 87% of the studies showed greater activity in the PRH compared to 27% of them showing increased activity in the HPC or the PHC[17].

Also, a study trying to look at what areas are related to recollection, familiarity and novelty using an ‘oldness’ function showed further distinction between the two systems. The ‘oldness’ function model assumes that areas showing familiarity will have an increasing activity based on how certain the participant is in the item’s oldness, whereas novelty related areas should show a decreasing pattern of activity. For recollection, the assumption is that the function will be flat for all low levels of confidence and will sharply increase when the subject is completely certain of the item’s oldness based on recollected contextual details. Indeed, this study revealed that the posterior HPC was more associated with recollection, the anterior HPC and PRH were associated with novelty and the PHC was associated with familiarity[46]. And as would be expected, reduction in the PRH BOLD activity, was associated with greater ‘oldness’ rating[47,48], thus underlining the importance of this area for familiarity.

1.3 The PRH

The part of the MTL that seems to be the most crucial in object recognition memory is the PRH. In monkeys, lesion to the PRH produced a deficit in visual object recognition memory[49–51]. For example, in one study[5], monkeys were trained on a delayed-non-matching-to-sample task (DNMS), after which they were divided into groups that went through either a PRH or a complete rhinal cortex ablation and a control group. Both the rhinal and specific PRH ablation produced impairment in the DNMS task, which can be a taken as an indication for PRH involvement in recognition memory. Similarly, lesion of the PRH in rats also produced recognition memory deficits[52,53]. In these studies, rats that underwent cytotoxic lesion to the PRH spent less time exploring a novel object in the spontaneous novel-object recognition task (NOR) after a 15-minute retention interval, indicating this area's involvement in recognition memory.

Studies exploring the neuronal activity of the PRH also showed this area's involvement in recognition memory. In monkeys performing a serial recognition task, the PRH multiunit activity decreased as they were presented with familiar stimuli compared to novel ones[54]. Similarly, in monkeys holding an item in working memory for a delayed matching-to-sample (DMS) task IT cortex (which includes the PRH) activity was attenuated to items that matched the ones held in memory (shorter span than the task before). In rats, PRH neurons respond by reducing their activity on presentation of a familiar stimulus both in anaesthetised[55] and conscious[56] conditions. A more thorough discussion of these results follows in section 1.3.5.

1.3.1 Lesion Studies

Several lesion studies in rhesus monkeys revealed that a lesion to PRH is sufficient to cause a deficit on the DNMS task with retention delays greater than 8 seconds[49,57]. Also, monkeys that underwent a neonatal neurotoxic lesion to the PRH at postnatal days 10-12, showed a deficit in the DNMS task similar to the one that adult lesioned animals showed[58]. In all of these cases, the performance was still markedly above chance level even for the longer retention intervals. This deficit is generalised to other tasks that are supposed to require recognition memory. Both adult[59] and neonatally[60] lesioned monkeys showed less preference

to novelty in the visual paired-comparison task, where the fixation time on simultaneously presented objects is measured.

Rats showed similar deficits to monkeys after PRH lesions. An excitotoxic lesion to the PRH and PHC produces a deficit in the novel object exploration task, such that rats with the lesion explored the novel object for almost as long as they explored the familiar one, thus not differentiating between the objects[61]. The deficit was only apparent in retention intervals of 15 minutes or more. More localised lesions targeting only the PRH produced similar deficits in object exploration[52,53]. The effects of the lesion were more pronounced in the rats than in the monkey, with the rats exploring the novel and familiar objects for the same amount of time.

However, some findings cast doubts on the theory that the PRH is the 'novelty' detection area of the brain, and suggest the PRH is at the pinnacle of the ventral visual stream and, thus, is mainly a visual discrimination area[62,63]. Indeed, more recent experiments showed that animals with a lesion to PRH presented deficit in perceptual discrimination, supporting this idea. In one such experiment, monkeys that underwent PRH lesion, showed deficits in associating a stimulus with a reward when the 'feature ambiguity', that is the similarity, between it and the unrewarded stimulus was high, but not when the two stimuli were very different, when the 'feature ambiguity' was low[64]. Other lesion studies also showed that the PRH is required for different visual discrimination tasks[65–67]. Interestingly, in one of the studies a PRH lesion caused deficit in an object discrimination task, while a TEv lesion did not[65].

A recent study seems to bolster the 'feature ambiguity' view of PRH function. In this study, instead of the usual novel exploration test where the rat is presented with the novel and familiar object simultaneously, the objects were presented sequentially. In this case, both control rats and rats with a PRH lesion showed the same preference, and explored the novel object more[68]. This might suggest that when both objects are presented together, the rat treats the familiar object as a novel object, not because of not remembering it saw it previously, but due to not being able to distinguish between the two objects. Indeed, a study looking at PRH-lesioned rats casts doubts on the novelty role of the PRH even further[51].

In this study, PRH-lesioned rats explored 2 identical objects, then after a retention interval spent either in their home cage or a visually restricted environment, explored 2 identical copies of a novel object. In the normal condition rats showed less exploration of the novel objects than controls, as shown previously. However, the same rats, after spending time in a visually restricted environment, i.e. with less visual interference, surprisingly showed normal exploration of the novel object, further suggesting that lesions to the PRH cause mostly visual disambiguation deficits rather than mnemonic recognition ones.

1.3.2 The difference between HPC and PRH function in recognition

As mentioned earlier, the HPC is the centre of the MTL memory system. The question is how does its contribution to recognition memory differs from that of the PRH. As mentioned above, fornix lesion causes deficits in 'context-dependent' recognition, i.e. recollection, while keeping familiarity, which depends on the PRH, intact[15,16].

Other lesion studies support the idea of the HPC being essential for place and context based recognition memory. Indeed, rats with lesions the HPC do not show exploration preference for objects in a novel location within a context, or to a novel object-context association, yet they show normal novel object preference in exploration[69,70]. Yet, one study showed that post-learning lesion to the HPC did cause a deficit in object recognition memory[71].

The unique role of the HPC in binding object with its context is further demonstrated by the fact that a lesion to the HPC and the contralateral PRH does not affect memory for location or object in isolation, but impairs memory for object in its location, or memory for the temporal order of object presentation[70]. This result shows that the PRH and HPC have their unique roles in recognition of objects and locations in isolation, but their interaction is important for binding the two together into a full recollection.

Results from monkeys further support the difference in function between the HPC and the PRH. Lesioning the PRH in monkeys impaired their performance on a DNMS task, showing impairment in object recognition, while damage to the HPC did not[72]. Similarly, results from a passive viewing task, in which recognition is inferred from the viewing time of the different items, and thus is very similar to

NOR, showed that PRH damage causes impairment in object recognition, while HPC damage did not[34]. Both, HPC and PRH damage impair object-in-place judgment. Interestingly, in both these studies and others[73,74] in monkeys, parahippocampal, rather than HPC damage, leads to impairments in location memory, suggesting a difference between the monkey and the rat.

1.3.3 The anatomy and neuronal population of the PRH

The PRH is composed of Brodmann's areas 35 and 36 (ref. 75), and can be recognised by the sparsity of layer IV in area 36 and its complete absence in area 35, and the complete absence of heavily-myelinated fibres[76]. In the rat, the PRH lies between the insular cortex at its rostral end, and the PHC at its caudal end. It is bordered ventrally by the LEC and dorsally by the somatosensory cortex at its rostral extent and by the ventral temporal association area (TEv) at its more central and caudal end[76] (**Figure 1.2**A). In the monkey, the PRH is located in the anterior part of the inferior temporal lobe around the rhinal sulcus. Area 35 lies at the fundus of the rhinal sulcus and is bordered laterally by the entorhinal cortex (EC)[77]. Area 36 lies laterally to the sulcus and extends laterally, and according to recent connection studies, it appears to lie almost all the way up to the fundus of the anterior middle temporal sulcus[77,78], over the area previously designated TE[79].

The cellular make-up of the PRH is different than other parts of the neocortex (**Figure 1.2**B). One difference is that the number of pyramidal neurons in this area is smaller than in the rest of the neocortex[80]. The PRH contains four major cell types: Regular-Spiking (RS), Fast-Spiking (FS), Burst-Spiking (BS) and late-spiking (LS) neurons and some Single-Spiking(SS) cells[81].

LS cells are abundant in layer II/III. Approximately 60% of the cells in these layers, and the rest are RS cells. LS cells in the PRH start spiking with a latency of 1 second of more in response to either a current injection[81] or synaptic stimulation[82]. After that, most of them maintain their spiking for the duration of the stimulation[82]. In this layer the LS cell morphology is indistinguishable from that of RS cells, with both having a small pyramidal cell morphology[81].

Layer V in the PRH contains an abundance of RS cells (77%). some BS cells (9%) and, unlike layer V in other regions, a relatively large population of LS cells

(14%). These have an exceptionally long response latency of up to 12 seconds[83]. The morphology of the cells in this layer is of a classical large pyramidal morphology, with the BS cells having the largest soma, and a thick apical dendrite[81]. The RS and BS neurons end in a tuft or bifurcate in layer I, and the LS cells look similar but terminate also within layer V itself[83].

Layer VI, again, unlike any other cortical region, shows almost no RS cells. The vast majority of the cells are LS (~86%), followed by SS (7%) and FS cells. Less than 1% of the cells in this layer are normal RS cells[84], which are the most abundant in other brain areas. The morphology of the cells in this layer is also different from other cortical areas. SS and LS cells cannot be readily morphologically differentiated. They are mostly non-pyramidal, of the pyramidal cells some are oriented horizontally. These neurons send projection to layer V and II/III[84]. The FS cells, in this layer, are the smallest in size, and are usually of a round small shape, and be readily differentiated from SS and LS cells[81]. As can be seen in **Table 1.1**, only a minority of the cells in the PRH are FS[81].

Cell Type	Layer II/III	Layer V	Layer VI
RS	40%	77%	**<1%**
LS	60%	**14%**	86%
BS	-	9%	-
SS	-	-	7%
FS	-	-	7%

Table 1.1. Neuronal types of the PRH*. The table summarises the neuronal populations of the PRH. The percentages in bold mark unique aspects of the PRH that are different to other cortical layers. Numbers were taken from refs. 81 and 84.*

1.3.4 Projections of the PRH

Unfortunately, there are only a few anatomical studies on the mouse, so most of the connectivity data in rodents is derived from rats. In general, the PRH receives most of its input from association areas. Thus, area 36 receives 16.5% and 71.4% of it connections from unimodal and polymodal association areas respectively, compared to only 5.1% of its afferents originating in primary sensory areas. The same goes for area 35 with 34.6% and 55.4% of its afferents coming from unimodal and polymodal association areas compared to only 1.6% from primary unimodal areas[85]. Cortical efferents from the PRH arrive mainly at frontal regions from the rostral part and to the temporal and piriform cortices from the caudal part, mostly from area 36[22] (**Figure 1.2**C).

Looking at visual areas, only 4.3% of the connections sent from the unimodal visual areas arrive in the PRH. These afferents originate from the visual association cortices, and end mainly on the posterior part of area 36 (ref. 85). Similarly, very few efferents from the PRH arrive at occipital areas[22]. The majority of the visual inputs to the MTL arrives at the PHC which receives strong projections from visual areas[22]. The PHC in turn projects to the entirety of areas 36 and 35, which might supply additional visual input to the PRH[86]. The majority of the input to area 36 arises from the temporal cortex, and there the majority of the projections arise from the TEv, which projects to the entirety of the area. Much fewer projections from the temporal area innervate area 35 (12.7% in area 35 compared to 49.7% in area 36). However, even there, the majority of the connections arrive from TEv[85]. This area in turn receives primary visual connections in its caudal part[87]. In the EC, the LEC projects to the PRH. The projections arrive mostly in area 35 (**Figure 1.2**C). The rostral LEC projects mainly to the rostral part of the PRH, while the caudal part sends connections to the entire extent of the PRH[88]. This might be another secondary source of visual input, as the lateral medial field (LM), which is one of the main targets of V1 sends strong projections to the LEC[89].

Area 36 is has a large amount of intrinsic connections, such that every area innervate the rest of the region as well as area 35[86]. In mice, similarly to the rat, the PRH receives afferent connections from the extra-striate cortex to area 36. However, while similarly to rats, the strongest afferents arrive at the posterior area 36, in mice afferents also arrive to area 35 and the anterior area 36[90].

The PRH also receives projections from HPC areas CA1-3[91] (**Figure 1.2**C). In turn, the PRH sends projections, to area CA1 and the subiculum[92]. However, these efferents are relatively weak compared to the very strong projections to the EC[22]. Yet, the EC in turn, projects heavily to the HPC through the perforant path[93].

1.3.5 Synaptic plasticity in the PRH

Any neuronal response to novelty, where neurons reduce their response to familiar visual stimuli requires some form of plasticity. Indeed, both long-term depression (LTD) and long-term potentiation (LTP) have been implicated in recognition memory. Thus, infusion of benzodiazepine, $GABA_A$ agonist in rat brain slices impaired both LTP and LTD. Injection of the same drug in the PRH of freely moving rats impairs NOR, while also abolishing the reduction in c-fos counts observed after exploration of familiar items[94]. The same behavioural and c-fos count effects are observed after injection of a L-type voltage gated channel antagonist[95] or transfection with a CREB inhibitor in the PRH[96]. Those are accompanied by abolishment of LTP when the CREB cycle is inhibted[96], and in LTD when the L-type channel was antagonised[95]. Thus, both LTP and LTD are involved in recognition as observed in NOR.

Both LTD and LTP in the PRH superficial layer I, induced by layer II/III stimulation, are NMDA receptor dependent, as can be seen by their blockade with AP5, and mGlu receptor independent at least in some cases, which is demonstrated by the lack of effect for their antagonist (*S*)-α-methyl-4-carboxyphenylglycine (MCPG)[97]. Interestingly, mGlu receptor agonists are sufficient to induce LTD in PRH[98], suggesting that this class or receptors is involved at least in one type of LTD. Indeed, low-frequency-stimulation-induced LTP depends on NMDA receptor when the cell is depolarised, and on an interaction between group II and III mGlu receptors at resting potential[99].

Evidence suggests that LTD in the PRH lies at the centre of recognition memory. Thus, PRH slices taken from rats exposed to familiar stimuli show normal LTP, while showing a reduction in LTD[100]. Also, attenuation of perineuronal-net formation enhances long-term object recognition memory, while also enhancing LTD in the PRH[101]. More direct evidence shows that an infusion of a peptide that blocks interactions between GluR2 and AP2, a clathrin adaptor that is crucial for AMPA receptor internalisation, abolishes LTD, while keeping LTP intact in slices[102]. In animals, viral transduction of this peptide to the PRH is accompanied by an impairment in recognition memory[102], which directly ties impairment in LTD in the PRH with recognition memory deficits. All in all, these results show that the PRH exhibits both LTD and LTP, and both in turn are necessary for recognition memory.

1.3.6 Neuronal responses to novelty in the PRH

Electrophysiological studies tried to answer the question of how the neurons in the PRH encode recognition memory. The majority of this work was done on monkeys, while much less was done in rodents. There appears to be no published work in mice. This section considers the evidence from single-unit studies of the PRH's response to novelty. A list of the data reviewed below, is presented in table 1.2.

One study requiring monkeys to do a discrimination task, showed that the response of 26 out of 95 visually responsive units were decreased upon repeated presentation of stimuli, compared to none of 395 units recorded in the HPC[103]. However, from looking at the recorded locations it seems that at most only 12 of the neurons were actually in the PRH itself[57]. Other studies also showed that units in the IT respond to familiarity[104–106]. An interesting and cautionary experiment showed that in commissural transected monkeys, while these neuronal recognition responses disappeared when switching from one eye to the other , recognition behaviour remained intact[107]. However, these studies did not specifically examine the PRH, and looked at the entire rhinal region.

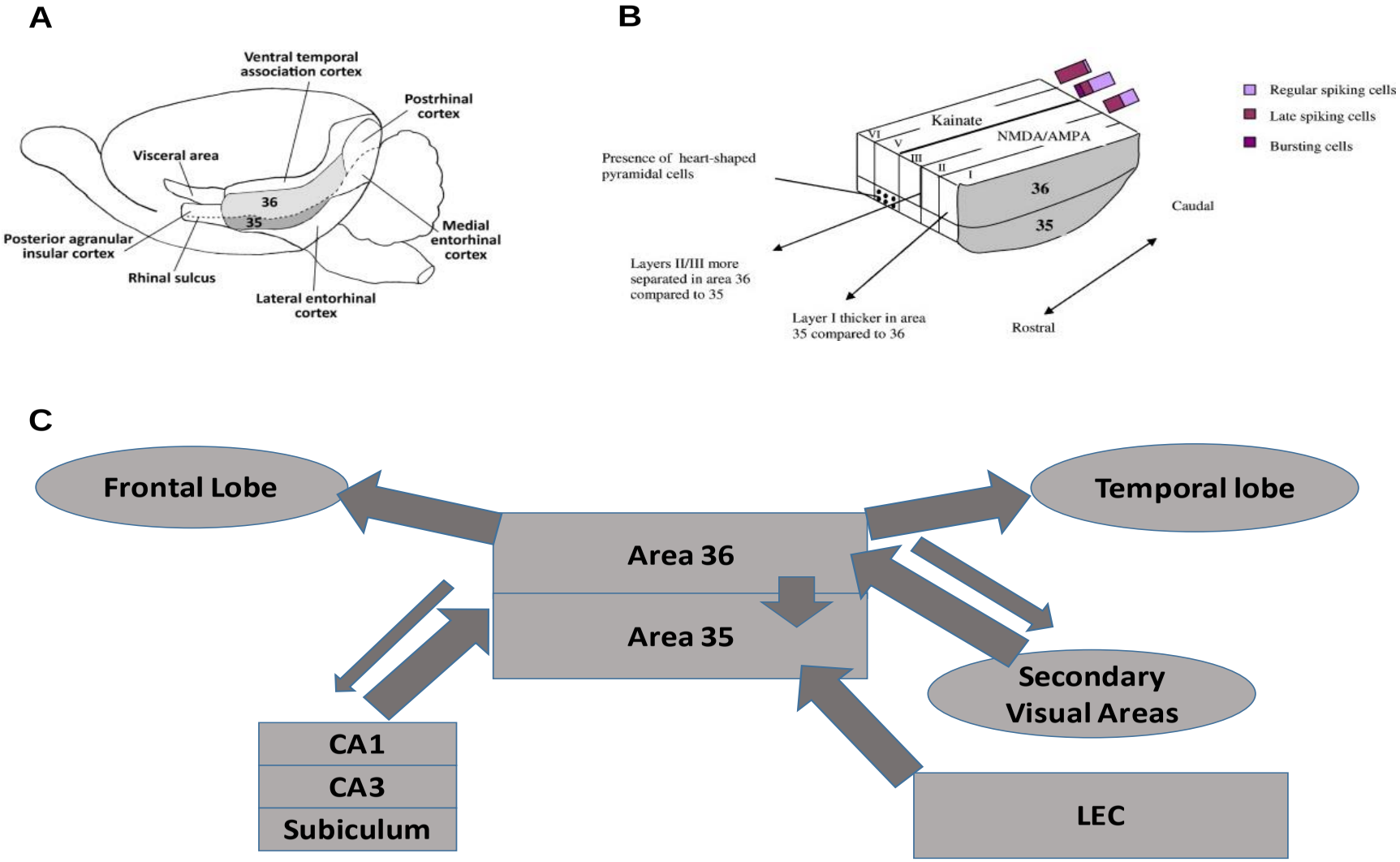

Figure 1.2. Anatomy and Physiology of the PRH. A) *The anatomical position of area 35 and area 36, that compose the PRH (Adapted from[171]).* ***B)*** *Summary of cell types in the different layers of the PRH (Adapted from[171]).* ***C)*** *Summary of the projections of the PRC. The size of the arrow depicts the strength of the connection.*

In a study looking specifically at the PRH, 25 out of 72 recorded neurons reduced their activity in response to a familiar object. 24 of those 'familiarity-responsive' neurons were stimulus specific, responding in that way to selective stimuli[108]. Thus, these neurons did not encode a more general non-specific familiarity signal. Other studies had similar results[109,110] (**Figure 1.3**A). According to the analysis presented thus far, it seems that a proportion of PRH neuronal population codes for familiarity. Yet, because their number is not different than in other region of the brain, this area might be receiving a familiarity signal from other areas.

Studies looking at PRH activity in rats looking at different objects, found a similar familiarity signal in the PRH in both anaesthetised[55] and awake condition[111](**Figure 1.3**B). However, the percentage of neurons showing the differential response was comparable to other areas, at least in awake rats. In this case, 25% of visually responsive neurons in the PRH showed a decrease in response to repeated stimuli, which was comparable to 33% in the EC, and 33% percent in the occipital cortex[111]. In anaesthetised rats, there are more familiarity encoding neurons in the PRH. However, not much more than other areas, especially if one considers neurons with increased response to familiar stimuli. Thus, in the PRH, 50% of the recorded neurons encoded familiarity (all of them with decreased response), compared to 30% in the HPC and 21% in the occipital cortex[55]. Thus, the familiarity signal might have arisen from any of these areas connected to the PRH.

Perhaps more troubling is the evidence from recent studies examining single-unit activity in freely moving rats that failed to find a unique novelty signal in the PRH. Thus, rats explored an environment with 4 identical objects. On a later session, a novel object was added to the environment. Examining the neuronal activity, 5/55 PRH neurons showed novelty-related change in response. However, that was similar to 13/98 LEC neurons that also responded to novelty[112]. In another study in which rats moved freely around a circular arena with either novel or familiar objects, found that while firing rate increased when the rats explored the

objects, there was no difference in firing rate between the novel or familiar objects, despite the rats' showing more exploration of novel objects[113].

In summary, the available evidence indicates that there are indeed familiarity encoding neurons in the PRH in both rats and monkeys. Yet, it seems that this neuronal response is a more widespread phenomenon, as novelty signals are found throughout the MTL and other parts of the cortex (e.g. the occipital lobe). Furthermore, the available evidence from freely-moving experiments, suggests that these types of neuronal responses in the PRH are relatively few in number.

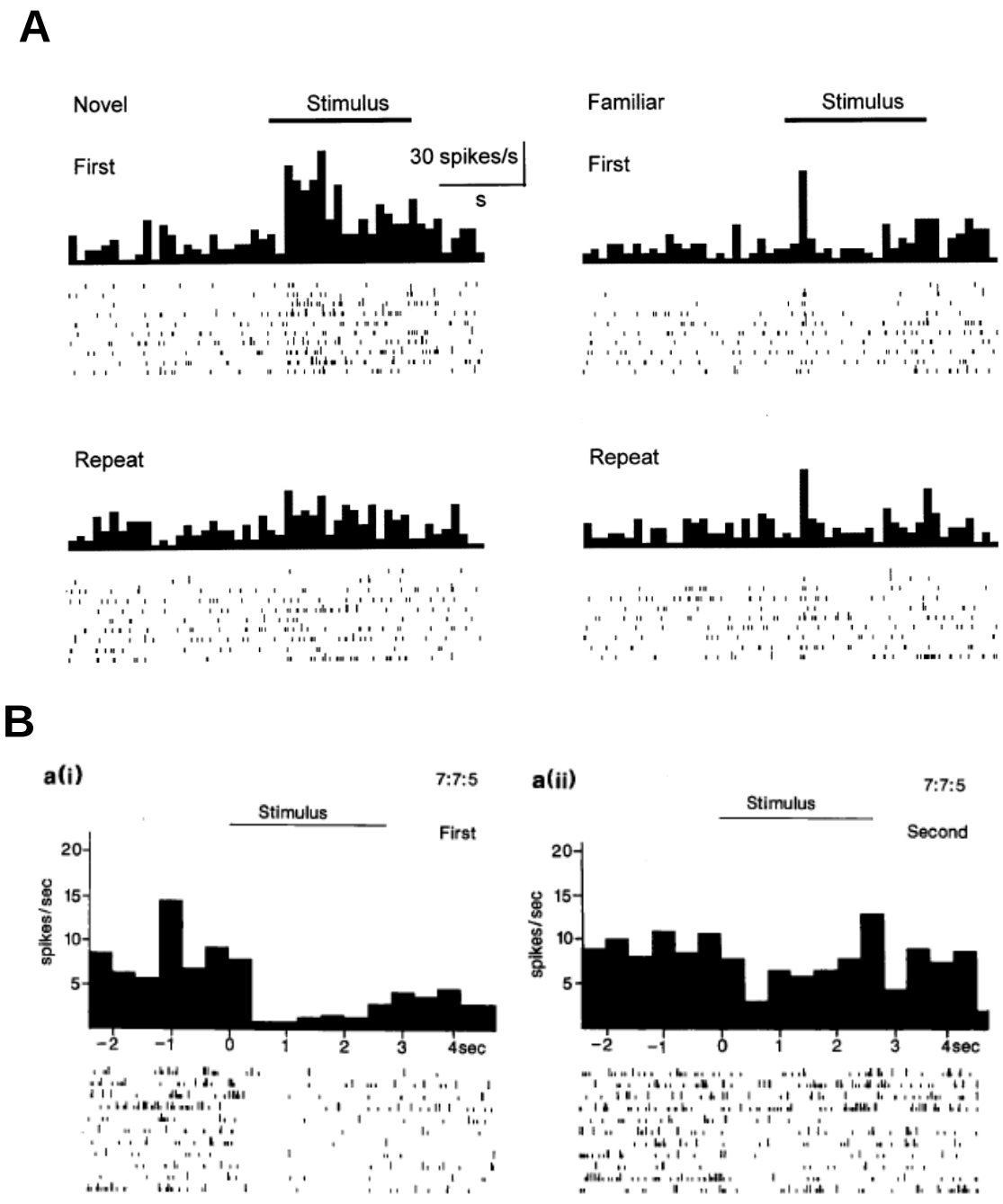

Figure 1.3. Examples of novelty-sensitive neurones in the PRH. A) *Novelty-sensitive neurone in the monkey PRH. The neurons response is reduced after a single exposure to an object, and stays lower during further presentations. Adapted from*[109]*.* ***B)*** *Novelty-sensitive neurone in the rat PRH. The neurone initially reduces its response with exposure to an object. Later, this reduction is decreased upon second presentation of the same object. Adapted from*[111]*.*

Ref	Species	Task	Area	Total Units	VR	FD	FI
103	Monkeys	DMS	IT (all of it)	173	55%	15%	0%
103	Monkeys	DMS	HPC	400	0%	0%	0%
114	Rhesus Monkeys	DMS	IT (PRH)	72	?	35%	18%
109	Monkeys	Serial recognition/ conditional value discrimination	EC	477	42%	8%	3%
109	Monkeys	Serial recognition/ conditional value discrimination	PRH	923	67%	9%	2%
109	Monkeys	Serial recognition/ conditional value discrimination	TE	344	61%	12%	5%
109	Monkeys	Serial recognition/ conditional value discrimination	HPC	283	14%	0%	6%
54	Monkeys	Serial recognition/ conditional value discrimination	EC	588	15%	1%	0%
54	Monkeys	Serial recognition/ conditional value discrimination	PRH	1016	54%	2%	0%
54	Monkeys	Serial recognition/ conditional value discrimination	TG	552	54%	3%	0%
54	Monkeys	Serial recognition/ conditional value discrimination	TE	439	66%	2%	0%
54	Monkeys	Serial recognition/ conditional value discrimination	Fundus STS	110	13%	0%	0%
55	Rat (A)	Passive Viewing	Occipital	70	27%	4%	1%
55	Rat (A)	Passive Viewing	TE	154	21%	4%	3%
55	Rat (A)	Passive Viewing	PRH	92	26%	13%	0%
55	Rat (A)	Passive Viewing	HPC	71	14%	3%	1%
111	Rat	Passive Viewing	Occipital	28	25%	0%	4%
111	Rat	Passive Viewing	TE	95	47%	11%	0%
111	Rat	Passive Viewing	Rhinal cortex	14	71%	7%	0%
111	Rat	Passive Viewing	HPC	16	31%	6%	13%
104	Monkeys	DMS	Lateral inferior temporal	27	63%	4%	0%
104	Monkeys	DMS	TE1	80	64%	4%	0%
104	Monkeys	DMS	Rhinal cortex	41	59%	20%	0%
104	Monkeys	DMS	DG	61	51%	0%	0%
104	Monkeys	DMS	CA3	20	25%	0%	0%
104	Monkeys	DMS	CA1	52	52%	0%	0%

104	Monkeys	DMS	subiculum	105	42%	0%	0%
104	Monkeys	DMS	parahippocampal gyrus	54	70%	0%	0%
104	Monkeys	Passive Viewing	Lateral inferior temporal	16	63%	0%	0%
104	Monkeys	Passive Viewing	TE1	80	80%	21%	0%
104	Monkeys	Passive Viewing	Rhinal cortex	66	36%	6%	0%
104	Monkeys	Passive Viewing	DG	19	16%	0%	0%
104	Monkeys	Passive Viewing	CA3	5	40%	0%	0%
104	Monkeys	Passive Viewing	CA1	25	36%	0%	0%
104	Monkeys	Passive Viewing	subiculum	36	47%	0%	0%
104	Monkeys	Viewing	PHC	24	63%	0%	0%

Table 1.2. Summary of electrophysiological findings in the PRH. *The table lists the species used and the areas tested along with the percentage of each neuronal response. '?' marks a non-stated datum. VR – Visually responsive neurons. FI – Familiarity increasing neurons, . FD – familiarity decreasing neurons. A – anaesthetised*

1.4 Memory and Mental illness

1.4.1 Schizophrenia and Autism

SZ and autism spectrum disorders (ASD) are highly debilitating diseases, with SZ affecting around 24 million people worldwide and about one child in 160 affected by ASD (World Health Organisation). The symptoms of SZ are typically divided into three categories: positive, which include hallucinations, delusions and disordered thoughts; negative, encompassing social withdrawal, reduced mobility and reduced motivation; and cognitive, which include impairments in attention, executive functions (i.e. decision making), and working and episodic memory[115–117]. Though positive symptoms are alleviated by typical and atypical antipsychotic medications, neither class of neuroleptics is effective in the treatment of cognitive deficits. ASD can be usually noticed during early childhood. Its symptoms include communication problems, and highly stereotypical behaviour. And it is quite frequently accompanied by some form of intellectual disability[118].

1.4.2 Models of Mental Illness

One approach to study mental illness is the pharmacological approach. This approach uses the fact that specific drugs reproduce symptoms that are reminiscent of SZ in humans, and to some extent, in animals. An example for such drug is phencyclidine (PCP). PCP seems to reproduce some of the symptoms observed in SZ in humans, including auditory hallucinations[119]. Indeed, in rats, PCP causes deficits in social interaction, which can be reversed by antipsychotic drugs[120]. PCP also induces increased locomotion and deficits in working memory[121]. The ability to reproduce these symptoms by a simple drug injection lead to an extensive use of PCP and other drugs in the study of SZ[122,123].

Another approach to model mental illness is the genetic approach. SZ is a highly heritable disease, with a heritability estimate of around 80%[124]. However, most SZ candidate genes confer only a slight increase in the odds ratio of the disease[125,126]. This suggests that SZ is the product of the cumulative effect of many different genes and an interaction with environmental factors. Similarly for ASD, heritability estimates run between 77%[127] and 91%[128]. Human genetic studies have identified numerous putative susceptibility genes for both diseases, and, interestingly, they both have a considerable genetic overlap[129].

Thus, a possible approach to investigating these mental illnesses is to look at individual endophenotypes[130]. That is, one can try to tease apart what phenotype, or symptom of this complex disease each gene confers. This approach has also been widely used in SZ research[131,132]. The various models for both diseases will be reviewed in chapter 5 and 6.

Since both ASD and SZ is such a complex disorder with many symptoms that are not always present in all human patients or animal models, it would be helpful if there was one feature that could indicate whether an animal model can be treated as a valid model of the disorder. It appears that an auditory deficit is one such symptom. Indeed, one of the most robust features of SZ are changes in auditory-evoked potentials (AEP)[133]. These are usually observed in the EEG signatures of patients in response to different auditory paradigms. In SZ there is usually a deficit in most AEP measures, specifically, in sensory gating[134–136], mismatch detection[137,138] and gamma entrainment[139–141]. All of these will be reviewed more thoroughly in chapter Chapter 5 .

This change in AEPs does not only appear in patients, but also appears in most common animal models of the disease[142–145]. This makes AEPs an important translational tool[146,147]. Thus, examining the AEP is an important part of characterising new potential models of SZ, and should be done as part of any set of experiments testing new models of the disease.

1.4.3 The CYFIP1 Model

One of the genes that show considerable overlap in both SZ and ASD is CYFIP1[148,149]. A meta-analysis found that 20% of the people having the 15q11.2 BP1-BP2 microdeletion – a region that spans the CYFIP1 gene - showed SZ symptoms and 27% had some form of ASD[150]. CYFIP1 codes for a protein that binds to the Fragile-X (FX) protein, FMRP. FX syndrome (FXS) is a syndrome which is associated with many intellectual and emotional disorders, and is frequently associated with ASD[151]. Accordingly, a new CYFIP1 haplo-insufficient mouse line has been developed to better understand the biological mechanisms disrupted by this mutation[152,153].

In order to explore the role of this gene further, a CYFIP1 deficient mouse line was produced[152]. The CYFIP1 mouse model is relatively new and not much

research on it has been published up to date. Slices taken from CYFIP1 haploinsufficient mice exhibit enhancement in both electrically and chemically induced LTD in the CA1 region of the HPC[152]. This enhancement is independent of protein synthesis and mTOR inhibition. These findings replicated earlier findings from *Fmr1*-KO mice[154]. Behaviourally, the same study found no difference between CYFIP1 deficient mice and WT controls in open-field analysis, light-dark transition and the elevated zero maze, nor in hippocampal-dependent tasks like the Y-maze and the Morris water maze. However, these animals showed a much larger extinction, by returning almost to baseline freezing after only 48 hrs, whereas controls showed only a minor reduction in freezing[154]. This was also similar to the *Fmr1*-KO mice[155].

CYFIP1 is enriched in hippocampal pyramidal neurons. normal CYFIP1 is more strongly expressed in excitatory synapses and in dendritic spines in the CA1 region of the HPC[153]. Pyramidal neurons in slices taken from CYFIP1 haploinsufficient mice have shorter dendrites and the dendrites themselves show less branching. Also, these dendrites exhibit an increase in immature spines. Together these results show that the CYFIP1 mice have enhanced LTD and fear extinction, similar to the *fmr1*-KO mice FX model[154,155], these animals also show a decrease in dendritic spines and spine formation.

1.4.4 Memory in mental illness

One of the common cognitive deficits observed in SZ are deficits in memory[156–158]. Thus, in a test asking patients to recognise pairs of objects presented earlier, their recognition was at chance level[158]. SZ patients also show deficits in delayed object and face recognition[159]. Accordingly, it is not surprising that a large meta-analysis looking at 70 studies, found strong evidence for memory deficits in SZ. Specifically, the analysis showed a significant recognition-memory deficit[160]. Interestingly, children with ASD show deficits in source memory, but have intact recognition memory[161,162]. Similarly, children with FXS have deficits only in free recall, and are comparable to controls in recognition memory[163]. Thus, recognition memory is one type of memory that can differentiate between the cognitive deficits in SZ and ASD.

The memory deficits seen in SZ can be replicated in to some extent in different models of the disease. Thus, rats who were sub-chronically injected with phenylcyclidine (PCP) did not show the typical increased novel object exploration, showing a deficit in recognition memory[164]. The same was true for mice repeatedly treated with PCP[165]. Also, in humans, ketamine, which is also used to model SZ, caused deficits in recognition of previously seen words[164]. Several genetic models of schizophrenia also show deficits in memory. Thus, DISC1 KO mice show deficits in fear learning, and spatial recognition, crucially with no deficit in recognition memory[166]. Neurgeulin-1 deficient mice show deficits in fear conditioning, while, similarly to the DISC-1 mice, showing normal recognition memory[145]. Yet, dysbindin-1 deficient mice show less exploration of a novel object, along with increased freezing in response to fear conditioning[167]. Thus, it seems that while the various animal models of SZ can some of the memory deficits, not every model shows the same deficits. That is to be expected due to the polygenetic nature of the disease. Interestingly, animal models of FXS show deficits in recognition memory, despite there being no apparent deficits in humans. Thus, the Fmr1-KO mice, which model FXS, explore a novel and a familiar object for the same amount of time, indicating a recognition memory deficit[168]. Testing the memory of each genetic animal model of mental illness is therefore required to establish the gene's involvement in the memory aspect of the disease, and might also help differentiate between different types of mental illnesses.

1.5 Thesis Aims

As described above, the exact way in which the PRH supports recognition memory is not fully understood. This thesis has two related parts. The first part is concerned mainly with the neural activity in the PRH as a function of stimulus familiarity in mice. In this part, I first investigated whether visual ERPs can be observed in the mouse PRH, similar to those observed in V1. Then, I examined whether the ERPs were modulated by familiarity. Secondly, I examined whether familiarity-related activity was observable at the single-unit resolution, thus adding more evidence to the current debate in the literature about familiarity responses in the PRH. The second part deals with the CYFIP1 hetero-insufficient mice, a putative new model for mental illness. I first examined whether this mouse

line has any AEP deficits similar to SZ and other mental illnesses, then I tried to use the data I gathered in the first part and apply it to this model, by first testing the memory of these mice, and then recording from their PRH and V1 and observing whether they presented any differences in ERPs compared to their WT littermates.

Chapter 2 Methods

All animal procedures were approved by the Home Office and carried out in accordance with the UK Animal Scientific Procedure Act (1986) and Cardiff University Ethical Committee guidelines. Great care was exercised to minimise the pain caused to the animals, and the number of animals used in the experiments.

2.1.1 Animals

C57BL/6N mice, sourced from Charles Rivers, were used for most experiments. The only exception were the experiments details in chapters Chapter 5 and Chapter 6 , where C57BL/6N-Atm1Brd Cyfip1$^{tm2a(EUCOMM)Wtsi}$/WtsiH (CYFIP1 heterozygous mice) sourced from The Mary Lyon Centre were used. The animals were bred and maintained in-house on a C57/B6 background. The animals were kept on a normal 12:12 hour light cycle, with lights on at 08:00, and were given access to food and water *ad libitum*. The housing room had a temperature of 19-21 °C and a relative humidity of 45-65%. Both female and male mice between the ages of 10-16 weeks were used for the experiments.

2.1.2 Anaesthesia

General anaesthesia was induced in an induction box with a delivery of 4% isoflurane in 2L/min 100% O_2. The animal was then transferred to a stereotaxic frame where it received 3% isoflurane, which was gradually reduced to 2%-1.5% during the course of the surgery, while ensuring that the animal remained anaesthetised and maintained a stable breathing pattern. The depth of anaesthesia was gauged during the surgery by checking the hind paw withdrawal and tail pinch reflexes. The temperature of the animal was monitored and maintained at 37 °C with a homoeothermic heat blanket (#507220F, Harvard Apparatus, Kent, UK).

2.1.3 Surgery

The animals head was shaved using electric clippers (Contura type HS61, Wella, UK). Then, the skin was disinfected with a povidone-iodine solution (Betadine, Betadine Inc., UK) to maintain a sterile surgical area. A paraffin-based eye lubricant (Lacrilube, Allegan Inc., USA) was applied to both eyes. Then, an incision was made to the scalp from the back of the skull to between the eyes

using surgical scissors. The connective tissue covering the skull was carefully removed using sterile surgical swabs. Bregma and lambda were then identified as the intersection between the front horizontal and posterior horizontal sutures, respectively, and the vertical suture; and their stereotaxic coordinates were measured using a needle held by a stereotaxic manipulator arm. Then, the mice were implanted with electrodes in the areas of interest according to a mouse brain atlas[169].

For chapter 3 and Chapter 6: two depth electrodes were implanted, one in the visual cortex (+0.8 AP, 2.8 ML relative to lambda, -0.5 DV; **Figure 2.1**B), and one in the perirhinal cortex (-3.3 AP, +4 ML relative to bregma -3.3 DV; **Figure 2.1**A,C). A ground/reference screw was placed above the frontal sinus. For the HPC and PRH recordings a pre-made pedestal of 2 electrodes of the same length and with 1 mm spacing was implanted in the PRH in the same coordinates and in the HPC (-3.3 AP, +3 ML relative to bregma -3.3 DV; **Figure 2.1**D).

For chapter Chapter 4 : a silicone probe was mounted onto a mini-drive and was implanted in the PRH (~-3.3 AP, ~-4 relative to bregma, ~-3.0 DV; **Figure 2.1**C). Then, postoperatively the probe was slowly lowered into the recording area. The implantation sight was in a radius of about 100 µm around the intended implantation area, depending on brain vasculature. Two screws placed above the cerebellum were used as ground and reference.

For chapter 5: a pedestal of 3 electrodes with 1 mm spacing and with the 2 front electrodes 1 mm shorter than the back was implanted at the following coordinates: HPC: AP -1.8, Ground: AP -0.8 and reference +0.2 AP. All were at -2.65 ML and the depth of HPC was -2.75 and -1.75 for the ground and reference, as was done is previous studies[144,170]. The animals were also implanted with screw electrodes above the frontal (~1 mm AP, 1 mm ML) and auditory (~-2.75 AP, ~-3.5 ML) cortices, with a ground electrode over the cerebellum.

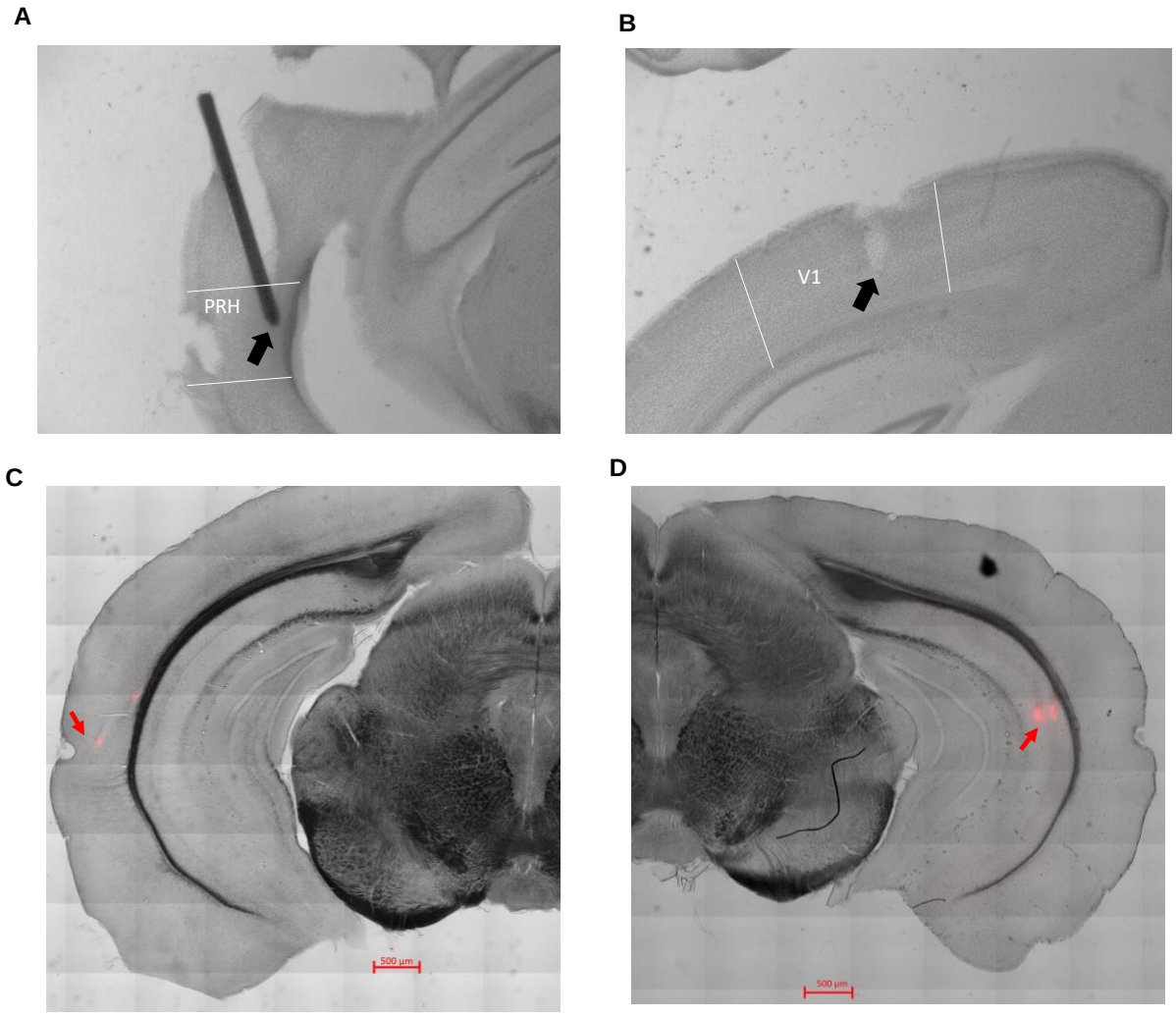

Figure 2.1. Localisation of silicon-probes. A) *An example of a stainless-steel electrode lodged in the PRH recording site* ***B)*** *An example of the V1 electrode track* ***C)*** *The PRH Dil-stained silicon-probe track* ***D)*** *The HPC Dil-stained silicon-probe track. Black arrows mark the location of an electrode, and red arrows mark the location of a silicon robe track. Slices were taken with the help of Tim Gould.*

2.2 Post-Surgical Care

Any loose skin flaps were sutured using braided 0.12 mm silk sutures. The wound area was then washed with saline an antiseptic powder (Battle Hayward and Bower Ltd, USA) was applied around the incision site. The anaesthetic flow was then ceased and the animal left to breathe pure oxygen for a few seconds, until it regained its pinch reflex. Then, the animal was carefully removed from the stereotaxic frame and allowed to recover under heating light until it regained its righting reflex. Then, it was moved back to the holding room. Animals were given a week to recover before any experimental procedure took place.

2.2.1 Electrodes

The electrodes used in the experiments were manufactured in-house. Different metal wires were used to make the electrodes. They were assembled in the appropriate constellation, and cut to length, according to the areas of interest and the position in the brain. For Chapter 5, 3 electrodes were aligned in a row and cemented prior to surgery, and for chapter 3: the HPC and PRH electrodes were also cemented together before implantation. The impedance of the electrodes was measured with an impedance metre (Table 2.1).

Metal	Width	Impedance
Pt/Ir	75 µM	~150 kΩ
Pt/Ir	50 µM	~250 kΩ
Pt/Ir	25 µM	~300 kΩ
stainless steel	75 µM	~50 kΩ
stainless steel	50 µM	~30 kΩ

*Table 2.1**. Impedance of the different wires used for electrodes assembly***

For chapter 4, a 32-channel silicone probe was used (ASSY-116 DBC-2-1; Cambridge Neurotech). The probes had 2 shanks, each shank containing 16 electrode sites. The distance between sites was 25 µm centre to centre. The recording sites were 11 X 15 μm^2 in area. The distance between the shanks was 250 µm.

2.2.2 Recording in freely moving animals

The animals were put in a Perspex box for the recordings. The exact procedure will be described in the appropriate chapters. Two different systems were used for signal acquisition.

For the ERP recordings, the recording rig was composed of a differential preamplifier (Supertech) with a 0.08 Hz high-pass filter and an impedance of 10 MOhm. The preamplifier in turn was connected to an analogue amplifier (BioAmp; Supertech). The amplifier was set to a gain of X1000 and a low-pass filter at 500 Hz. The amplifier was connected to a CED 1401 Digitiser (Micro3 D.130; Cambridge Instruments) and the signal was digitised at an acquisition rate of 1000 Hz. The signal was then visualised and recorded using Spike2 7.3 (Cambridge Instruments).

For silicon probe recordings, the system was composed of a Plexon Recorder/64 amplifier connected to a Plexon ADQ board. To acquire the data a X20 gain headstage (HST/32V-G20-LN; Plexon) was plugged directly to the electrode connector and to the amplifier. The signal was recorded and visualised using Plexon Recorder software. The system was also connected to an AVT Stingray camera (80 FPS). Video recordings were made using the Cineplex recording software (Plexon).

2.2.3 Head Restrained Recordings

After implantation, the animals' heads were fixed to a holder with a pre-implanted head plate, by attaching the head-plate to the holder and screwing it on both ends. The animals were free to run on a foam wheel. Two screens were placed on both sides of the animal. However, only one of them presented the stimulus at each time. Before starting any behavioural procedure, the animals were habituated for sessions of 10 minutes for 2 days prior to the first experimental session.

2.2.4 Electrode Placement Verification

Electrode placement was verified using several different methods. For the stainless-steel electrodes, the track was usually visible after the normal histological procedure. For some of the animals, a small lesion was made by injecting a 30 μA current for about 10 sec through the electrode. As the silicone

probe was too small to leave a prominent track, used, it was immersed in a 1% DiI Stain (1,1'-Dioctadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiIC18(3); Thermo-Fisher) solution for half an hour before implantation. Then after slicing the brain, the track was imaged using a fluorescent microscope (Nikon E100).

2.2.5 Histological Procedures

Animals were deeply anaesthetised with 200 mg/kg sodium pentobarbital (Euthetal, Merial Animal Health Ltd, Essex, UK). Then, the animals were perfused intracardially using a peristaltic pump with phosphate-buffered saline (PBS, Sigma) with a 20 ml/min flow rate, followed by a 4% paraformaldehyde In 0.1M PBS, pH 7.4 with a 10 ml/min flow rate for 10 mins. The brain was then removed from the skull and left in the paraformaldehyde solution for 8 more hours. The brains were then moved to a PBS solution and kept at 4°C until they were cut. 80-100 µm slices were then obtained using a sliding microtome (Leica VT 1000S vibratome). The slices were then mounted on glass slides, and dried overnight at room temperature. The slides were put through a series of preparation steps as follows: they were defatted for 1 h in a mixture of chloroform/ethanol 100% (1:1), and then rehydrated through a graded series of ethanol, twice for 2 minutes in 100% ethanol, 2 min in 96% ethanol, 2 min in 70% ethanol, 2 min in 50% ethanol, 2 min in dH_2O, and stained 30 seconds in a 0.125% thionin (Fisher Scientific) solution, dehydrated and cover slipped with DPX (BDH Laboratory supplies, Poole, England).

2.2.6 Statistics

ANOVA was used whenever multiple tests were needed. The parameters for the ANOVA are described in the results chapters. Where significant differences were found the appropriate multiple comparisons were conducted using Bonferroni correction.

Chapter 3 Familiarity-Related ERPs in the PRH, V1 and HPC

3.1 Introduction

As described in chapter Chapter 1 , the PRH has long been implicated in recognition memory[27,62,171–174]. The question is, then, what computations occur in this area to support this type of memory. The way to answer this question is to record the activity of neurons in this area and find which aspects of the environment they encode. In humans, due to its location, it is difficult to record from the PRH using regular EEG caps, without sophisticated triangulation techniques[175,176]. That is why human recordings in this area come from epileptic patients. These patients have electrodes implanted in their brains in order to pre-surgically identify the exact locus of their seizures. Since focal epilepsy usually starts from the HPC area, these patients are asked to participate in experiments studying the areas around the HPC, which conveniently encompasses most of the MTL structures.

Studies in humans have identified the AMTL-N400, a negative-going component of the ERP in the anterior medial temporal lobe (AMTL)[177,178]. A study in humans with MTL epilepsy found a reduction in the ERP amplitude with repetition of either verbal or non-verbal stimuli[179]. Looking specifically at the PRH in epileptic patients, correctly recognising a familiar word in a continuous word recognition paradigm was correlated with a higher N400 than correctly recognised novel words[180–183]. An interesting contrast was that while, the change of the HPC ERP was dependent on the subject consciously and explicitly remembering the word, the PRH showed no such sensitivity and showed a decreased response to a repeated word regardless of the subject consciously remembering the word[184].

A study comparing the ERPs in the PRH and the HPC in a word free-recall task found that for common words, subsequent recall of the word was associated with a higher N400 signal in both the PRH and the HPC. However, for less common words, while the HPC showed a lower response for forgotten words, the PRH showed no recall-related difference in the N400 amplitude[10].

Most of these studies focus on verbal stimuli. However, in one study, no familiarity-related difference could be observed in the PRH when pictures were used instead of words[186]. Yet, other studies found repetition effects to pictorial as well as to word stimuli, but it was smaller than word stimuli, and, had a floor effect when used to determine seizure laterality, whereas word stimuli were big enough to help detect seizure laterality[172,187]. Similar studies looking at the ERPs evoked by famous or non-famous faces found that they evoked an ERP in the PRH, and that it was modulated by repetition. However, in both studies non-famous faces evoked a lower AMTL-N400 than famous faces, and showed no repetition related modulation[188,189].

In animals, all studies looking at neuronal response during recognition memory tasks approached this topic employing the single unit approach[55,109,110,190]. However, as described above, studies in humans have mainly used the ERP approach, albeit invasively. Further, future and, indeed, current development might mean that deep structures such as the MTL can be recorded non-invasively[191,192]. Thus, recording ERPs in the PRH will enlighten future research as it will contribute towards a fuller understanding of how neuronal processes in the MTL generalise across different species, and of how well rodents can model processes in the MTL. More importantly, while single-unit studies are important for isolating and defining populations of neurons, ERPs give an insight as to how an area responds to a certain stimulus as a whole. To the best of my knowledge, no study tried to look at evoked potentials in animals. The current study tries to bridge this gap in research.

Thus, this study looks at ERPs in the mouse PRH. Since NOR studies are usually based on exploration of objects and thus are mainly visual, the following experiments used visual stimuli to study the ERPs in the PRH. Since studies in humans found repetition response in the HPC as well[184,185], ERPs in this area were also recorded. First, following studies examining visual responses in V1[193,194], the ERPs in response to simple gratings were recorded in both the PRH and V1. Alongside the simple grating stimuli, pictures of objects were also used, following the paradigm used in the single-unit studies of the PRH[55,109,110,190]. Since, at least in the NOR task, following a PRH lesion, rats show deficits only for intervals greater than 15 minutes[52,53,61](section 1.3.1) both short (2 min) and

long (24 hrs) time-intervals were used to see whether there is any time-dependent difference in the natural function of the area. Surprisingly, in this study, no effect for novelty could be observed in the PRH, and no ERP was observed in the HPC.

3.2 Methods

All surgery, post-surgery, care and recording methods are described in chapter 2.

3.2.1 Visual Evoked Potentials (VEP)

After implantation, rest and habituation, the animals were placed on a running wheel. The sessions were 20 minutes long and comprised of presentation of visual stimuli on the screen to the left of the mouse. The stimuli were presented for one second with one second inter-stimulus interval. All the sessions were comprised of the presentation of 500 stimuli. The stimuli were either horizontal and vertical gratings (**Figure 3.1**A) or full sized black and white pictures of different objects (**Figure 3.1**B). The contrast and frequency of the gratings was chosen as the one eliciting the strongest response in previous studies[193,194]. Each trial consisted of 2 stages. At the first stage a stimulus, called the 'control' stimulus – either a stationary grating or a picture were presented 500 times. After a retention interval of either 2 minutes or 24 hours, at the second stage the stimulus from the first stage, now designated the 'familiar' stimulus, was presented 250 times, interleaved with a novel stimulus (either a grating with a different orientation, or a novel picture) that was presented 250 times. If pictures were used, another test consisted of a slightly different second stage, where the familiar stimulus was presented 250 times interleaved with 50 cases of different novel pictures. For the 2-minute retention period the mouse stayed in the apparatus, with the screen turned on but without any stimulus. For the 24-hour retention interval, the mouse was returned to its home cage. During the inter-stimulus interval, the screen was of constant light grey colour.

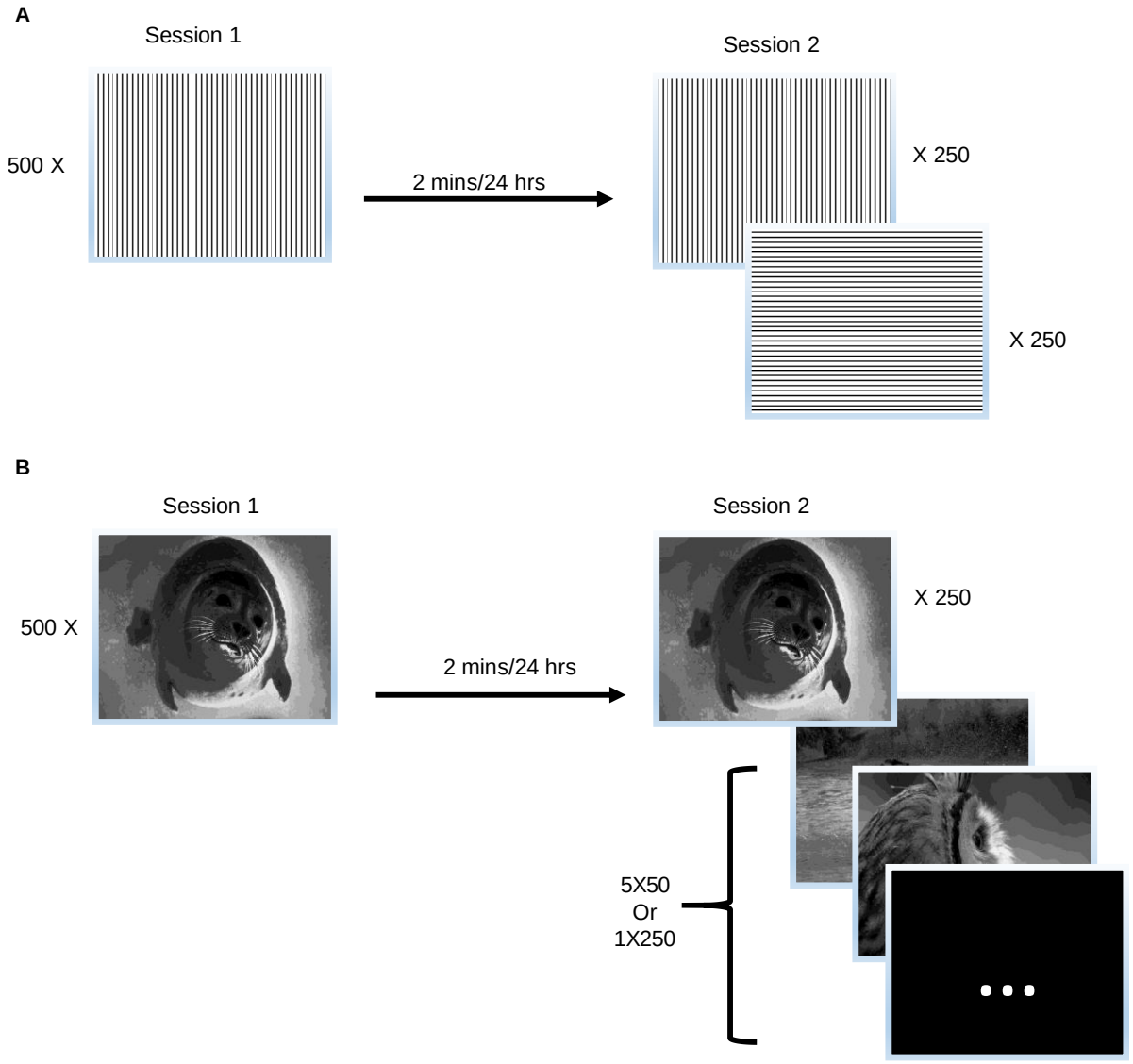

***Figure 3.1. The general VEP procedure**. **A)** The grating procedure consisted of 500 presentations of a grating in one orientation followed after 2 minutes or 24 hours by 250 presentations of gratings in the same orientation and 250 presentations in a 90° rotated grating. **B)** The picture procedure consisted of 500 presentations of one complex image followed by a retention interval of either 2 mins or 24 hrs by 250 presentations of the same picture interleaved with either 50 presentations of 5 different novel pictures in one procedure, or by 250 presentations of 1 novel picture, similar to the grating procedure.*

3.2.2 Visual Stimuli

Object images were drawn from a standardised image bank[195]. Natural images were taken from a free stock photo website (http://www.freeimages.co.uk). Care was taken that images were not too similar when they were used for the same task, in terms of general contour and texture patterns. The images were resized to fit the presentation screen so it covered the entire screen.

3.2.3 VEP Analysis

A custom-made automatic script was used to find the evoked potentials in both V1 and the PRH. All results were later verified visually. The average signal for all the trials in the different cases was averaged (250 trials) for each animal. For V1 (**Figure 3.2**A), the most prominent trough was identified. The time of this trough relative to presentation onset was defined as the latency and the amplitude of the evoked potential was defined as the difference in amplitude between this trough and the peak directly preceding it. In the PRH (**Figure 3.2**B), the first prominent peak was identified. The latency of this peak relative to stimulus onset was defined as the evoked-potential latency and its amplitude was defined as the difference between this peaks amplitude and the trough immediately preceding it.

3.2.4 Movement Analysis

Movement was recorded by a motion detector attached to the wheel on which the animal was placed. The movement recorded was the angular rotation of the wheel. To get the normalised movement, the movement in 1 sec of stimulus presentation was divided by the movement in the 1 sec before the presentation for each stimulus presentation.

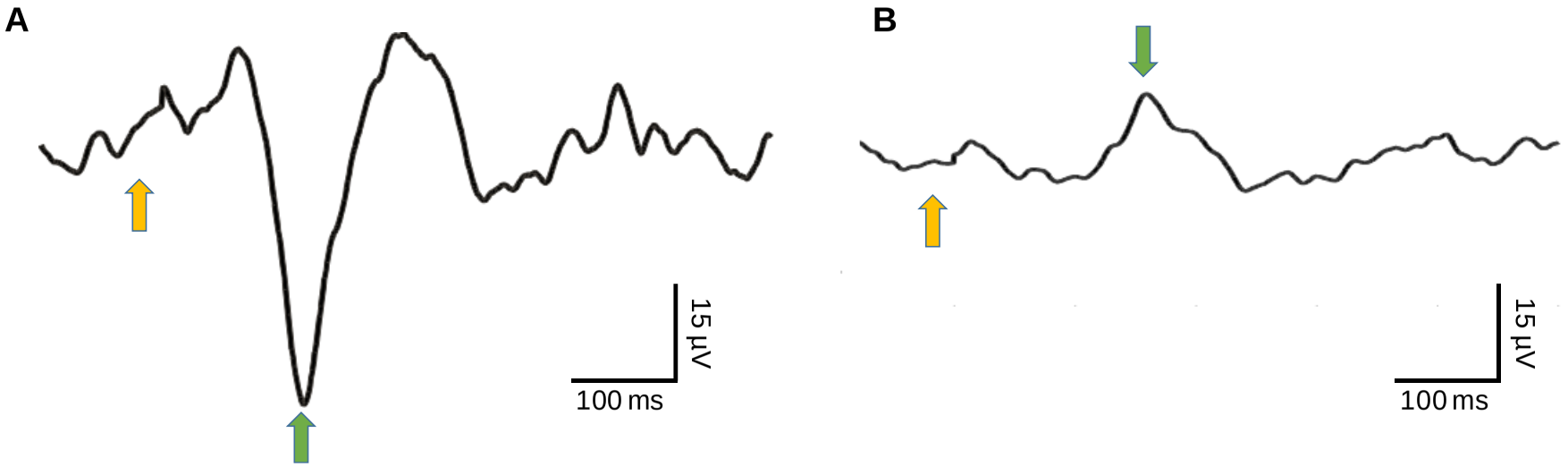

Figure 3.2. Visual evoked potentials A) VEP in V1. *The yellow arrow marks the stimulus onset. The green arrow represents the first identified trough. The Amplitude of the evoked potential was defined as the difference between this trough and the first peak preceding it.* ***B)*** *VEP in the PRH. The green arrow shows the first identified peak. The evoked potential amplitude was defined as the difference between the peak and the first trough preceding it. In both cases the latency is the time when the signal reaches its maximum/or minimum, where the green arrow is pointing.*

3.3 Results

3.3.1 Short term Familiarity

To first verify that the PRH responds to simple visual stimuli, simple stationary horizontal or vertical gratings were used (**Figure 3.1**A). The gratings evoked a negative deflection in V1 (-50.89 μV ± 5.66 μV) with a latency of 128.5 ms ± 5.22 ms. This indicated that the mouse was able to see the gratings. Then, the response in the PRH was examined. Indeed, the gratings evoked a response in this area too. Like other studies the first prominent response was quantified[179,180,184]. The evoked response was positive going, and its amplitude of 18.49 μV ± 0.95 μV, was smaller than the primary visual cortical response ($t(18)=5.64$, $p<0.001$; n=10). Its latency, as would be expected from a higher order area, appeared later than the V1 response, at 169 ± 5.74 ms ($t(18) = 5.22$, $p<0.001$; n=10).

After the presence of a visual response was established, the influence of stimulus novelty and familiarity was examined. To determine if there were differences in the visual response within the first day itself, the stimulus data from the first day were halved and the response to the first half of the presentation (presentation 1-250, Control 1 in the figures) was compared to the second half of the presentations (presentations 251-500, Control 2 in figures). These responses were further compared to the 250 presentations of the same stimulus, after a 2-minute retention interval and 250 presentations of a novel orientation. In V1, the difference in familiarity between cases did not elicit any change in response amplitude ($F(3, 27) = 0.08$, $p=0.97$, n=10; **Figure 3.4**D,E) or latency ($F(3, 27) = 1.77$, $p=0.19$, n=10; **Figure 3.4**D,F). The results were the same for the PRH, where an ANOVA revealed no familiarity/novelty-related difference in the evoked response ($F(3,27) = 2.11$, $p = 0.14$, n=10; **Figure 3.3**A,B). There was, also, no significant difference in latency of the different responses ($F(3,27) = 0.81$, $p=0.49$, n=10; **Figure 3.3**A,C).

Recordings were also taken from the ventral HPC. However, no ERPs could be identified in this area, for either gratings or complex pictures (**Figure 3.4**, n=6).

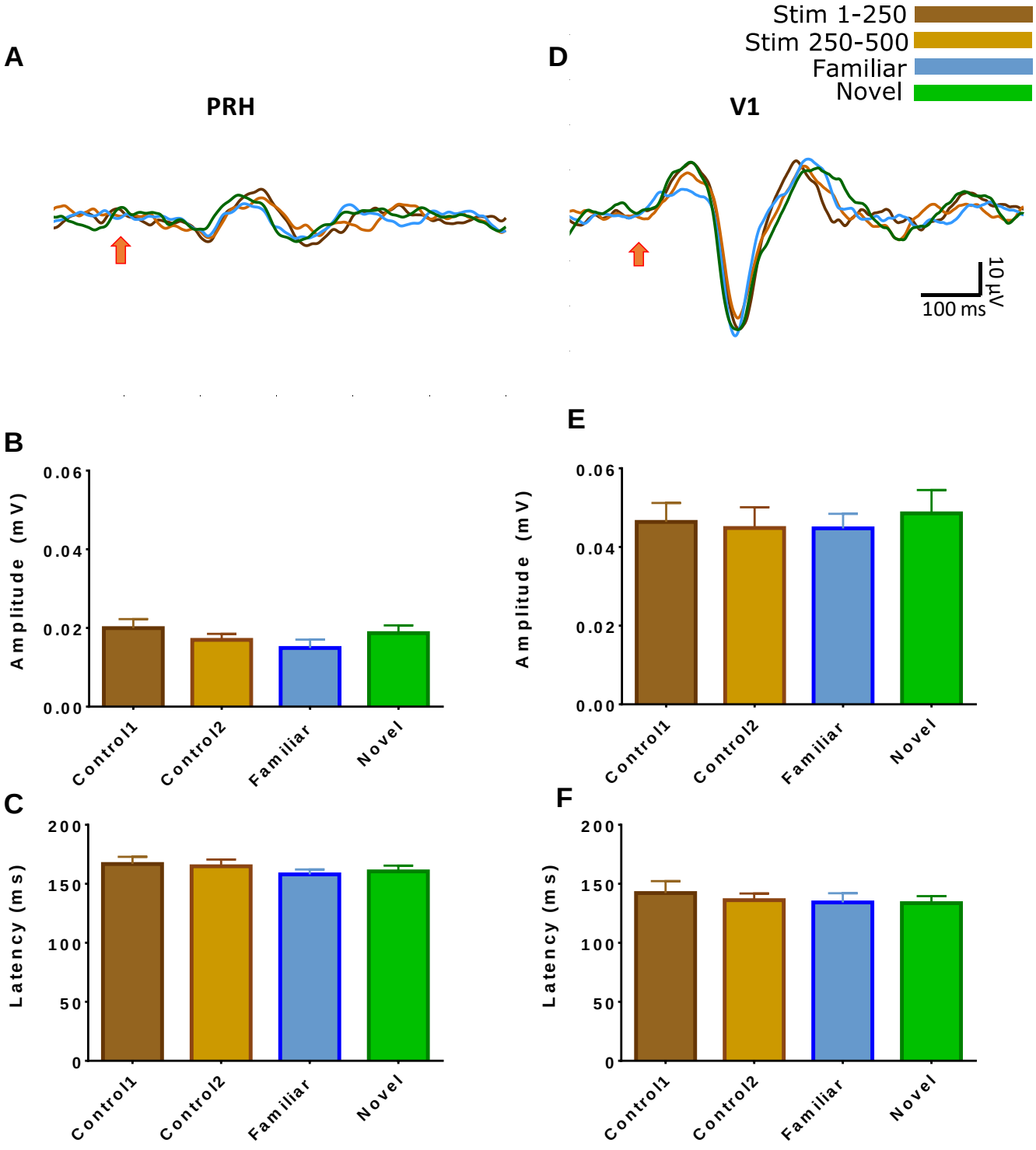

Figure 3.3. Short-term response to gratings. ***A,B,C*** *described the PRH response and* ***D,E,F*** *describe the V1 response* ***A)*** *average PRH group response* ***B)*** *Summary of mean PRH response amplitude.* ***C)*** *Summary of mean PRH response latency.* ***D)*** *average V1 group response* ***E)*** *Summary of mean V1 response amplitude.* ***F)*** *Summary of mean response latency.* ***Control 1(brown)*** *sums the response for the first 250 presentation within the first session.* ***Control 2(yellow)*** *sums the response for the last 250 presentations within the first session.* ***Familiar(blue)*** *sums the response for the 250 presentations of the familiar orientation in the second session.* ***Novel(green)*** *sums the response for 250 presentations of a novel orientation in the second session. Red arrow marks the stimulus onset.*

The lack of a modulation effect in the ERP recorded from the PRH might have stemmed from the stimulus being too simple. To try to match past studies using more complex stimuli[55,109,110,190], the same procedure was repeated with complex pictures of objects as the stimulus (**Figure 3.1**B). An evoked response could be observed in both V1 (**Figure 3.5**D) and the PRH (**Figure 3.5**A) in this case as well. After the existence of the response was verified, the response to complex stimuli was tested in the same way, with a first presentation of an image, followed by a presentation of the same image and a novel image after a 2-minute retention period. There was no significant familiarity difference in the V1 response amplitude ($F(3,33) = 0.098$, $p=0.96$, $n=12$; **Figure 3.5**E) or latency ($F(3,33) = 0.316$, $p=0.81$,$n=12$; **Figure 3.5**F). In the PRH, in this case, there was no difference in response amplitude ($F(3,33) = 0.66$, $p=0.58$, $n=12$; **Figure 3.5**B) or latency($F(3,33) = 1.28$, $p=0.29$, $n=12$; **Figure 3.5**C) induced by familiarity.

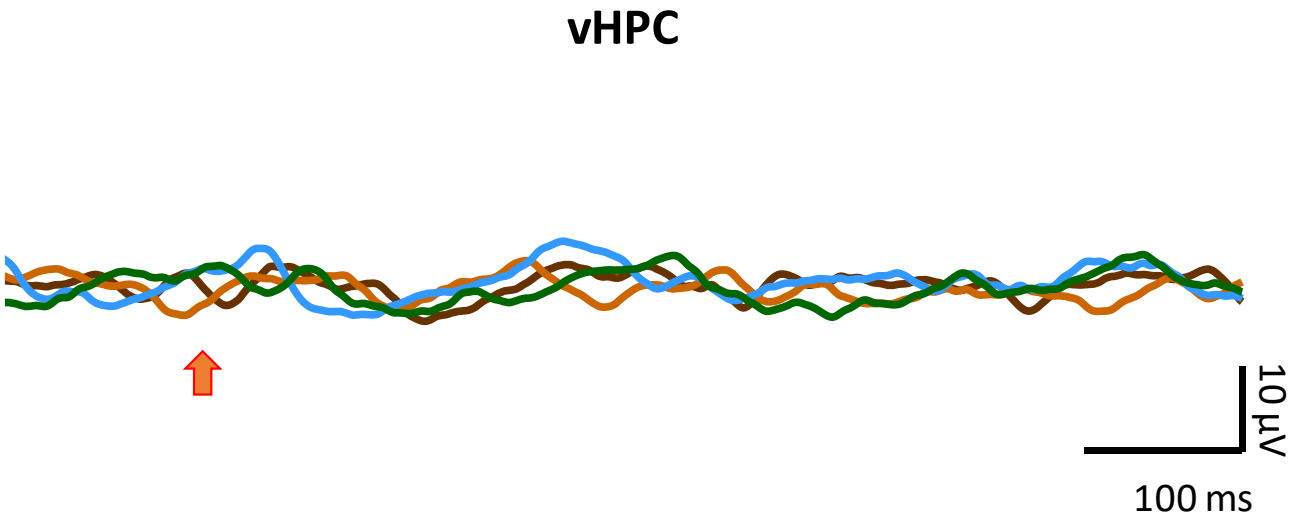

Figure 3.4. Lack of vHPC evoked potential. *The vHPC response to gratings no visual response could be identified. Red arrow marks the stimulus onset.*

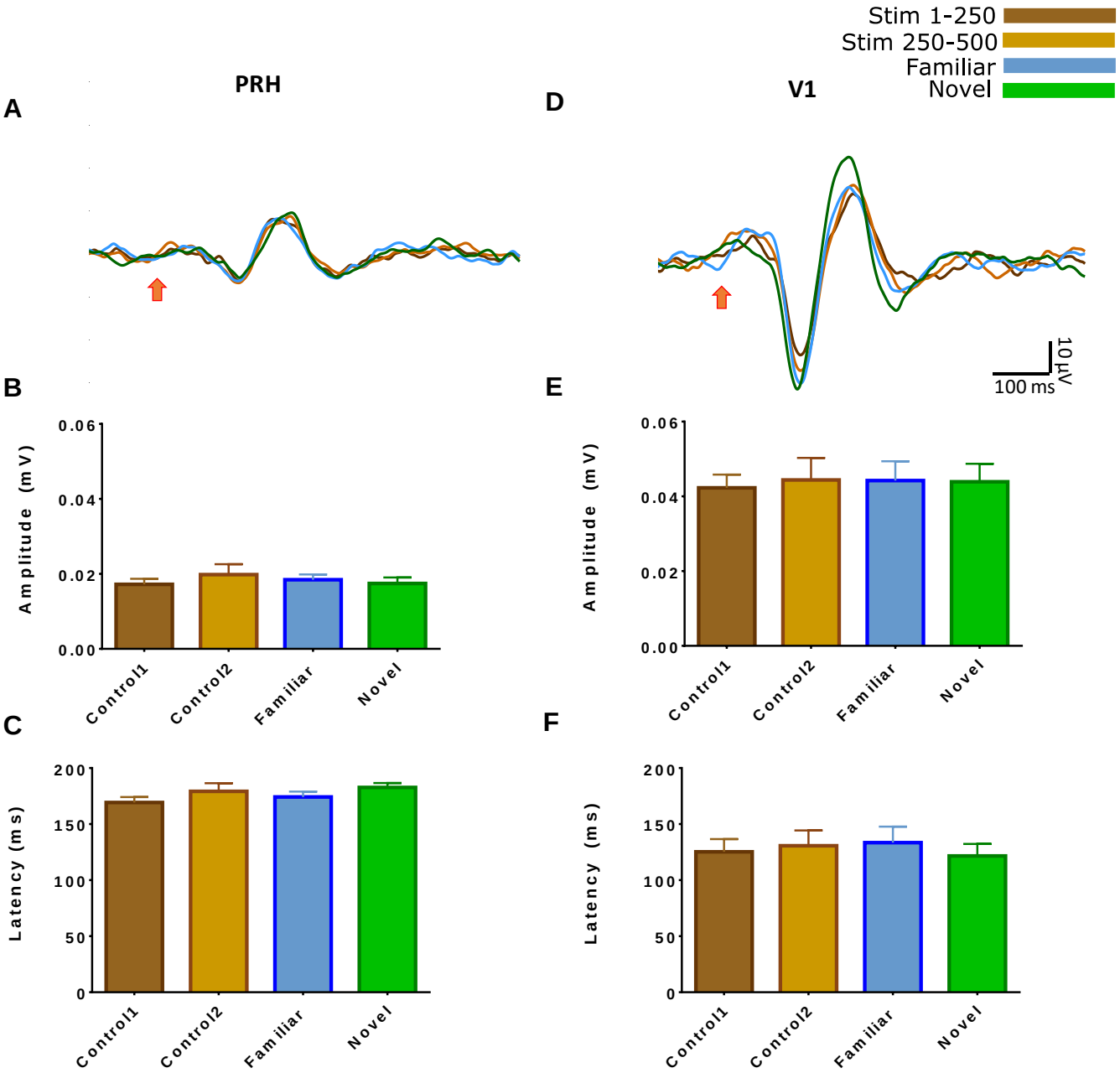

***Figure 3.5. Short term response to pictures of objects**. **A,B,C** described the PRH response and **D,E,F** describe the V1 response **A)** average PRH group response **B)** Summary of mean PRH response amplitude. **C)** Summary of mean PRH response latency. **D)** average V1 group response **E)** Summary of mean V1 response amplitude. **F)** Summary of mean response latency. **Control 1(brown)** sums the response for the first 250 presentation within the first session. **Control 2(yellow)** sums the response for the last 250 presentations within the first session. **Familiar(blue)** sums the response for the 250 presentations of the familiar picture in the second session. **Novel(green)** sums the response for 250 presentations of a novel picture in the second session. Red arrow marks the stimulus onset.*

It might be that after being exposed to the same image, even the item designated as ‘novel’ already becomes ‘familiar’ after just a few exposures. Further, it may have had sufficient elements in common with the ‘familiar’ object to evoke a ‘familiar’ response. To try to increase ‘novelty’ even further, instead of just 1 novel object presented 250 times, 5 novel objects were used on the second trial, each presented 50 times. Interestingly, V1 did show a familiarity-related modulation in the ERP ($F(3,36) = 5.28$, $p<0.01$, $n = 13$; **Figure 3.6**D,E). Multiple comparisons showed that both the later presentation of the stimulus on the first trial (85.52 μV ± 20.64 μV, $p<0.01$) and the familiar stimulus (81.11 μV ± 20.49 μV, $p<0.05$) were both significantly bigger than the response amplitude to the novel stimuli (44.81 μV ± 5.093 μV). Yet, despite a difference in the V1 evoked response, there was no significant difference in amplitude ($F(3,36) = 1.79$, $p=0.17$, $n= 13$;**Figure 3.6**A,B) or latency ($F(3,36) = 0.43$, $p=0.70$, $n= 13$; **Figure 3.6**A,C) between the different stimuli groups in the PRH. Nor was there a significant difference in latency in V1 ($F(3,36) = 0.07$, $p=0.81$, $n = 13$; **Figure 3.6**A,F).

To summarise, in the case of a 2-minute retention interval, the present study found no evidence of a modulation in the ERP in the PRH as a function of familiarity or novelty of a visual stimulus. Furthermore, even under conditions where the V1 response was modulated by novelty, there was no change in the PRH. Also, in all of the conditions the HPC showed no ERP. Yet, it might be the case that changes in evoked-response happen after a longer period of time post stimulus presentation, due to plasticity effects. To determine if that is a case a longer retention interval was then used.

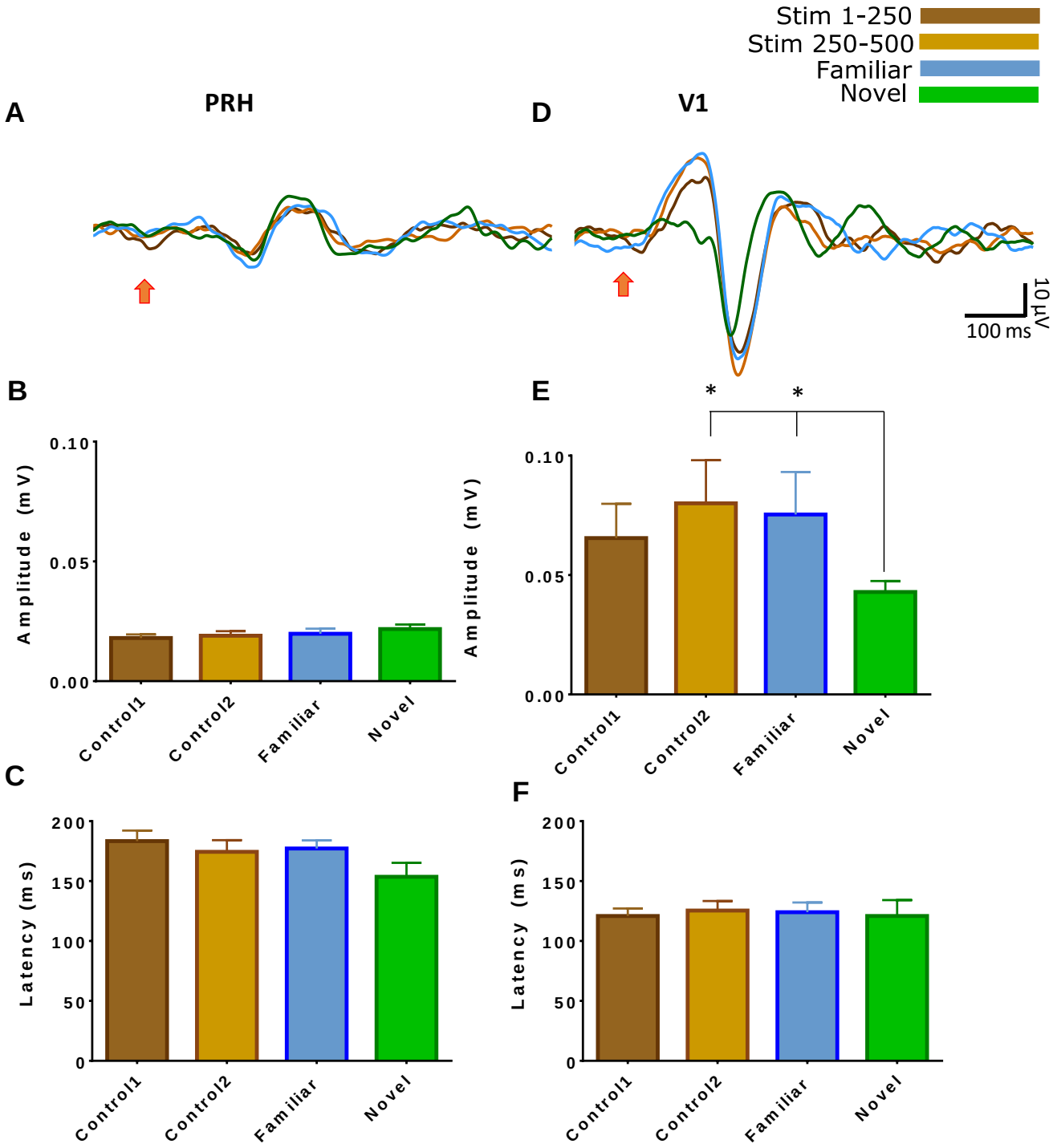

***Figure 3.6. Response to pictures of objects (5 novel objects)**. **A,B,C** described the PRH response and **D,E,F** describe the V1 response **A)** average PRH group response **B)**Summary of mean PRH response amplitude. **C)** Summary of mean PRH response latency. **D)** average V1 group response **E)** Summary of mean V1 response amplitude. **F)** Summary of mean response latency. **Control 1(brown)** sums the response for the first 250 presentation within the first session. **Control 2(yellow)** sums the response for the last 250 presentations within the first session. **Familiar(blue)** sums the response for the 250 presentations of the familiar orientation in the second session. **Novel(green)** sums the response for 5X50 presentation of novel pictures in the second session. The red arrow in the traces marks the stimulus onset. Red arrow marks the stimulus onset. Brackets represent a significant difference (*p<0.05)*

3.3.2 Long term Familiarity

To explore if there is a long-term familiarity response, the same tests were repeated with a 24-hours retention interval, so that if there is any form of a long-term plasticity taking place its effects could be observed. As before, there was no difference in either amplitude ($F(3,42) = 0.15$, $p=0.93$, $n= 15$; **Figure 3.7**D,E) or latency ($F(3,42) = 0.71$, $p=0.48$, $n= 15$; **Figure 3.7**D,F) to gratings in V1. Similarly to the short-term case, there was no difference in the PRH response for the longer retention interval, not in amplitude ($F(3,42) = 0.71$, $p=0.93$, $n= 15$; **Figure 3.7**A,B), nor in latency ($F(3,42) = 0.32$, $p=0.81$, $n= 15$; **Figure 3.7**A,C).

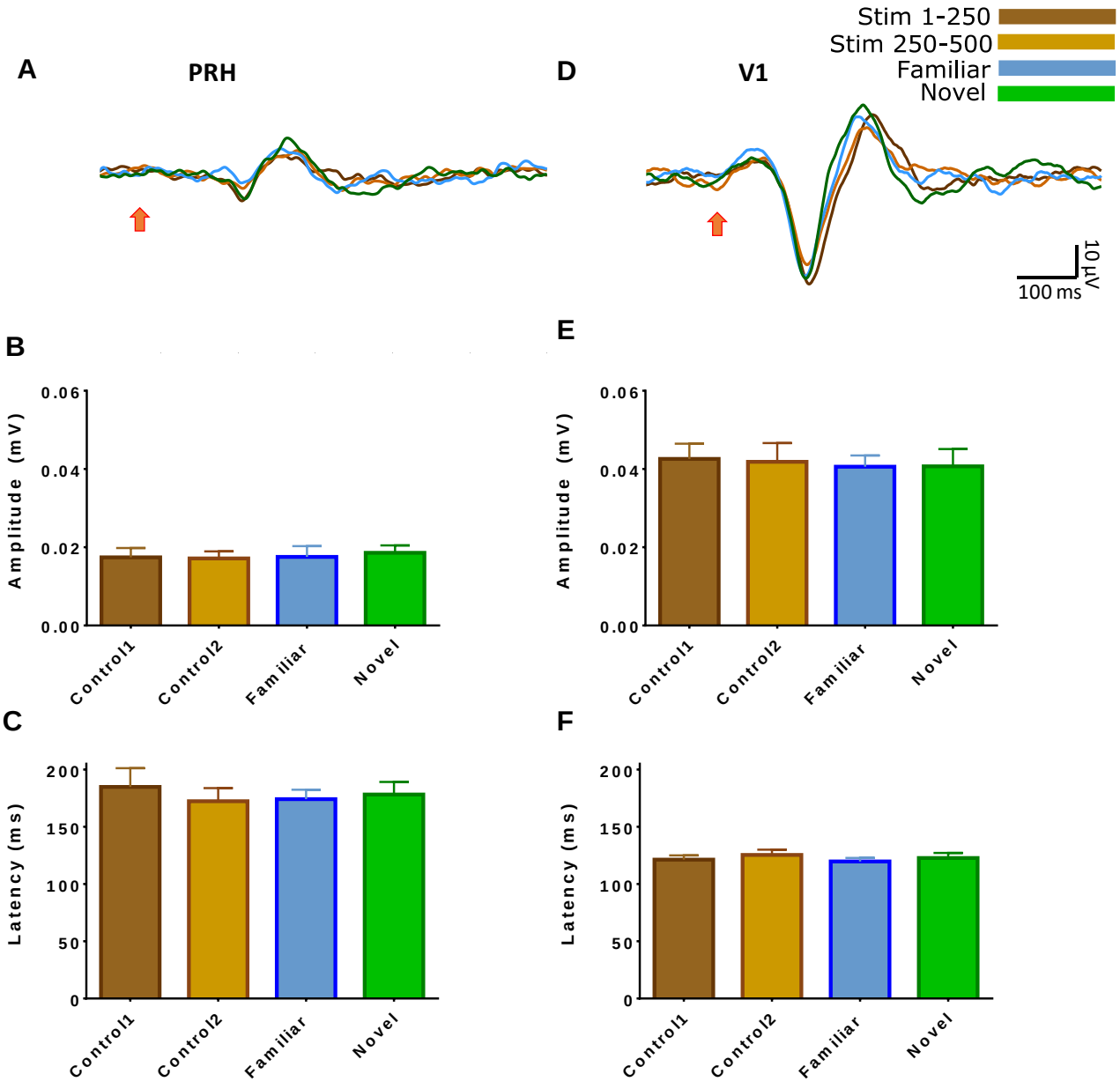

***Figure 3.7. Long-term response to gratings**. **A,B,C** described the PRH response and **D,E,F** describe the V1 response **A)** average PRH group response **B)** Summary of mean PRH response amplitude. **C)** Summary of mean PRH response latency. **D)** Average V1 group response **E)** Summary of mean V1 response amplitude. **F)** Summary of mean response latency. **Control 1 (brown)** sums the response for the first 250 presentation within the first session. **Control 2 (yellow)** sums the response for the last 250 presentations within the first session. **Familiar (blue)** sums the response for the 250 presentations of the familiar picture in the second session. **Novel (green)** sums the response for 250 presentations of a novel picture in the second session. Red arrow marks the stimulus onset.*

Similarly, when using images of objects, there was no difference in response amplitude ($F(3,33) = 1.31$, $p=0.28$, $n= 12$;**Figure 3.8**D,E) or latency ($F(3,33) = 0.89$, $p=0.38$, $n= 12$;**Figure 3.8**D,F) in V1. Nor was there a difference in amplitude ($F(3,27) = 1.23$, $p=0.31$, $n = 10$;**Figure 3.8**A,B) or latency ($F(3,27) = 0.08$, $p=0.92$, $n = 10$;**Figure 3.8**A,C) in the PRH.

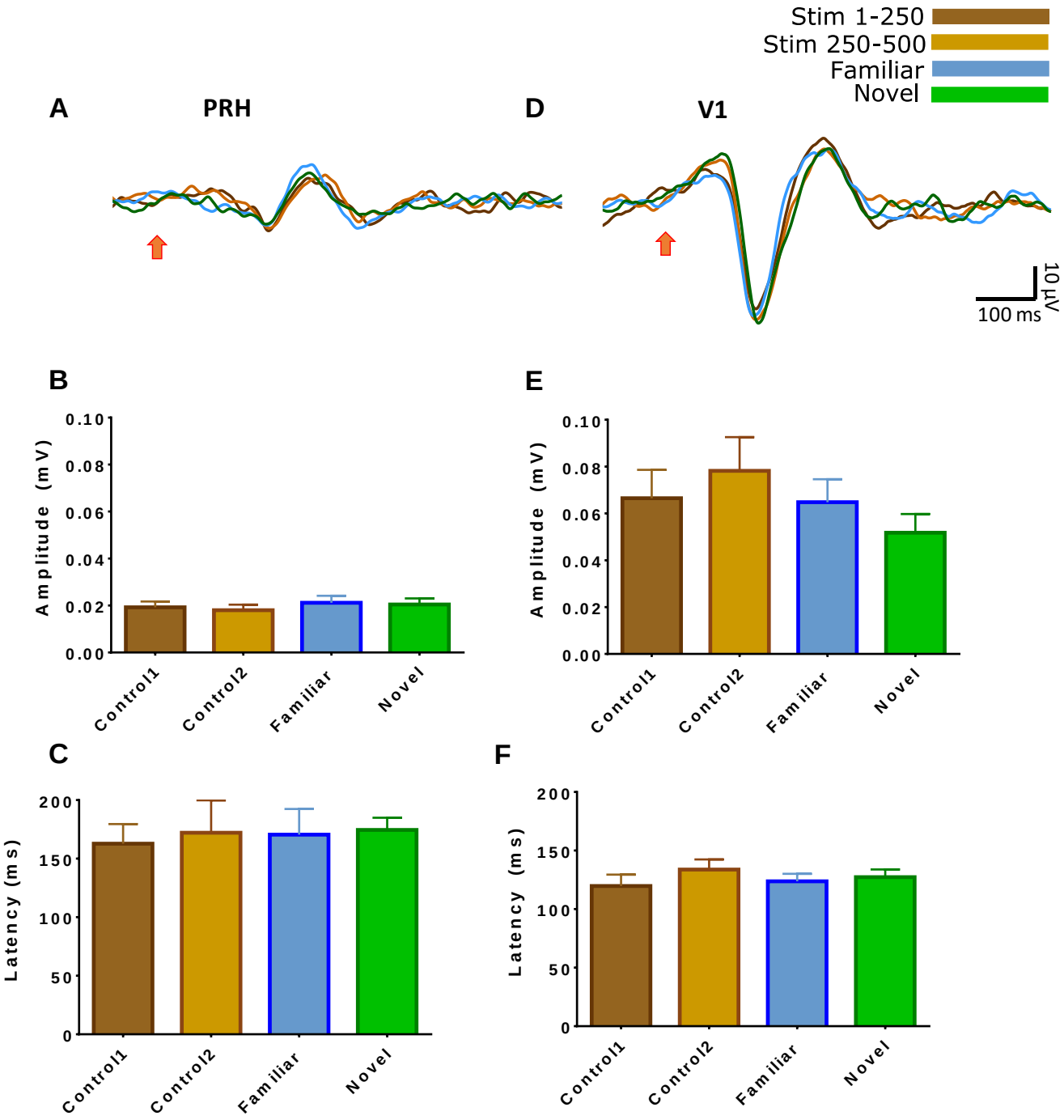

***Figure 3.8. Long-term response to object pictures**. **A,B,C** described the PRH response and **D,E,F** describe the V1 response **A)** average PRH group response **B)** Summary of mean PRH response amplitude. **C)** Summary of mean PRH response latency. **D)** average V1 group response **E)** Summary of mean V1 response amplitude. **F)** Summary of mean response latency. **Control 1(brown)** sums the response for the first 250 presentation within the first session. **Control 2(yellow)** sums the response for the last 250 presentations within the first session. **Familiar(blue)** sums the response for the 250 presentations of the familiar orientation in the second session. **Novel(green)** sums the response for 5X50 presentation of novel pictures in the second session. The red arrow in the traces marks the stimulus onset. Red arrow marks the stimulus onset.*

When using 5 novel objects on the second trial, a pattern similar to the one observed with the 2-minute retention interval emerged in V1. Thus, there was a difference in response amplitude according to novelty ($F(3,51) = 4.73$, $p<0.01$, $n = 18$;Figure 3.9D,E). Simple comparisons showed that the difference was between the second half of the presentations of the stimulus on the first trial (78.17 μV ± 14.39 μV), and the response to the novel stimuli on the second (52.59 μV ± 7.85 μV; $t(18) = 3.81$; $p<0.01$ $n=18$). No difference in latency was observed in V1 ($F(3,51) = 0.95$, $p=0.37$, $n=18$;**Figure 3.9**D,F). Similarly to the PRH response following a short-term retention interval, there was no familiarity-related difference with a long-term retention interval, neither in amplitude ($F(3,42) = 1.81$, $p=0.16$, , $n= 15$;**Figure 3.9**A,B), nor in latency ($F(3,42) = 0.66$, $p=0.53$, $n= 15$;**Figure 3.9**A,C).

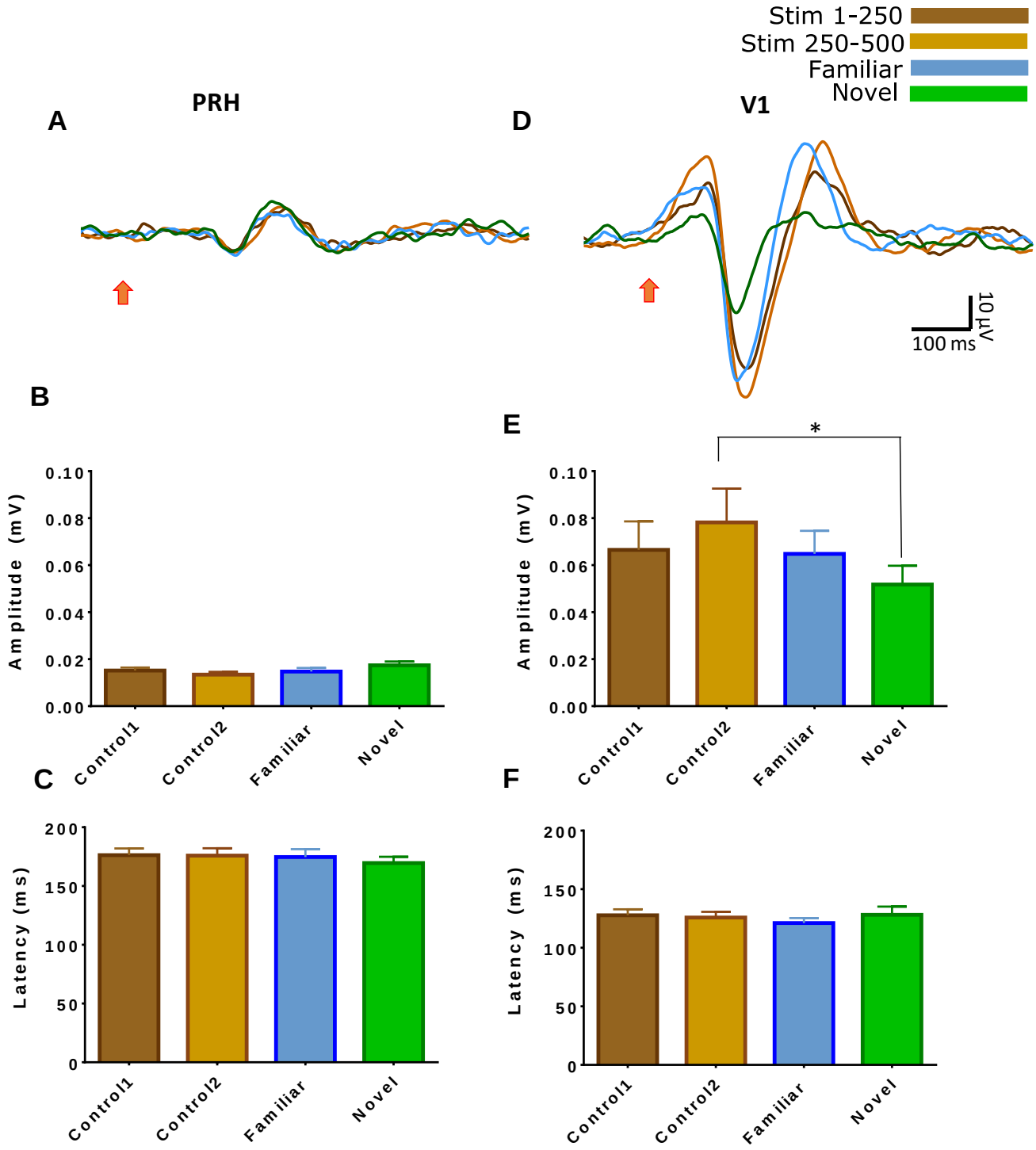

Figure 3.9. ***Long-term response to object-pictures (5 novel). A,B,C*** *described the PRH response and* ***D,E,F*** *describe the V1 response* ***A)*** *average PRH group response* ***B)*** *Summary of mean PRH response amplitude.* ***C)*** *Summary of mean PRH response latency.* ***D)*** *average V1 group response* ***E)*** *Summary of mean V1 response amplitude.* ***F)*** *Summary of mean response latency.* ***Control 1 (brown)*** *sums the response for the first 250 presentation within the first session.* ***Control 2 (yellow)*** *sums the response for the last 250 presentations within the first session.* ***Familiar (blue)*** *sums the response for the 250 presentations of the familiar orientation in the second session.* ***Novel(green)*** *sums the response for 5X50 presentation of novel pictures in the second session. The red arrow in the traces marks the stimulus onset. Red arrow marks the stimulus onset. Brackets represent a significant difference (*p<0.05)*

3.3.3 Movement

Since this task involved only passive viewings, the mouse's movement was analysed to determine if there was a locomotor reaction to novelty, as is the case in the freely-moving NOR task. An ANOVA with the same factors as described above but with movement as the dependent variable was conducted for all experiments. No novelty-related difference in movement was observed in the case of short-term memory for grating($F(3,51) = 0.244$, $p=0.87$, $n = 18$; **Figure 3.10**A), or in the case of short term ($F(3,48) = 1.2$, $p=0.32$; **Figure 3.10**C) and long-term term ($F(3,45) = 2.334$, $p=0.087$, $n=16$;**Figure 3.10**D) memory for pictures, when one novel picture was presented. No difference in movement was seen even when the novel phase involved 5 novel stimuli instead of one both for short($F(3,45) = 1.043$, $p=0.38$, $n = 16$; **Figure 3.10**E) and long ($F(3,51) = 1.33$, $p=0.28$, $n = 16$; **Figure 3.10**F) retention interval. The only case where the was a difference was the long term grating orientation memory ($F(3,75) = 6.191$, $p=0.0008$, $n =26$; **Figure 3.10**B). Multiple comparisons showed the difference was between the movement in the first 250 stimuli and both the novel ($t(75) = 3.237$, $p=0.009$) and familiar ($t(75) = 3.989$, $p=0.0009$) stimuli.

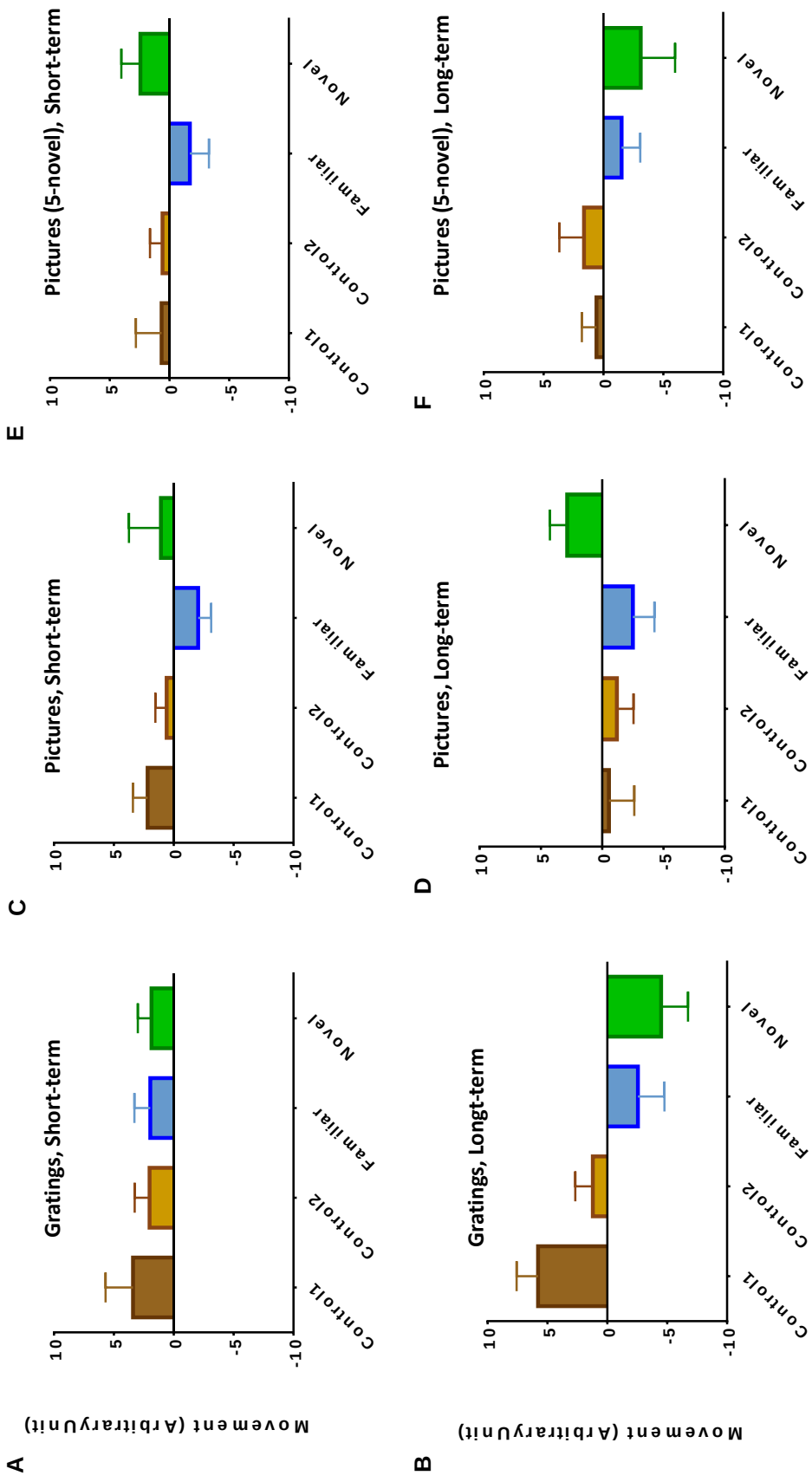

Figure 3.10. Average normalised movement. *The movement of the animals while the stimuls was presented for **A**) Gratings-Short term condition,, **B**) Gratings -Long-term condition, **C**) Pictures in the short-term conditon, **D**) Pictures in the long-term condition, **E**) Pictures with 5 novel stimuli in the short-term conditon and **F**) Pcitures with 5 novel stimuli in the long-trerm condition. Control1 and Control 2 are the first and last 250 preentations of the same stimuls, familiar is the Control stimulus after the retention interval and novel is a new stimulus or stimuli.*

3.4 Discussion

The experiments described in this chapter showed, for the first time, the presence of a visual evoked response in the mouse PRH. The response was similar in waveform to the intracranial response shown in humans[177,178]. The latency of the response was shorter than in humans. The human response latency is around 300 ms, whereas in our recordings the latency in the mouse was around 130 ms. However, this difference is to be expected considering the brain size, and this 0.43 ratio matches the 0.4 ratio in the HPC observed in auditory evoked potential studies[170]. The polarity was also different. The humans' polarity recorded in the electrodes was negative-going, while in the current case the polarity was positive-going. This difference in response polarity might be due to the different orientation of the PRH in rodents compared to humans[196]. Another factor that might have impacted the polarity is the reference electrode. In order to have a proper visual cortical control, the reference electrode was positioned in the prefrontal cortex, following from previous experiments recoding ERPs in V1[197], whereas in the human PRH recordings, the reference is usually the mastoid processes[177,178].

Despite the presence of a visually evoked response, no general difference in evoked potentials was found in the PRH in this set of experiments. This is contrary to what has been observed in humans, at least in the case of verbal[177–179] or pictorial stimuli[172,187]. In these experiments, epileptic patients were presented with a series of stimuli and then at a later session they were presented again with stimuli, a proportion of which had been encountered before. One crucial difference between the previous human experiments and this chapter's mouse experiments is that in the human experiments, the trials were compared only for correctly recognised 'new' or 'old' items, whereas in the current experiments only the familiarity-valence of the stimuli was being compared, without any independent evidence for the detection of novelty or familiarity by the animal. Thus, it might be that the inability to know when the mouse truly recognised a stimulus as familiar affected the results.

These experiments looked at passive recognition, that is, whether the 'novelty' value of a visual stimulus changed the ERP, regardless of the behavioural outcome. Since the animals' movement does not correlate with familiarity, it cannot be taken as measure of recognition. However, the unit-recording

experiments in rats used the passive-viewing paradigm, which also does not involve a response, and yet single cells that changed their response to familiarity could be observed, despite the task involving only passive viewing[55,111]. Since there was an evoked-response for complex-object stimuli, future experiments can use the same paradigm, while training the animals to respond differently to novel and familiar stimuli. These experiments might reveal a difference in the evoked responses similar to the human studies.

Animals, including mice, doing the NOR task in freely moving settings explore a novel object more than a familiar one, thus presenting a behavioural indication of familiarity/novelty detection[198]. However, during this task the animals receive tactile/olfactory input on top of the visual input to the PRH. The combination of visual tactile and olfactory stimuli might then, lead to a difference in the evoked response. Thus, it might be that a visual input alone in the mouse is not sufficient to evoke a familiarity/novelty response.

It might be the case that for a familiarity-related modulation of the ERP in the PRH, a certain threshold must be reached. In the human studies, it might be the case that a familiarity response is presented because only these trials, where the threshold was crossed are taken into account. It might be, that in the current experiments, not many trials passed the threshold, because of the lack of tactile sensory experience, and thus on average no response could be seen. That could explain the discrepancy between the lack of modulation in the ERP, with the presence of familiarity encoding cells described in previous experiments.

What can these results mean? First of all, to the best of my knowledge, it is the first time an ERP in higher order area has been shown in mice. Interestingly, whereas the PRH showed visual ERPs for all stimuli, the vHPC showed none. This might suggest that the PRH is the end of the visual stream, at least in the mouse, whereby it is the last area that responds synchronously to visual stimuli. Secondly, as discussed, the failure to find a modulation in this visual response means that there is no simple relation between the novelty/familiarity properties of a visual stimulus and PRH activity. This seems to agree with the view seeing the PRH as an area responsible for object identification[62,199,200]. Although these results cannot corroborate or disprove the idea, at the very least they show that

both simple and, more importantly, complex stimuli evoke a response in this area, and thus can be employed is process of discrimination. Future studies might use the same experimental method to test how object features affect the ERP in the PRH to further test whether object features have a direct effect on the PRH's ERP and thus test the role of the PRH in object identification.

As suggested, modulation of the evoked response in the PRH may depend on having more than a simple visual input. It may also reflect the requirement for active attention to be paid to cues. Thus, future experiments in which the animal can behaviourally demonstrate recognition would be helpful, as a behavioural response to novelty would confirm stimulus detection and subsequent processing. The results from the present study, nevertheless, suggests that the evoked response in PRH does not simply habituate in response to a previously encountered stimulus, i.e. PRH activity may be necessary but is not sufficient for a familiarity or novelty signal to propagate.

Recordings from V1 in these experiments were done mainly as a control, verifying that indeed at least a basic visual response could be recorded. Previous research found that the ERP in layer 4 in V1 increases in response to repeated presentations of a grating stimulus[193,194], a surprising results considering that single neurons habituate with repeated presentations. This results was not observed in the current experiments for stationary gratings. The absence of an increase in responses might be due to slight differences in procedure. In the previous experiments[193,194], the researchers used a drifting stimulus, while the mouse was still, whereas in the current experiments the mouse could move on a wheel and the stimuli were stationary. Since the mice were largely inactive in the current experiments, the most likely reason for the difference between studies is the movement of the stimulus. However, further experiments are required to evaluate whether stimulus movement alone or in combination with novelty is sufficient to modulate the evoked response in the V1.

Another important addition to the literature from the present set of experiments was that using complex images produced a similar visual ERP in V1 compared to simple gratings. This might be exploited further for more experiments on the visual system. An interesting observation was that when 5 novel pictures were

used instead of 1 in the second session, an increase in the response to the familiar stimulus could be observed compared to the novel picture stimuli. When a 2-minute retention interval was used between sessions, the response to the second 250 presentations of a familiar picture, and to another 250 presentations of the same picture on the second session was bigger than the response to the novel stimuli. A similar response was observed when a 24 hours delay was used between sessions. However, the response to the familiar stimulus was not significantly larger relative to the novel stimuli, probably because of decrease in modulation with time.

Drifting gratings evoke a familiarity-related change in the V1 response[193,194]. Also, in some cases complex images of objects produce a similar modulation in response in V1, as seen in the current experiments. However, stationary gratings do not evoke any familiarity-related change in ERP. Taken together it might mean that there a certain level of saliency, achieved by either movement or image complexity, that produces modulation in the ERP recorded from V1. Although not the main topic of the current research, it is an interesting idea that can be pursued further in future research into how stimulus saliency affects modulation of the ERPs observed in V1. However, the most crucial point of this results is that even when familiarity-related changes could be observed in V1, none could be seen in the PRH.

The experiments described in this chapter looked at gross synchronous activity in the PRH as recorded by ERP. This method might overlook asynchronous activity and the response of single isolated cells rather than larger populations. Furhter, the longer latencies even in V1 suggest that all the quicker single cell response is missed. Thus, the next step would be to use the same method and see how single cells respond to visual input and familiarity in the PRH with a single cell resolution. This will enable to more fully understand what happens in the PRH when a visual stimulus is presented, and to find out whether or not familiarity response occurs in that area in the mouse.

Chapter 4 Unit responses in the PRH and HPC

4.1 Introduction

Previous research in primates showed that the PRH contains cells that change their response according to stimulus familiarity[103,109,114]. This finding was later replicated in rats using the paired-viewing paradigm, in which rats passivelyviewed an object presented to them while stationary[55,111]. However, more recent findings examining the neuronal activity of rats exploring objects in an arena failed to find any familiarity-induced modulation of neuronal response in the PRH, despite the recorded neurons changing their firing-rate in response to the animal exploring objects[113], thus showing sensitivity to object exploration, but not to the object's novelty-valence.

A recent paper tried to resolve the controversy by using the same recording method of tetrodes in rats, while doing both the paired-viewing paradigm and freely exploring objects[201]. Indeed, in this experiment, whereas familiarity-modulating neurons could be found in the paired-viewing paradigm, none were detected in the freely moving condition. This paper although using the same recording technique still has two completely different paradigms. That makes it hard to draw any general conclusion, especially since both paradigms produced completely different results.

In the following experiments, this problem was addressed by using just one experimental procedure that combines features of both the paired-viewing paradigm and the NOR task. This procedure is the one described in the previous chapter. In this procedure the mouse, although not freely moving, can still run on a foam wheel. Also, like in the NOR task a limited number of items is presented for the mouse the recognise. However, like in the paired-viewing paradigm, the stimulus delivery time and properties can be tightly controlled. Thus, using this paradigm, an attempt is made to find whether any neuronal response in the PRH is related to the familiarity-detection presented in the NOR task.

In the present study, no visually evoked response in the PRH could be observed, despite the presence of ERPs in the area. Evoked responses record synchronous change in large neuronal populations. Thus, it is possible that no ERP could be observed, despite the existence of a response at the single-cell level, albeit a

non-synchronous one. Previous research in monkeys showed that while familiarity neurons can be observed in the PRH, none could be observed in the HPC[103,109], making the HPC a useful control. Thus, in this chapter single units were recorded in the PRH and the HPC, while using the same visual procedures used to record visually-evoked potentials. Intriguingly, while visually responsive neurons were observed in both areas, no neurons changed their response as a function of familiarity.

4.2 Methods

All surgery, post-surgery, care and recording methods are described in chapter 2.

4.2.1 Visual procedure

The same as in section 3.2.1-3.2.3

4.2.2 Spike Sorting

Spike sorting was done using the pre-written KlustaSuite[202]. In trials that spanned several recordings, in order to keep the spike sorting procedure consistent, the recordings were merged, and the spike sorting was run on one merged recording. After filtering the signal using a 500 Hz high-pass Butterworth filter, spikes were detected using a strong (θ_s) and weak thresholds (θ_w), Their values were: θ_s=4 s.ds and θ_w=2 s.ds of mean signal amplitude. Spikes were defined as points in the filtered signal crossing θ_w for all of the putative spike's duration, and crossing θ_s for at least one point in the spike (**Figure 4.1**A1 and A2 for examples of detected spikes). Then, the putative spikes were aligned, and 3 principle components of their features were extracted using principle component analysis (PCA). Then using a EM clustering algorithm[202], the PCA components and the temporal and spatial data of the spikes were used to cluster the spikes automatically. After automatic clustering, the spikes were re-clustered manually, if needed. This was done, first, by examining the spike shapes and channels on which they appeared (**Figure 4.1**B). The auto-correlogram of all putative clusters were inspected and only those with a clear refractory period were chosen for further analysis (**Figure 4.1**C), and the rest were treated as 'multi-unit' activity. Then, the cross-correlogram of all chosen cluster were inspected further, to see whether the show a refractory period suggesting that they are the same cluster. These suspected clusters were analysed further and if the spike shapes were similar and were shown on the same channels and their PCA features overlapped, they were merged to a single cluster[203]. Only spikes that had clearly distinct feature were defined as separate spikes (**Figure 4.1**D).

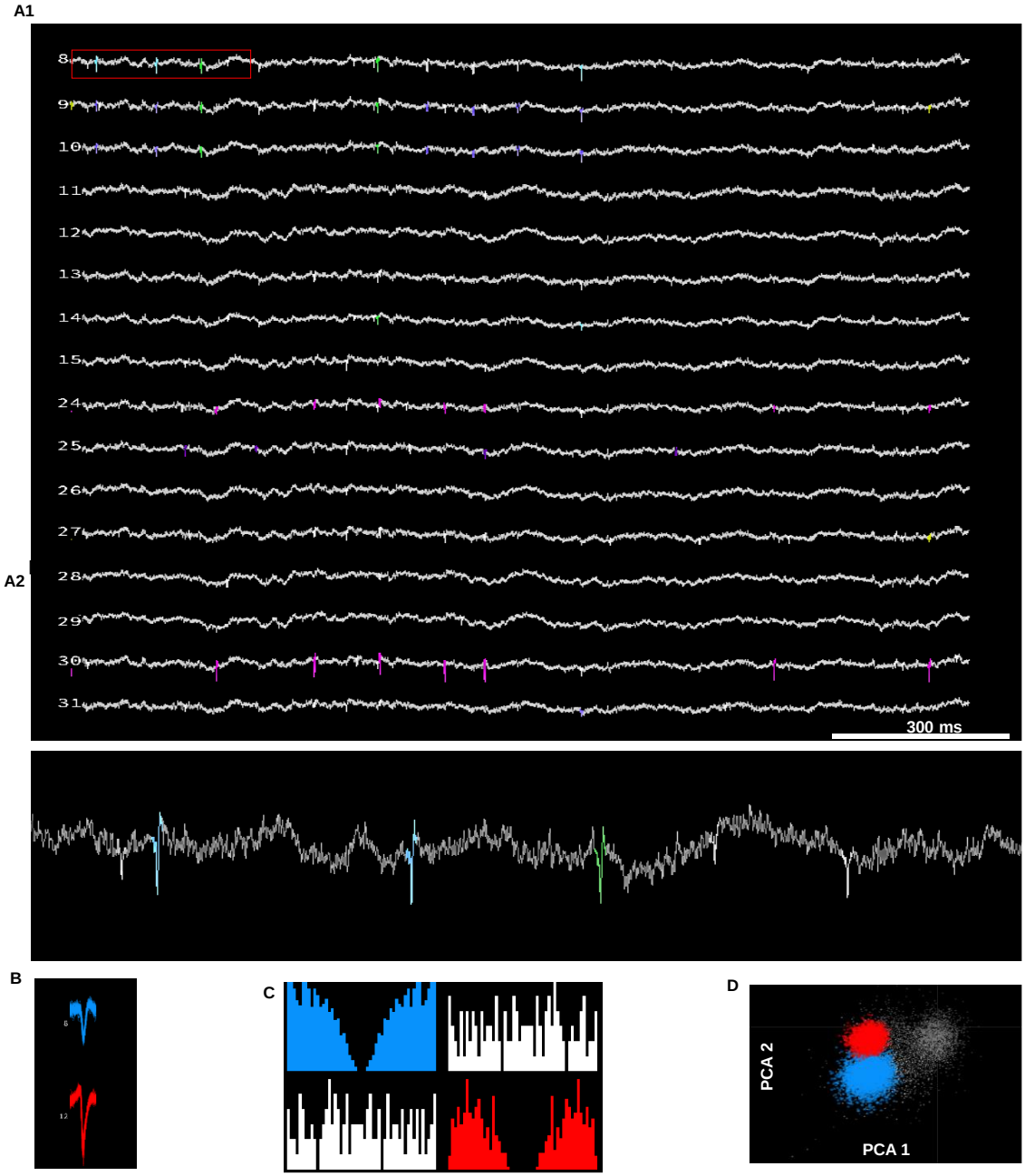

***Figure 4.1**. **Spike sorting raw data. A1**) 1500 ms recording from 32 channels with detected spikes coloured in. The red box marks the 300 ms snippet shown in A2 **A2)** A 300 ms trace with detected spikes. **B**) two different spikes from two distinct clusters. **C**) The cross and auto-correlograms of the two clusters. A clear refractory period could be observed as well as no correlation in the cross-correlogram. **D)** The spikes are clearly distinct on the PCA graph.*

4.2.3 Responsiveness detection

A peri-stimulus histogram (PSTH) was derived for each of the isolated units, using 250 ms. Then, using a 2-way ANOVA of time and novelty factors, the average firing rate in the 1 second periods preceding and following the stimulus presentation were compared over the series of the first (Control1) and second (Control 2) 250 presentations of a stimulus in the first trial, and the 250 presentations of the same stimulus (Familiar) and 250 presentations of the novel stimulus (Novel). If there was a significant difference in firing rate while the stimulus was on, or if there was a significant interaction between stimulus and time bin, the neuron was classified as visually responsive. The visually responsive neurons were further analysed by looking at the individual time bins compared to the pre-stimulus time bin to determine the first bin to respond to the stimulus, in that case, too, a 2-way ANOVA performed to determine whether there is an interaction in the single time-bin level. In all the visually-responsive cases, the response itself was further classified as increasing or decreasing. In all cases significance was defined as $p<0.05$.

4.3 Results

The neuronal activity of single units was recorded over all the trials described in chapter 3. Overall, 218 units in the PRH and 97 units in the HPC were isolated in successfully in all the different trials (6 trials) for both the short-term (**Figure 4.2**A) and long-term retention intervals (**Figure 4.2**B) (n=2 animals for each area).

Then, the visual-responsiveness of these isolated units was addressed. On average 19.17%±2.651% (Median of 22%) responsive neurons were observed in the PRH, and 19.17%±5.375% (Median 23%) responsive neurons were observed in the HPC, revealing no difference in the responsiveness of units in these 2 areas (see **Figure 4.2**A,B;n = 6 session).

Averaged across all sessions, 68%±14.75% of the responsive neurons in the PRH increased their firing rate after stimulus onset (visually-excitable neurons; VE) (**Figure 4.3**A1 for a trace sample, **Figure 4.4**A1 for a corresponding PSTH), while the rest decreased it (visually-inhibited neurons; VI) (**Figure 4.3**B1 for a trace sample, **Figure 4.4**B1 for a corresponding PSTH). In the HPC, 72.60%±8.1% of the neurons were VE (**Figure 4.3**A2 for a trace sample, **Figure 4.4**A2 for a corresponding PSTH), while the rest were VI (**Figure 4.3**B2 for a trace sample, **Figure 4.4**B2 for a corresponding PSTH). In both areas, neurons that exhibited no change in activity with stimulus onset were classified as non-responsive (**Figure 4.3**C1,C2 for a trace sample, **Figure 4.4**C1,C2 for a corresponding PSTH)

An ANOVA with the recording site and response-type as factors revealed no difference between the areas in terms of the neuronal response type ($F(1,18) = 0.1142$, $p=0.74$, n=6 sessions). However, it confirmed that the were more neurons that responded by an increase in activity, rather than with a decrease over both areas ($F(1,18) = 10.51$, $p<0.01$, n=6 sessions). None of the observed neurons changed their response with stimulus familiarity (For a complete breakdown of the neuronal responses see **Figure 4.5**)

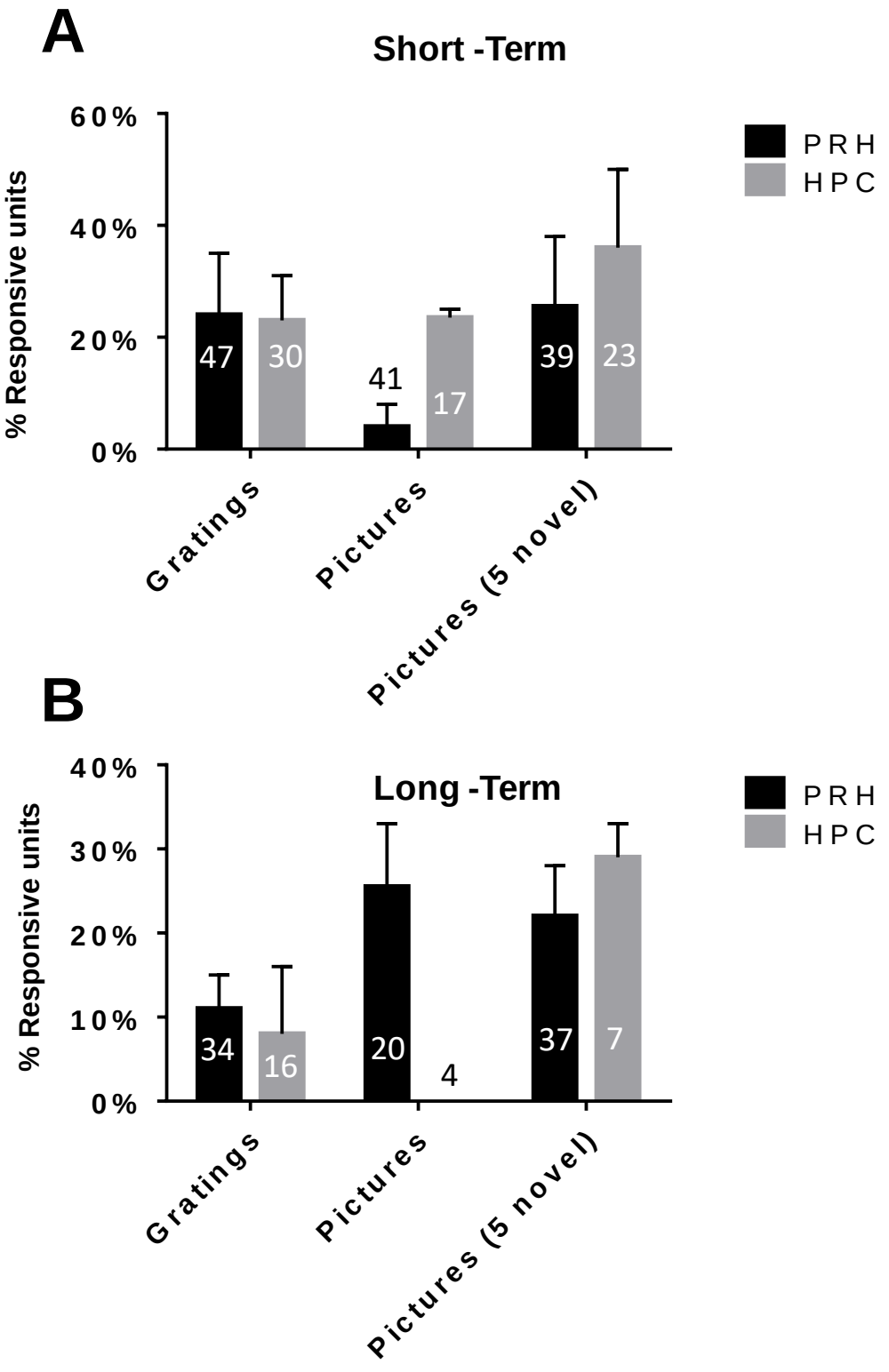

Figure 4.2 Visual responsiveness percentages. A) *The number of responsive neurons in the short-term condition.* ***B)*** *The number of responsive neurons in the long-term condition. The number inside the bar is the number of isolated units, while the bar shows the percentage units defined as visually responsive. The rest of the neurons were classified as non-responsive. Data exressed as mean ± SEM.*

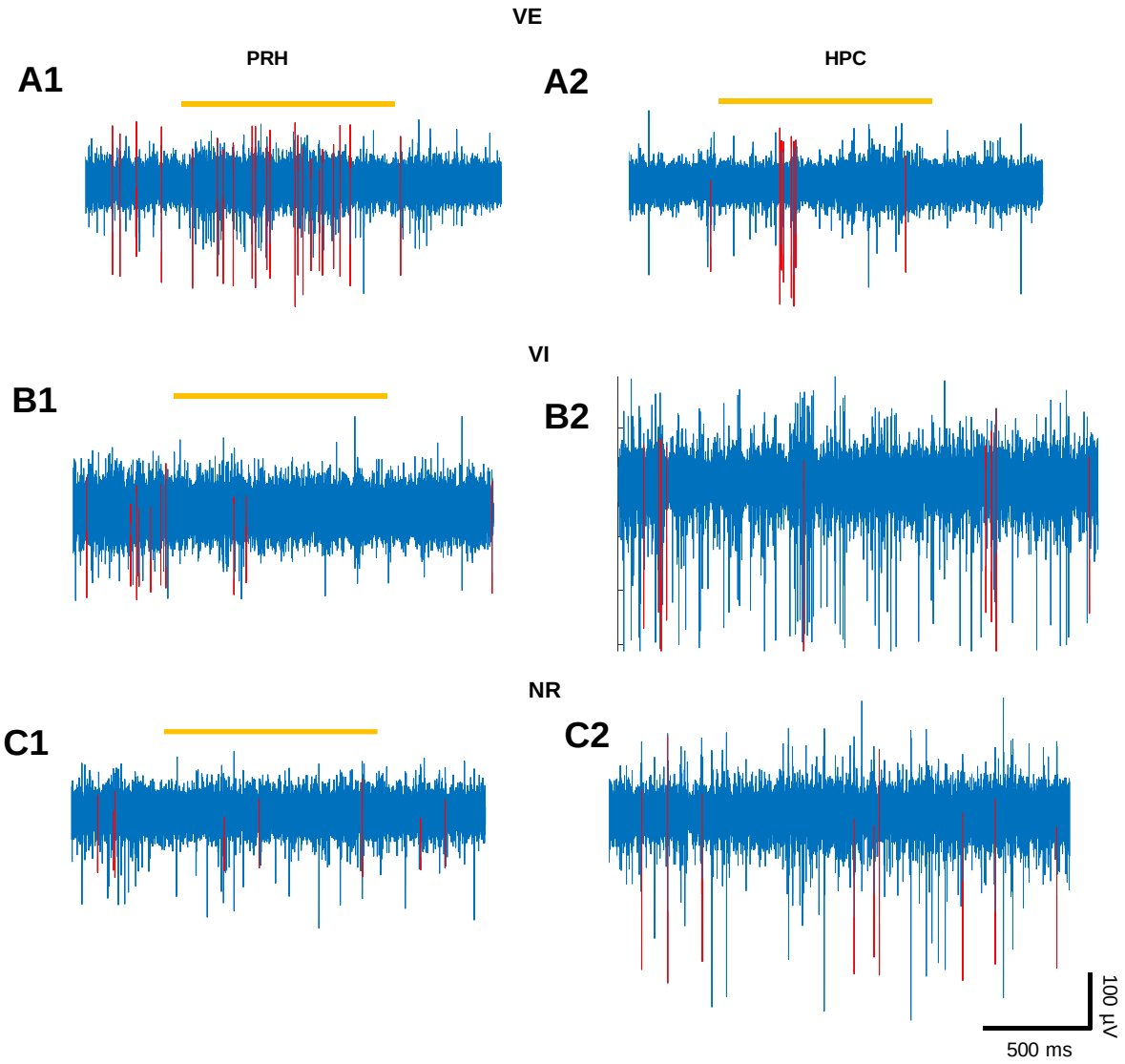

Figure 4.3. Trace examples for all identified response types. A1,A2) *Traces from a single trial of a visually-excited neuron in the PRH and the HPC.* ***B1,B2)*** *Traces from a single trial of a visually-inhibited neuron in the PRH and HPC.* ***C1,C2)*** *Traces from a single trial of a non-responsive neuron in the PRH and HPC. The yellow line marks the stimulus presentation. Red spikes are spikes belonging to an isolated unit.*

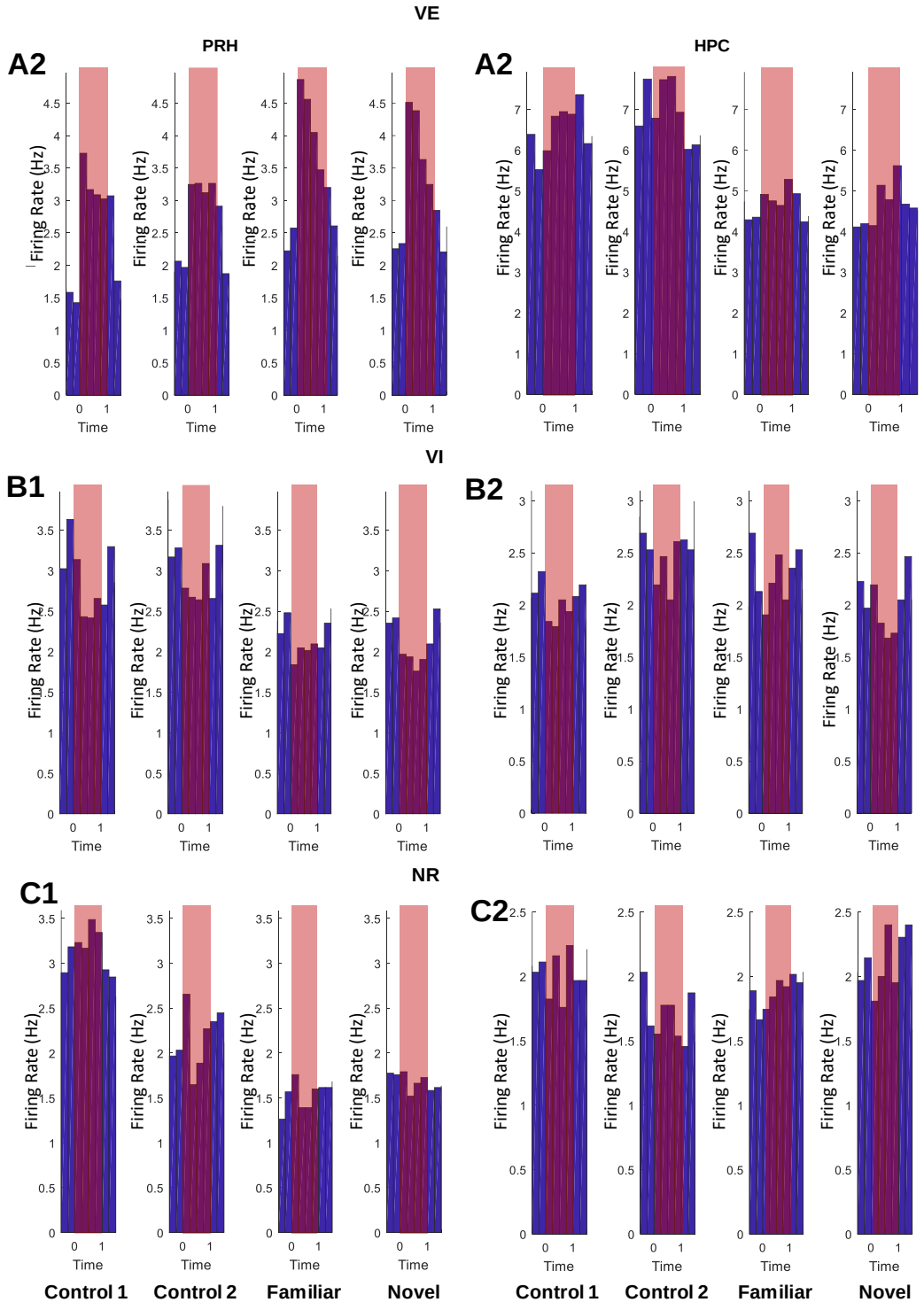

Figure 4.4. Example PSTH diagrams of neuronal response types. *Classification of neurons to non-responsive, responsive-increasing and responsive-decreasing in the PRH and HPC. (continued)*

A) *examples of VE neurons in the PRH (ANOVA bin: F(4,3984)=11.60, p<0.001, interaction: F(9,3984) = 0.21, p=0.89) on the left, and the HPC (ANOVA bin: F(4,3984)=32.82, p<0.001, interaction: F(9,3984) = 0.60, p=0.79), on the right, that increased their firing-rate with stimulus onset.* ***B)*** *examples of VI neurons in the PRH (ANOVA bin: F(4,3984)=32.82, p<0.001, interaction: F(9,3984) = 0.60, p=0.79), on the left, and the HPC (ANOVA bin: F(4,3984)=3.86, p<0.05, interaction: F(9,3984) = 0.07, p=0.97), on the right that decreased their firing-rate with stimulus onset.* ***C)*** *examples of NR neurons in the PRH (ANOVA bin: F(4,3984)=1.02, p=0.31, interaction: F(9,3984) = 0.84, p=0.47), on the left, and the HPC (ANOVA bin: F(4,3984)=0.48, p=0.48, interaction: F(9,3984) = 0.36, p=0.78), on the right that did not change their activity with stimulus onset. In the PSTH each bin is the average frequency over a 250 ms time window averaged over 250 trials. Red shadow marks the stimulus presntation.*

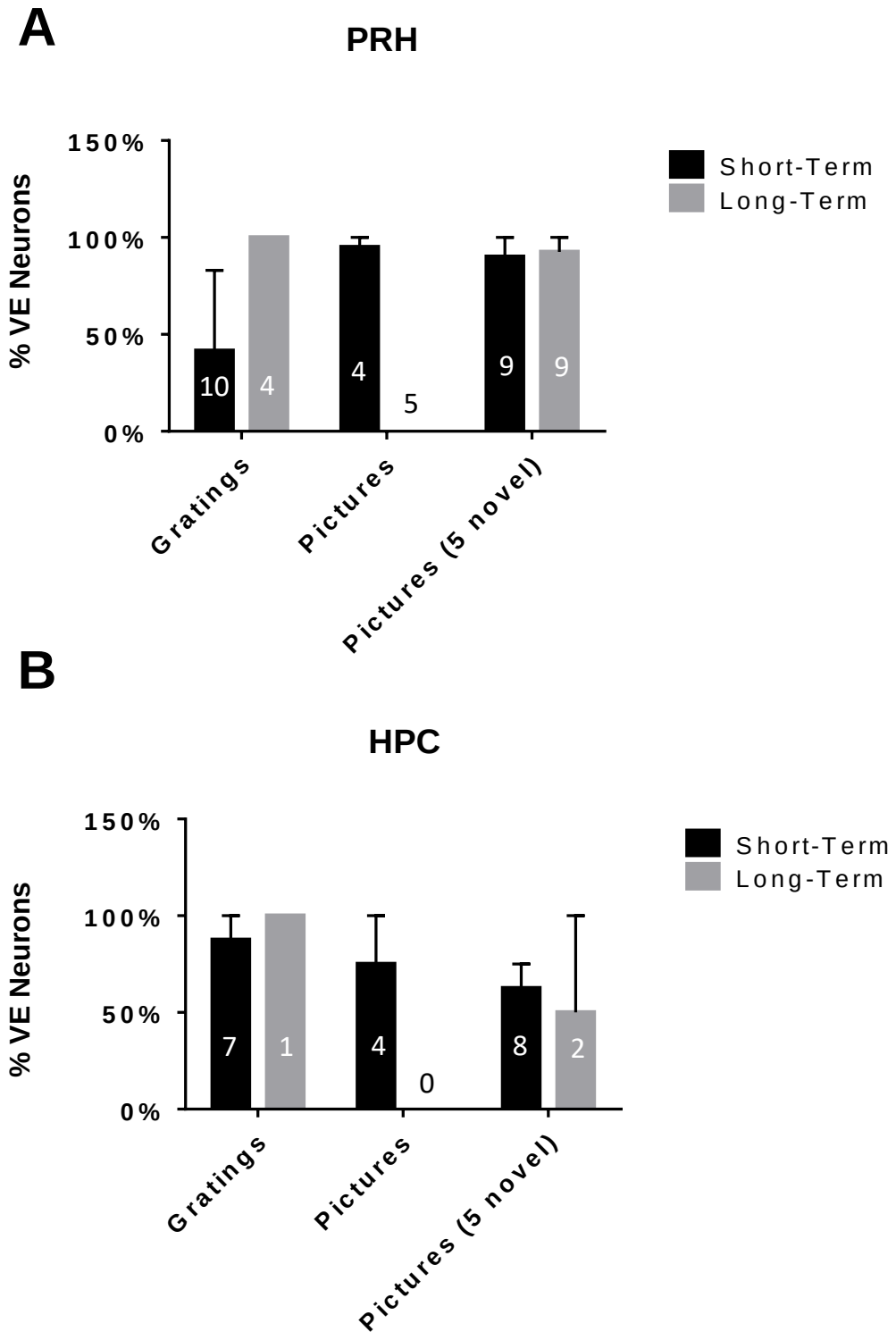

Figure 4.5. VE neuron percentages. A) *The response type breakdown in the short-term and long-term conditions in the PRH.* ***B)*** *The response type breakdown in the short-term and long-term conditions in the HPC. The total number of responsive neurons in each condition is presented on the bars, while the bars show the percentage of VE neurons out of this total. The rest were VI neurons. Data are presented as mean ± SEM.*

Still, the question remains of whether neurons with different response types (i.e. NR, VE or VI) differs from each other within the areas and between them. The first question was whether the baseline activity before the presentation of the stimulus determined the type of neuronal response that followed. it could well be that the VE baseline activity is lower and thus it tends to be excited, whereas VI baseline activity is higher and then it tends to be inhibited by visual stimuli. To answer this question, an ANOVA was run on the baseline activity in both areas. However, no significant difference was detected between the different response types in both the PRH ($F(2,204) = 1.142$, $p=0.32$, N = NR: 170, VE:26 VI:11; **Figure 4.6**A) and the HPC ($F(2,88) = 0.56$, $p=0.57$, N = NR: 71, VE:14 VI:6; **Figure 4.6**B).

Then, the latency and magnitude of the response of both VE and VI neurons in both the PRH and HPC were examined. Looking at the response latency, an ANOVA with the area and response type revealed an interaction between the area and the latency ($F(1,55) = 36.80$, $P<0.001$; PRH: VE: N = 27, VI: N = 12, HPC: VE: N = 14, VI: N = 6 ; **Figure 4.7**A). Interestingly, in the PRH the latency of the VE neurons was shorter than the VI neurons ($t(55) = 3.375$, $p<0.01$). However, in the HPC the reverse was true with the VI neurons preceding the VE response ($t(55) = 5.044$, $p<0.001$). Between the areas, the peak response of the PRH VE neurons preceded the HPC's VE neurons' peak response ($t(55) = 2.061$, $p<0.05$), while the HPC's VI neurons' peak-response preceded the PRH's VI neurons' response ($t(55) = 5.906$, $p<0.001$). Looking at the magnitude of the change in frequency of the peak-response, did not reveal a similar interaction ($F(1,55) = 0.1$, $p=0.75$; **Figure 4.7**B).

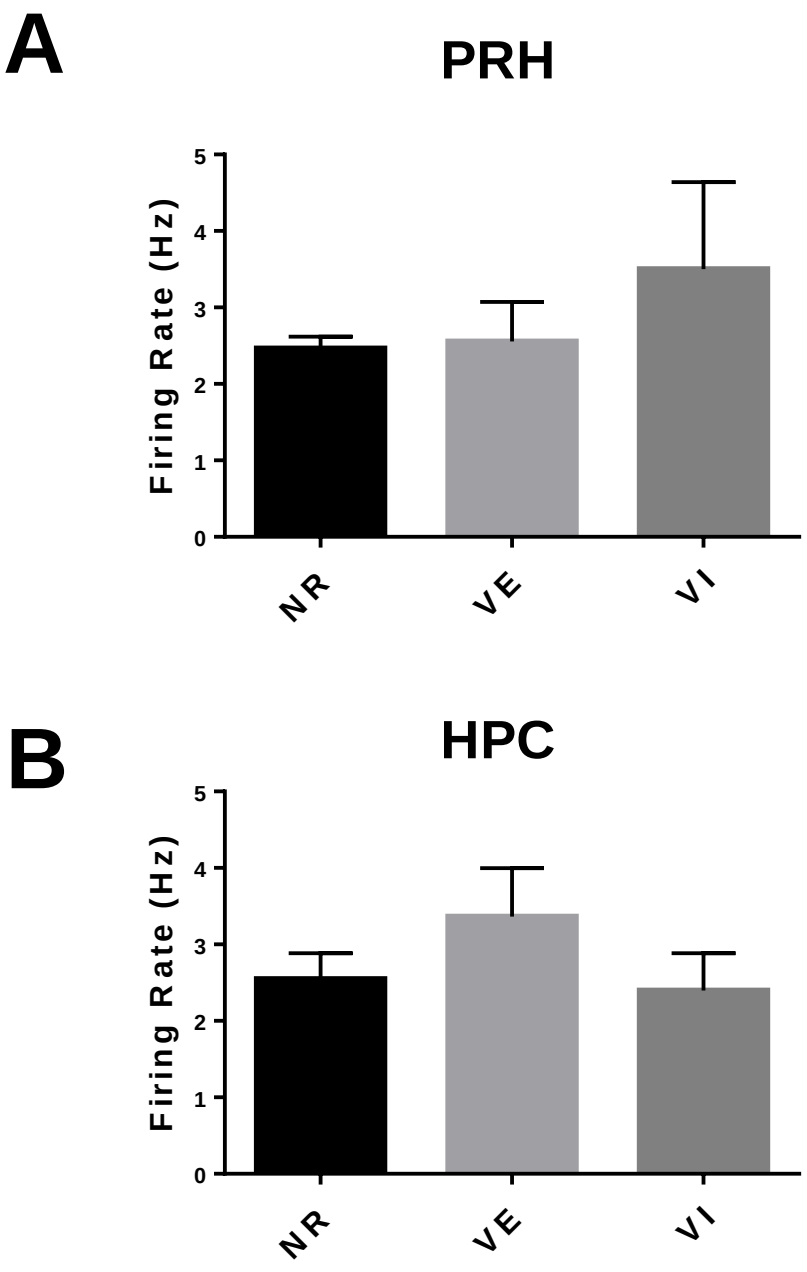

Figure 4.6. Baseline activity of neuronal types. A) The baseline firing rate of NR, VE and VI neurons in the PRH. ***B)*** The baseline firing rate of NR, VE and VI neurons in the HPC. NR = Non-responsive neuron, VE = visually excited neuron, VI = visually inhibited neuron. Data are expressed as mean ± SEM/

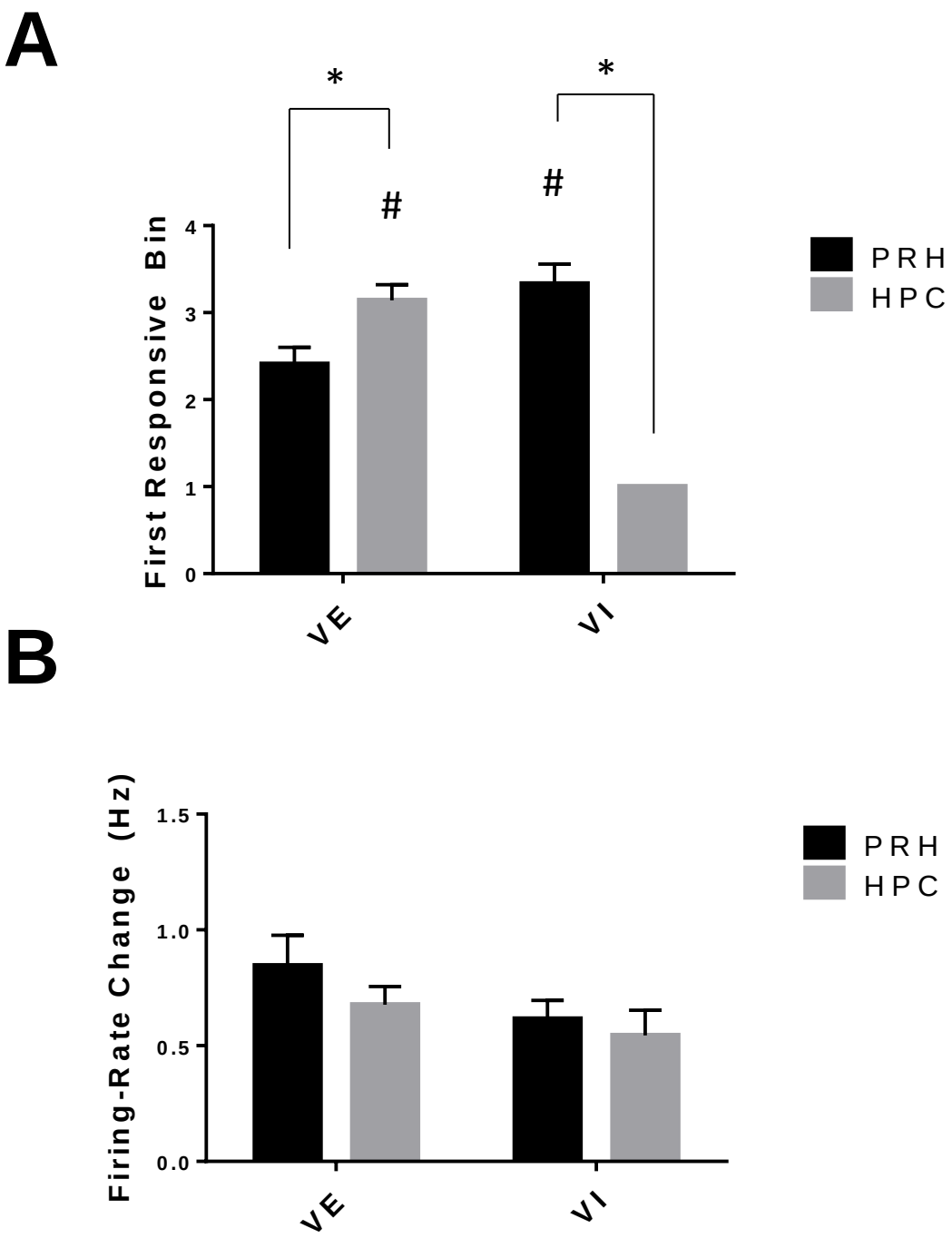

Figure 4.7. Peak response properties. A) *The peak response latency of increasing and decreasing neurons in the PRH and HPC.* ***B)*** *The change in firing-rate between the peak-response and base-line for increasing and decreasing neurons. # marks a significant difference between neurons that increase and neurons that decrease their activity within an area ($p<0.05$). * marks a difference between areas within a neuronal response sub-type ($p<0.05$). NR = Non-responsive neuron, VE = visually excited neuron, VI = visually inhibited neuron.*

4.4 Discussion

In these experiments, single units were isolated from the PRH and HPC while animals were doing the same visual tasks described in the previous chapter. Even though visually-responsive units could be identified in both areas, none of the isolated units changed its visual response with novelty.

Visually responsive neurons could be observed in both the PRH and the HPC. In the PRH, the number of responsive neurons was lower than the number reported for rats. In the current experiments, on average 19% of the neurons were found to be responsive whereas in the rest of literature the number of visually responsive neurons was slightly higher with 26% (anaesthetised)[55], 25%[201] and even 51%[111] visually responsive neurons reported in previous trials. In the HPC the 19% visually responsive neurons lies in the middle between the other values reported in rats of 14% (anaesthetised)[55] and 31%[111]. A reason for the discrepancy might be to do with the experimental methods. In the previous experiments, the neurons are recorded on a single trial in which the neurons are determined to be responsive or not, whereas the current experiments employed a stricter condition, in which only neurons whose signal remained stable across the two sessions (either after 2 min or 24 hrs) were considered for further analysis. Another factor is species differences between the rat and mouse. For instance, many more visually responsive neurons could be found in monkeys than in rats, with around 55% responsive neurons in the PRH reported previosuly[54,103,104,109,114], with a more mixed picture in the HPC with as low as 14%[109] visually responsive neurons[109] to about 52%[104]. Yet, since rats and mice are more similar to each other than primates and rodents, the most likely explanation is method difference. Nevertheless, despite the presence of visually responsive neurons, no familiarity neurons could be found.

There is a disagreement in the literature as to the presence of familiarity neurons in the PRH. As mentioned previously, older papers reported finding a population of familiarity sensitive neurons in the PRH in both monkeys[54,103,104,109,114] and rats[55,111]. However, more recent studies failed to find any familiarity-modulating neurons in the PRH of rats freely exploring an arena with familiar and novel objects[113], despite the presence of neurons that changed their activity in the presence of an

object[112,113]. A recent study, from the same group that originally identified the 'familiarity neurons' in the rat PRH, showed that although familiarity neurons can be found in the paired-viewing paradigm in rats, none can be found while they perform a freely-moving NOR task[201]. The methodology used in this chapter lies somewhere in-between the completely freely moving tasks and the paired-viewing procedure. In the experiments performed in this chapter the mouse is head-restrained, yet it can run on a wheel. Also, like the paired-viewing, the stimuli are presented directly in front of the mouse's eye, which enables control on when the stimuli are viewed, unlike the completely-freely-moving task.

An important, and maybe crucial difference, between this task and the previous studies, is the complete lack of reward. Unlike the experiments reported here, in the vast majority of the previous experiments the animals received a reward if not for the task itself, for attending the objects[54,103,104,109,114]. The only exception was an anaesthetised experiment in the rat[55]. Yet it is not known if the animals were exposed to reward for attending the stimuli before the anaesthetised experiment. In the more recent freely-moving NOR experiments, the animals also received a reward, but for running around a circular arena, rather than for looking at or exploring the objects[112,113]. The presence of reward that is directly associated with the objects, might cause the activation of the reward pathway[204] and release dopamine (DA) into the PRH. Indeed, the rat PRH shows a moderate DAergic innervation in all layers[205]. Their functionality can be demonstrated by the fact that cocaine and d-amphetamine in the rat, induce the secretion of DA in the PRH[206]. Crucially, after infusion of the D1-receptor antagonist SCH23390 to the PRH, rats did not differentiate between novel and familiar object in a long-term NOR tasks, while an infusion of the D1 agonist SKF38393 improved the recognition index in the same task, compared to vehicle infused rats[207]. Thus, the release of DA, activated by reward or reward seeking, might, at least partially, explain the difference in results.

Another important difference between the current experiments and others, is that like in the recent NOR tasks[112,113], the animals encountered the 'familiar' objects first in the session before the retention interval. However, in the previously reported experiments in both monkeys and rats, the 'familiar' stimuli had been encountered by the animals for many days before the recordings[54,55,103,104,109,111,114].

This leads to the possibility that the effects are not due to a wider system-wide habituation to the stimuli, the fact that most reported units decreased their response (**Table 1.2**) might support this notion. Also, habituation to a stimulus causes changes in the response even in V1[193,197], which is at the very start of the visual stream. Not much is known about how such changes affect other areas downstream of this region. But one cannot dismiss the idea that a long-term repetitive exposure to objects or visual stimuli will cause changes in V1, and further affect neuronal populations further downstream from it. One of these areas, that might be affected by such repetitive exposure is indeed the PRH. Thus, the possibility that the familiarity effects previously observed in the PRH are a result of changes at the beginning of the visual stream (e.g. V1) should be addressed in future experiments.

4.4.1 Communication between the HPC and PRH

When examining the responses to objects in both the PRH and the HPC, the majority of the responsive units responded by increasing their activity, while a lesser number of neurons responded by decreasing their activity level. When the latency of their response was examined it was found that the response of the VE neurons in the PRH preceded those of the HPC, while the reverse was true in the case of the VI neurons. These might suggest that neuronal sub-population are visually activated by two different streams. One stream, would be from visual areas, through the PRH and into the HPC. The most likely pathway for the second stream would be through the MEC. This area, like the LEC receives visual input through the PHC[85]. However, unlike the LEC, the MEC also receives quite an extensive direct visual input from the occipital lobe[85]. Since the response of the activity-decreasing neurons was much quicker, it would suggest that these neurons are of the second putative stream, while the activity-increasing neurons are of the first. Further anatomical evidence for the relation of the activity-decreasing neurons to the MEC stream is that the proximal area of CA1, from which the recordings were taken receives most of its entorhinal cortex input from the MEC[208].

The MEC exhibits spatial coding[209], and this is believed to contribute to contextual encoding of events, rather than item coding that is believed to happen in the LEC and PRH[210]. Thus, it might be the case that the interplay between the two

neuronal populations is what encodes a complete engram of context as encoded by the VI neurons and item as encoded by the VE neurons.

4.4.2 The role of the PRH

In light of the results presented here and previous research, the question still remains of what the PRH actually does. First of all, there is a population of neurons in the PRH that responds to visual stimuli. This would mean that visual processing does occur in this area. More recent views on PRH function, see it as responsible for visual discrimination alongside object recognition[211,212]. Indeed another complementary approach does away with the modular view of the PRH and looks at it as the apex of the visual stream[200]. According to this view, the more rostral the area in the visual stream the more complex the stimuli being processed there, in terms of conjunction of properties. Thus, according to this model, the PRH encodes the most complex stimuli in the stream. Admittedly, the experiments reported here cannot conclusively verify this view. But they do join the other experiments that show no familiarity response, under non-rewarded conditions, while showing at least some level of processing of visual information in the area that, seems to be, then, further transmitted to the HPC.

Chapter 5 Auditory responses in a putative animal model of mental illness

5.1 Introduction

SZ and autism spectrum disorders (ASD) are highly debilitating diseases, with SZ affecting around 24 million people worldwide and about one child in 160 affected by ASD (World Health Organisation). The symptoms of SZ are typically divided into three categories: positive, which include hallucinations, delusions and disordered thoughts; negative, encompassing social withdrawal, reduced mobility and reduced motivation; and cognitive, which include impairments in attention, executive functions (i.e. decision making), and working and episodic memory[115–117]. Though positive symptoms are alleviated by typical and atypical antipsychotic medications, neither class of neuroleptics is effective in the treatment of cognitive deficits. ASD can be usually noticed during early childhood. Its symptoms include communication problems, and highly stereotypical behaviour, and it is frequently accompanied by some form of intellectual disability[118].

One of the symptoms prevalent in SZ[133] and to some extent in ASD[213] is a dysfunction in the processing of auditory stimuli. Usually these auditory evoked potentials (AEPs) are measured in response to different auditory paradigms, such as the paired-click, oddball and auditory steady-state response (ASSR), which will be discussed below. Another important factor is that the difference in AEP responses can also be observed in animal models of these disorders[147,214,215]. This similarity in response between patients and animal models, make AEPs particularly useful for translational studies. Importantly, the deficit in AEP response in each of the paradigms mentioned above is not always similar in SZ and ASD. The differences between them will be discussed below.

5.1.1 Auditory Sensory Gating

The ability to filter repeated information in the environment is crucial. One way to test this ability is the paired click paradigm[216]. In this paradigm pairs of identical tones are played with a short inter-stimulus interval. In normal controls, the first positive peak of the AEP after the tone (P1) and first the negative peak after the tone (N1), as measured by EEG recordings (**Figure 5.1**), are diminished after the

second click[217–220]. This diminished response is supposed to reflect an attentional inhibitory filtering mechanism[221].

SZ patients usually exhibit deficits in their responses to this auditory paradigm. They usually show less suppression in both P1 and N1[219,220,222–224]. Several meta-analyses have suggested this deficit is present in the majority of SZ cases compared to controls[134–136]. Indeed, the suppression deficit has even been suggested as an early biomarker for SZ[225]. However, this reduced response is present in other mental disorders as well, like Alzheimer's disease [217], bipolar disorder[226–228] and low-functioning ASD[229] . Yet, some studies have failed to find a consistent diminished response to the second tone in normal controls[222,230]. Also, it was found that the response to a paired-click paradigm depends on the vigilance state of the subjects[231]. However, the majority of studies did find a difference between controls and SZ patients for this measure.

In ASD patients, deficits in sensory gating are not usually observed. For example Magnée et al. (2009) found no difference in sensory gating between a group of highly functioning ASD patients and normal individuals, whereas they did find a difference between a control group and a group of highly functioning SZ patients, which agrees with previous findings[233]. However, another study looking at high functioning autistic children did find a decrease in sensory gating, but, only in those with mental retardation[229].

5.1.2 Mismatch Negativity

When a series of similar tones is played, the response evoked by each tone diminishes. When a deviant tone is played the average evoked response shows a large negative deflection. The difference between this response and the response to the tone preceding the deviant tone is termed mismatch negativity (MMN). SZ patients typically show a reduced response to the deviant tone compared to control populations[137,138]. Specifically, a meta-analysis showed the MMN is a robust feature of the illness[234]. MMN deficits also appear in ASD. Children have a reduction in the mismatch response to the deviant tone, but only when they are not actively attending to the tone[235]. Also, autistic children with mental retardation show an enhanced MMN compared to controls[236].

5.1.3 Gamma Band Auditory Steady-State Response

Playing a 40 Hz click train elicits a 40 Hz (gamma band) response in the auditory cortex in humans[237,238]. In contrast, a reduction in the power of the gamma response to a 40 Hz click train is observed in patients with SZ [139–141]. Moreover, the response is specific to this frequency as in all of these experiments, the strongest reduction was in response to the 40 Hz frequency compared to all other tested frequencies. This puts the ASSR response as a robust feature of the illness. Similarly, children with ASD fail to show an increased gamma response, measured by MEG, elicited by a 40 Hz click train compared to normal controls[239]. Interestingly, a similar pattern is found when comparing parents of autistic children with controls[240]. This suggests an underlying genetic mechanism for this specific ASD phenotype.

5.1.4 Translational Potential of AEPs

As mentioned before, the main importance of these AEPs is that they are readily translatable to animal models. Thus, the normal gating response is apparent in rats[241] and mice[170]. Furthermore, rat[242–244] and mouse[170,245] models of SZ exhibit analogous deficits in their suppression of the EEG response to the second tone in the paired-click paradigm. These facts make this measure a useful translational tool to test the validity of SZ animal models[246,247]. It is important to note that the difference in response is found with electrodes placed near the CA3 region of the HPC, referenced to the cortex above it, which is different to the recordings in humans which are usually taken from the scalp. Interestingly, atypical antipsychotic medicine seems to return the auditory gating in schizophrenic patients to normal[248]. Similarly, in DBA/2 mice, which show a similar gating deficit to that presented in SZ, clozapine restores to normal the decrease in the P1/N1 ratio in response to the second tone in a paired-click paradigm[144]. Similarly in rats, clozapine restores the gating response in PCP treated animals[249], and also attenuates the decrease in gating in d-amphetamine treated rats[250], both of which are models of SZ. As for ASD models, *fmr1*-KO mice, which model FX, demonstrate enhanced gating compared to controls as measured by prepulse inhibition (PPI) response[251]. Interestingly, this is the complete opposite of FX patients carrying a full mutation of this gene, who show an almost complete abolition of gating[251].

Similarly to auditory gating, the changes in MMN can also be observed in animal models of SZ. Thus, a study looking at an isolation rat model of SZ found a reduction in the MMN response to the oddball tone[244]. Importantly, the difference was observed in the cortex, rather than in the CA3 area in the HPC. Conversely, in mice the injection of ketamine, which is known to produce an SZ-like phenotype, reduced the MMN response in CA3 hippocampal area[252]. Also, mice with haploinsufficiency of neuregulin-1 show a decrease in MMN, also in the CA3 area[145].

5.1.5 Current Study

In this study AEPs, which are both robust and translatable features of ASD and SZ, are used to explore the CYFIP1 mice phenotypes further, and to determine which deficits, if any, present themselves in this animal model. The results suggest that CYFIP1 mice present deficits in AEPs that relate to both ASD with mental retardation and SZ.

5.2 Methods

All surgery, post-surgery, care and recording methods are described in chapter Chapter 2

5.2.1 Auditory Brainstem Response

Mice were anaesthetised using pentobarbital (6 mg/kg in a 6 mg/ml concentration). When the mouse was fully anaesthetised needles were put in both the right and left auditory bullas for ground and reference, and on the vertex. The signal was acquired through a fine 25 µm silver wire attached to a stainless-steel pole that was threaded through the needles. After implanting the wires, 500 tone trains were played. Each train consisted of 17 clicks going from 85 dB to 0 dB in steps of 5 dB. Each click was 5 ms long, and the intertone interval was 50 ms and there was a 500 ms interval between click trains. In order to get the auditory brainstem response (ABR), the signal triggered by the tones was averaged. The threshold was defined as the volume 5 dB below before the response reached its maximum amplitude, as described previously[253].

5.2.2 Auditory evoked potentials (AEP)

After 1 week of recovery, the animals were put in a transparent Perspex box. They went through paired click, oddball and theta and gamma wave entrainment trials played in a pseudo-random order from a speaker hanging above the box. Each trail was played three times. The sensory-gating trains consisted of 30 steps of two 1500 Hz pips with 500 ms inter-stimulus interval (ISI) and 8 seconds interval between each tone pair. The oddball paradigm consisted of 30 trains with a 500 ms ISI of 23 1500 Hz tones followed by a single 3000 Hz ‘oddball’ tone. The gamma and theta entrainment trials consisted of 30 trials of 1 second 40 Hz or 10 Hz tones, respectively, with a 1 second ISI. Each train of stimuli was played three times in a random order. If no prominent P1 and N1 could be clearly detected the recording was discarded.

5.2.3 AEP Analysis

The signal was locked to the delivery of the auditory stimulus and averaged across trials. Then it was baseline corrected to the mean amplitude 200 ms before each stimulus by subtracting the mean voltage during that period from the signal. The P1 response was defined as the maximal positive peak occurring between 5

and 40 ms after the stimulus for the hippocampal recordings and 10 and 45 ms for the cortical recordings, and the N1 response was defined as the maximal negative peak between 20 and 60 ms for the HPC and 30 and 70 ms for the cortex (**Figure 5.1**), as in previous studies[244,254]. Sensory gating was tested by measuring the Test to Control (T/C) ratio, which is the ratio between the peak to trough amplitude of the second tone, to that of the first tone. MMN was calculated by measuring the difference between the peak to trough amplitude of the oddball tone and the tone immediately preceding it (effectively the 23rd tone in a 24-tone train). The ASSR response was measured as the ratio of the power between 39 and 41 Hz during the 40 Hz click train relative to 1 second before the train was played. The power in the gamma band was calculated by a Fourier transform on the time period when the stimulus was delivered and taking the integral under the curve in the specific 39-41 Hz band. A similar analysis was done for the theta train with the power taken between 9 and 11 Hz. All analysis was done using a custom-made script in Matlab.

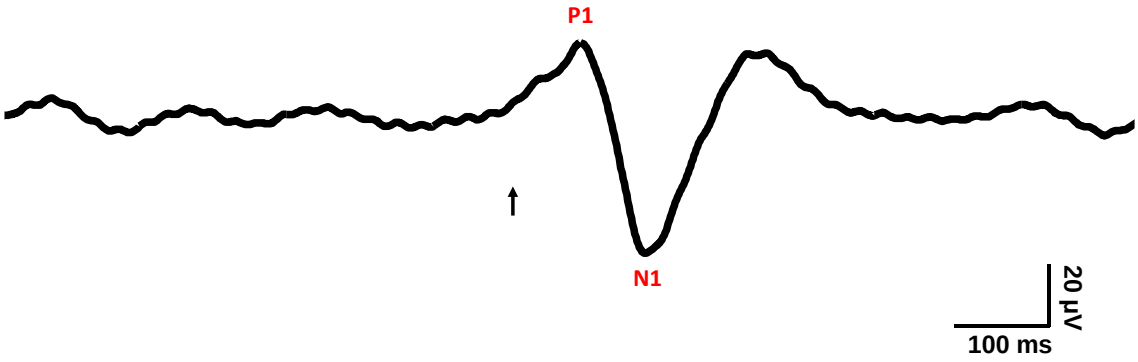

Figure 5.1. A typical trace of an auditory evoked potential. *The averaged response (n=200 presentations) of a single mouse to an auditory stimulus. The black arrow indicates the time when the tone was delivered. The first positive deflection after approximately 20 ms and the negative response after 40 ms can be clearly seen, and are marked as P1 and N1, respectively.*

5.3 Results

5.3.1 Auditory Brainstem Response

Before the evoked potential responses were tested, the animals' hearing was verified by examining their auditory brainstem responses (ABR)[255] to make sure that any effect observed is not due to a different hearing threshold. The WT had a response threshold of 23.8 ± 1.3 dB and the CYFIP1 mice had a threshold of 25.00 ± 0.0 dB (**Figure 5.2**), revealing no significant difference between the strains ($t(6)=1.000$, $p= 0.3559$, $n=4$ for both groups).

5.3.2 Sensory gating

In the cortex (**Figure 5.3**A&C), there was no difference in amplitude in the responses between the genotypes for the first and second tones (**Table 5.1**). There was, also, no apparent difference in the T/C gating ratio between CYFIP1 and WT animals (CYFIP1: 67.21%±12.47%; n=10, WT: 53.96%±5.67%;n=13, $t(18)=0.96$, $p=0.34$)(**Figure 5.3**E). However, there was a difference in latency between the genotypes for both the first ($F(1,21) = 5.407$, $p<0.05$; WT: n=13, CYFIP:n= 10) and second tone ($F(1,21)=4.611$, $p<0.05$; WT: n=13, CYFIP:n= 10**; Table 5.1**). In CYFIP mice the latency of P1 was delayed by 6.42 ms, and by 5.46 ms in response to the first and second tone, respectively, compared to the WT controls (**Table 5.1**).

In the frontal-hippocampal response (**Figure 5.3**B&D) there was also no significant difference in the response amplitude after both the first and second tone (**Table 5.1**). However, whereas the WT mice had a T/C ratio of 43.7%±9%, the CYFIP1 mice showed a much milder decrease in response, with a T/C ratio of 76%±8.9% ($t(10)=2.554$,$p<0.05$; n=6 for both groups **Figure 5.3**F). Contrary to the cortical signal, there were no difference in latency between the CYFIP1 and WT mice (**Table 5.1**).

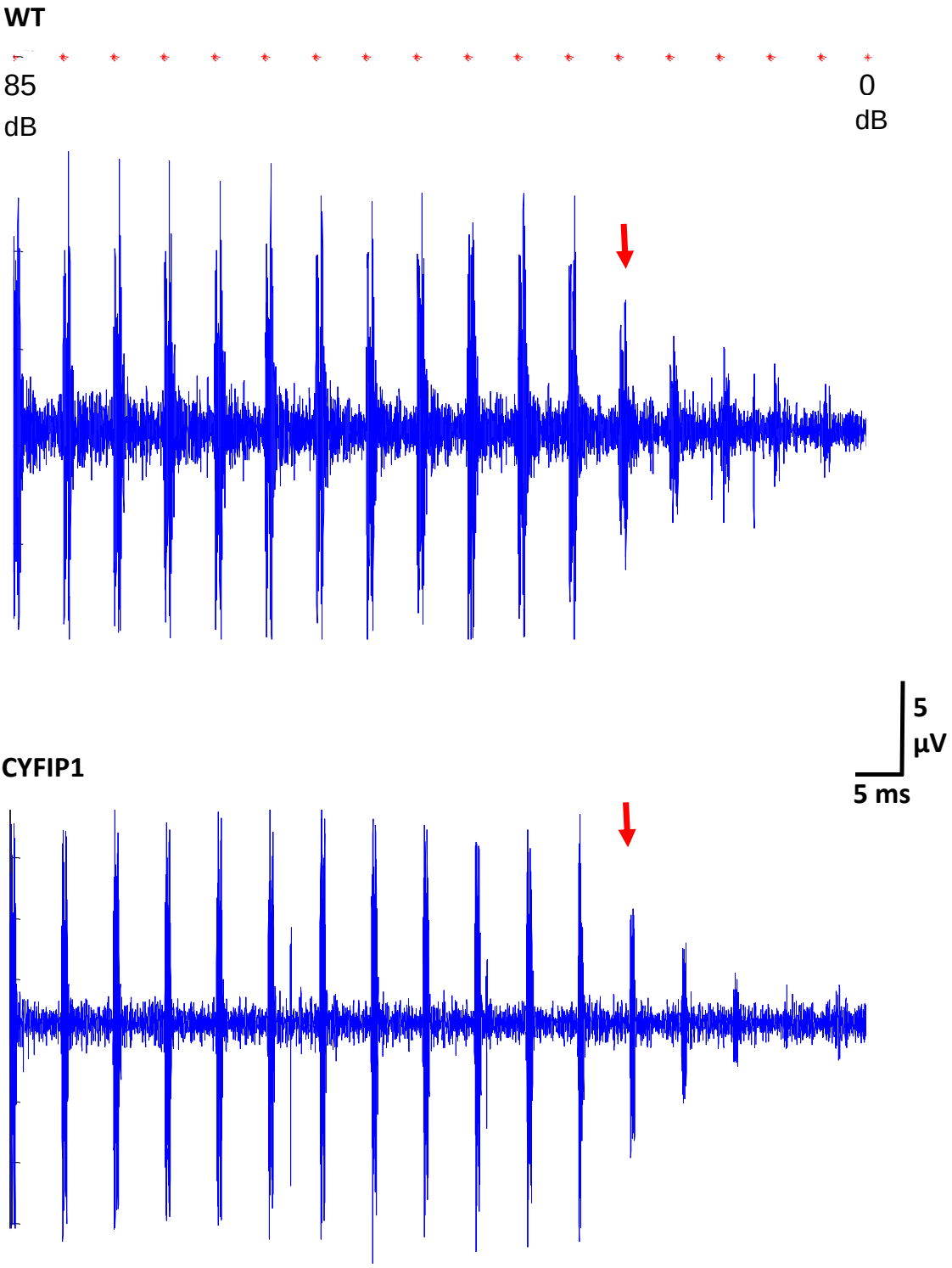

Figure 5.2. Representative brainstem response traces from a WT and a CYFIP1 heterozygous mice*. The tones (marked by stars), were given in a series going from 85 dB to 0 dB. The red arrow marks the hearing threshold, determined by being the one before the response to the click stabilises on a certain amplitude.*

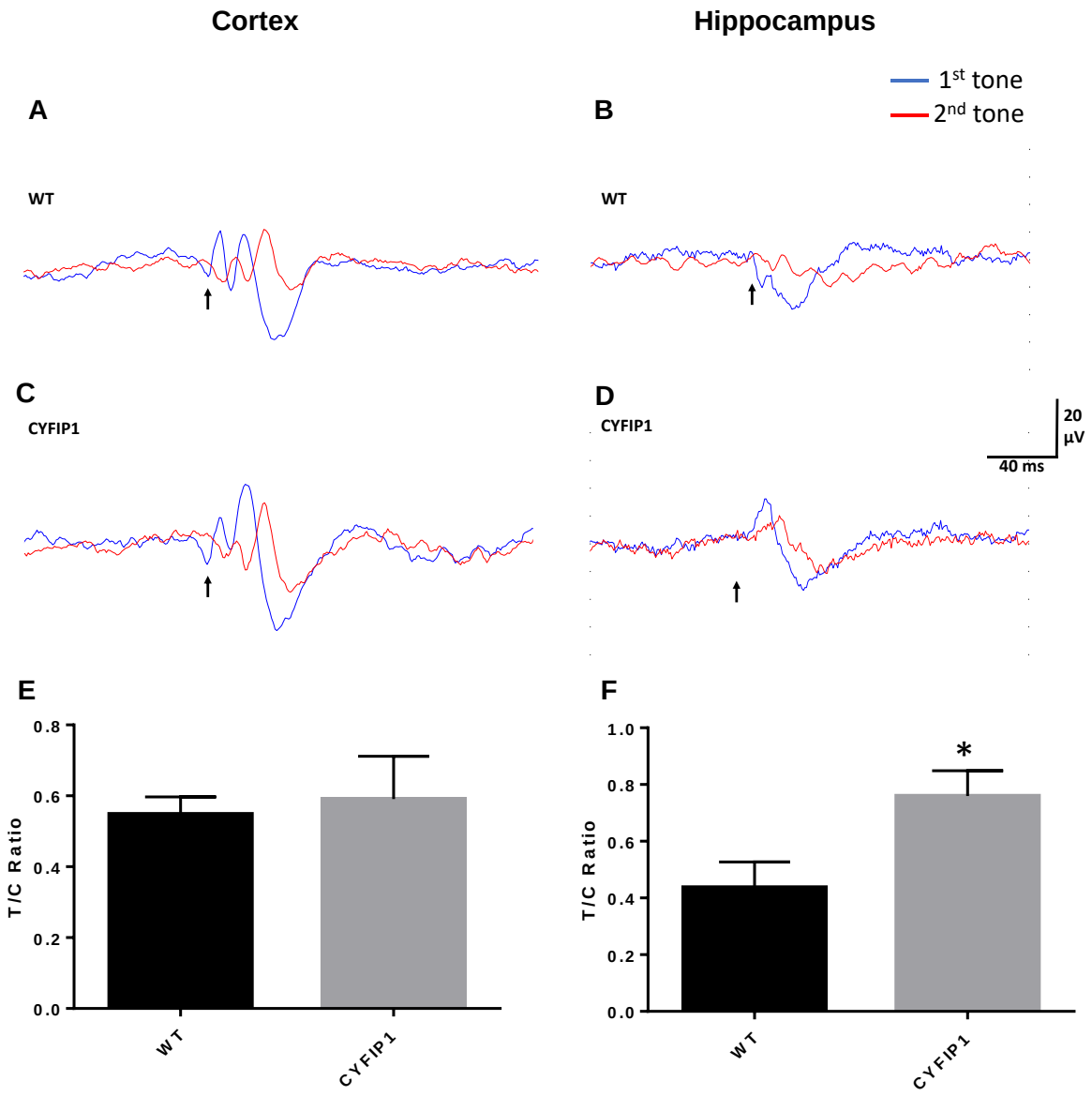

Figure 5.3. Sensory gating in the cortex and HPC of CYFIP1 mice*. **(A-D)** show group average of response to a pair of tones. The response to the first tone is in blue, and the response to the second tone is in red.. **A and C** show the cortical response in WT (**A**) and in CYFIP1 mice **(C)**. **B and D** show the hippocampal response to the tone pair in WT (**B**) and CYFIP1 mice (**D**). **(E-F)** show the summed average of the T/C ratio in the cortex (WT: n = 13, CYFIP: n = 10) **(E)** and HPC (n=6 in both groups) **(F)**. The bars show the means. Error bars represent SEM. *p<0.05*

		WT	CYFIP1	WT	CYFIP1	WT	CYFIP1	WT	CYFIP1
	Tone	**P1 Amplitude (μV)**	**P1 Amplitude (μV)**	**P1 Latency (s)**	**P1 Latency (s)**	**N1 Amplitude (μV)**	**N1 Amplitude (μV)**	**N1 Latency (s)**	**N1 Latency (s)**
CTX	**1**	11.91 ±4	12 ± 3	17.08 ± 1.97	23.50 ± 2.89 *	-22.73 ± 4	-26.19 ± 3	52 ± 2.9	59 ± 2.88
	2	7.011 ± 2	5.638 ± 2	26.54 ± 3.2	32 ± 3.9 *	-12.15 ± 2	-14.77 ± 3	55.23 ±3.9	68.20 ± 2.7
HPC	**1**	24.3 ± 5	36.8 ± 6	15 ± 3.7	16.17 ± 4.3	-39 ± 14	-43 ± 9	47 ±4.8	42.3 ±4.2
	2	21.34 ± 9	14.66 ± 15	16.3 ± 3.6	21.7 ± 4	-30.8 ± 7	-41.1 ± 6	42.7 ±7.3	41.7 ±5.6

Table 5.1. The amplitude and latency of the cortical and hippocampal responses to the first and second tone. *The only difference was in the latency of the N1 response in the cortex, which was longer in the CYFIP1 mice. Significance is in difference between the strains. *p<0.05 compared to WT. for the cortex WT: n =13, CYFIP: n =10. in the HPC n=6 for both groups. The values are means with the SEM in parentheses. CTX = cortex, HPC = hippocampus.*

5.3.3 Mismatch Negativity

In the fronto-auditory ERP (**Figure 5.4**A&C), there was no significant difference in amplitude between genotypes in the 23rd Tone before the oddball (**Table 5.2**). However, in response to the 24th tone, there was a significant interaction between genotypes and deflection ($F(2,42) = 9.068$, $p<0.001$; WT: n = 13, CYFIP: n = 10), which was due to the N1 response of the CYFIP1 mice being 0.02±0.004 µV stronger than in the WT mice. Accordingly, the CYFIP1 mice showed an enhanced response to the 'oddball' tone played after a series of similar tones. The N1 response following the mismatching tone was twice as strong in the CYFIP1 mice as their WT littermates (CYFIP1: 40.63 µV; n = 10, WT: 22.26±7.05 µV; n = 13, $t(18)= 2.124$, $p<0.05$), which also expressed itself in difference in the MMN (WT: 22.47± 5.66 µV; CYFIP1: 41.10±4.74 µV) (**Figure 5.4** E). The CYFIP1 mice also showed a delay of 8.80±3.63 ms in the N1 response for the oddball tone ($p<0.05$) (**Table 5.2**).

The frontal-hippocampal AEP (**Figure 5.4**B&D), did not exhibit any significant difference between the individual response amplitudes for the regular and oddball tone. However, there was a difference in the peak to trough MMN response to the oddball tone. Whereas, the WT mice had a response magnitude of 25.45±7.26 µV, the CYFIP1 mice had a much bigger response of 61.13+-13.33 µV ($t(10)=2.35$, $p<0.05$;n = 6 for both groups) (**Figure 5.4**F). Interestingly, in this case too, there were no latency differences between the CYFIP1 and WT mice.

5.3.4 Gamma Entrainment, Auditory Steady-State Response (ASSR)

After playing a 1 sec-long 40 Hz (gamma band) click pulse there was an increase of 221%+-38.07% in the 40 Hz frequency power recorded from WT mice. When the same tone was played for the CYFIP1 mice, no enhancement could be observed (120.9%±14.03%, $t(18)=2.468$, $p<0.005$; WT: n = 13, CYFIP1: n = 10) (**Figure 5.5**A-B). When only the narrow 10 Hz band was considered, no such difference could be observed when a 10 Hz (theta band) train was played (WT: 120%±15.24%; CYFIP1:120.9%±19.70%, $t(21) = 0.037$, $p=0.971$). However, looking at the entire theta band (5-11 Hz) there was a difference between the WT and the CYFIP1 mice, who showed much smaller increase in the band in response to the tone train (WT: 493.3%±26.23%; CYFIP: 367.2±41.38%, $t(21) = 0.014$,$p<0.05$) (**Figure 5.5**C-D).

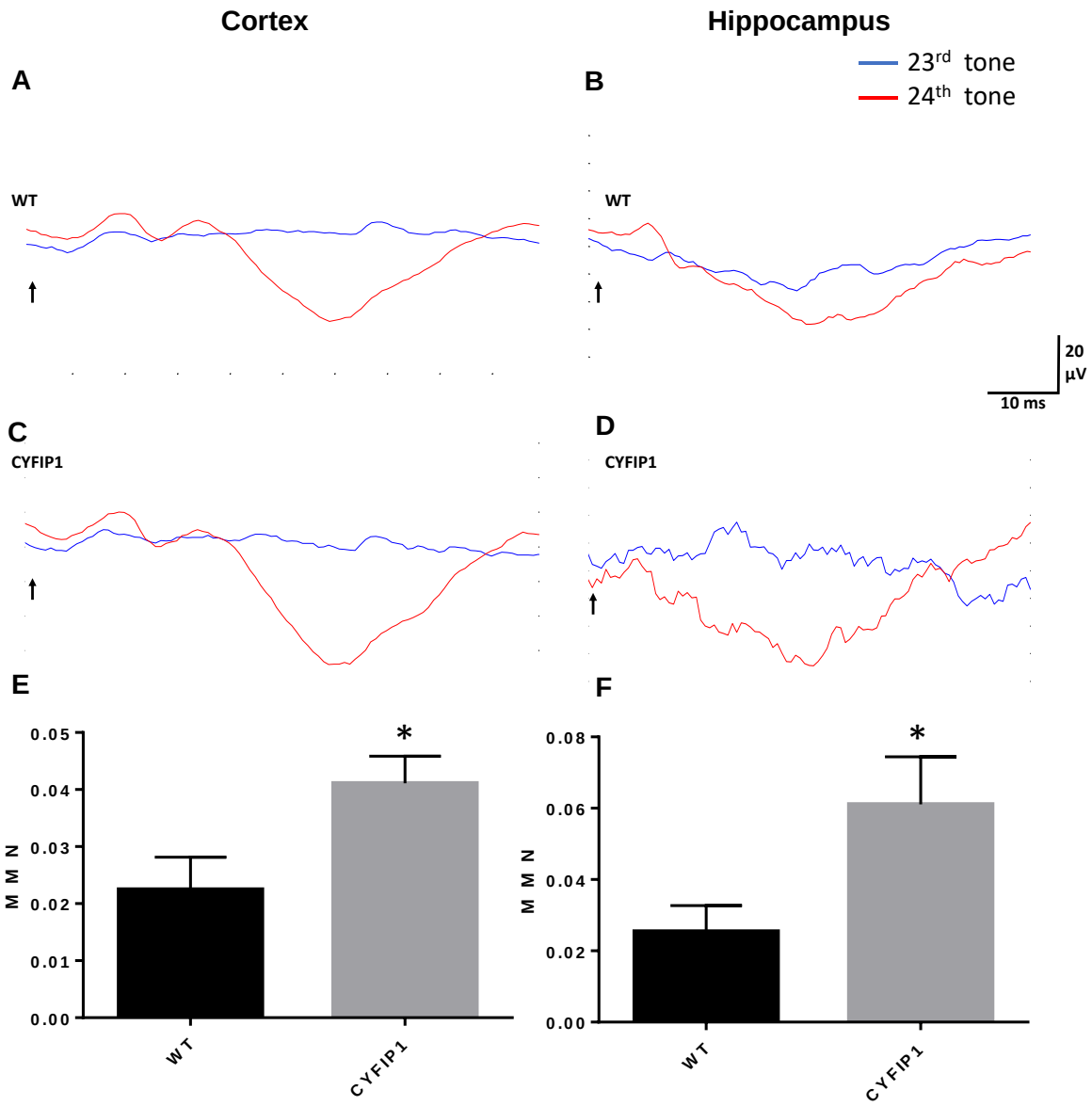

Figure 5.4. MMN in the cortex and HPC of CYFIP1 mice*. **(A-D)** show group average of response to the last regular and to an oddball tone. The response to the regular tone is in blue, and the response to the oddball tone is in red. **A and C** show the cortical response in WT (**A)** and in CYFIP1 mice **(C)**. **B and D** show the hippocampal response to the tone pair in WT (**B)** and CYFIP1 mice (**D). (E-F)** show the summed average of the MMN in the cortex (WT: n = 13, CYFIP: n = 10) **(E)** and HPC (n=6 in both groups) **(F)**. The bars show the means. Error bars represent SEM. *p<0.05.*

		WT	CYFIP1	WT	CYFIP1	WT	CYFIP1	WT	CYFIP1
	Tone	**P1 Amplitude (μV)**	**P1 Amplitude (μV)**	**P1 Latency (s)**	**P1 Latency (s)**	**N1 Amplitude (μV)**	**N1 Amplitude (μV)**	**N1 Latency (s)**	**N1 Latency (s)**
CTX	**23**	3.076 ± 5	2.764 ± 6	26 ± 2.9	19.80 ± 3.8	1.691±14	2.378 ± 9	56.8±3.6	66.30 ± 5.5*****
	24	12.40 ± 3	6.889 ± 3	24.31 ± 2.8	19.90 ± 3	-17.19 ± 3	-37.43 ± 3******	55.69 ±3.3	64.50 ±1.4*****
HPC	**23**	19.9 ± 7	11.82 ± 6	14.5 ± 2.9	17.2 ± 3.8	-35.02 ± 7	- 15.57±11	47.5 ±3.63	50.2±5.5
	24	4.8 ± 3	12.5 ± 5	16.7 ± 3.1	18.2 ± 4.1	-29.1 ± 8	-15.6±5	49.2 ± 3.3	48.7 ±5.3

Table 5.2. The amplitude and latency of the cortical and hippocampal responses to a regular and oddball tone. *The latency and amplitude were different in the CYFIP1 mice cortex. The values were compared between CYFIP1 and controls. *p<0.05 **p<0.0001 both compared to WT. WT: n =13, CYFIP: n =10. in the HPC n=6 for both groups. The values are means with the SEM in parentheses. CTX = cortex, HPC = hippocampus*

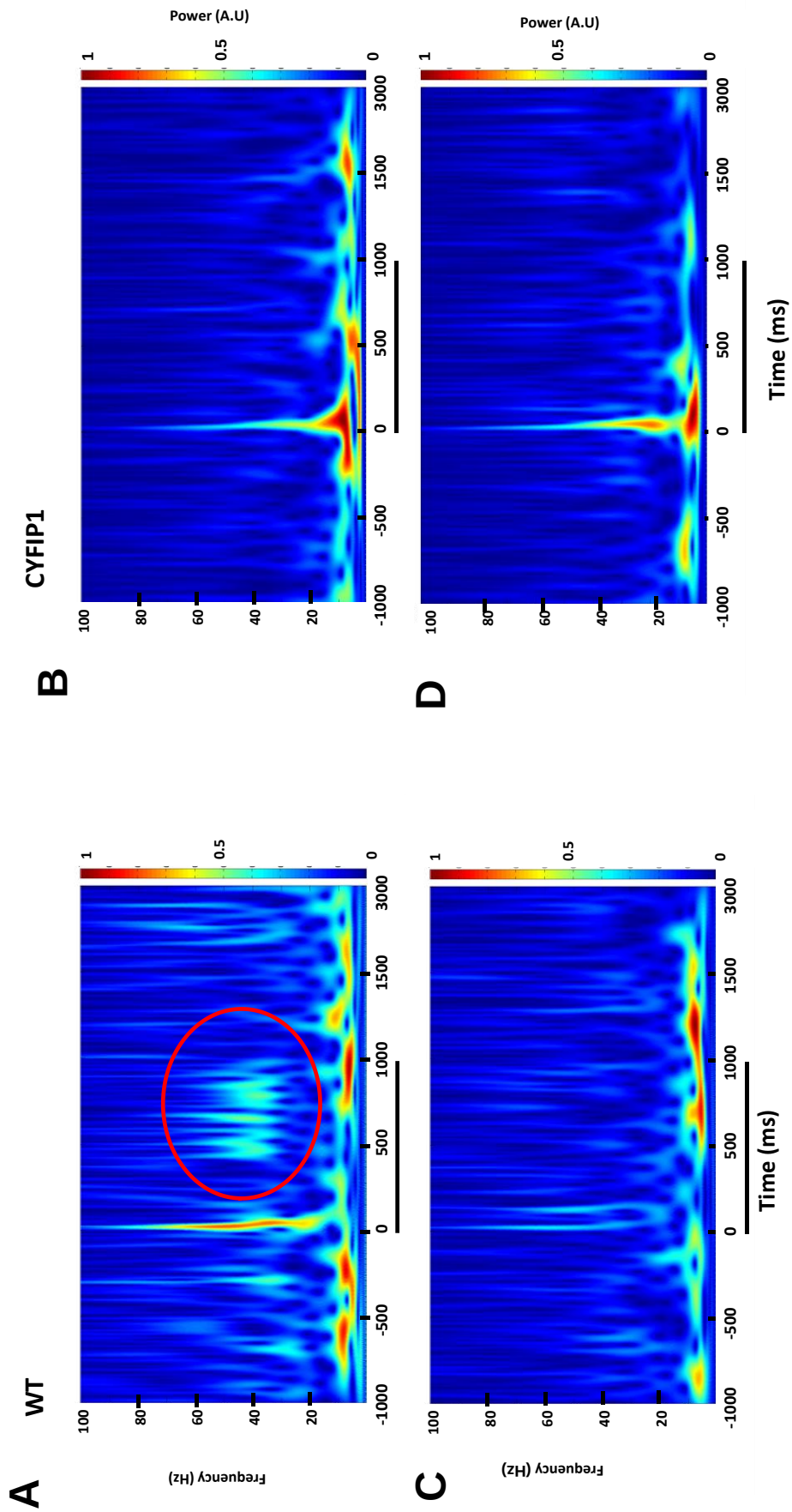

Figure 5.5. Typical averaged spectrograms from a WT and a CYFIP1 mice. *The WT mice presented an increase in the gamma frequency range after hearing a 40 Hz tone* ***(A)****, which is clearly lacking in the CYFIP1 mice* ***(B)****. No such difference was recorded in the theta range when a 10 Hz tone was played between WT* ***(C)*** *and CYFIP1* ***(D)*** *mice. The black line marks a 1 second 40 Hz or a 10 Hz tone. The red circle marks an induced increase in the gamma range in the WT, which is missing in the CYFIP1 mice. The spectrogram is an average over 90 trials.*

5.4 Discussion

In this set of experiments various auditory paradigms were used to investigate potential differences in the AEPs of CYFIP1 mice compared to their WT littermates. The eventual goal of these experiments was to find out whether their response to these stimuli is similar to any known mental illness phenotype in humans, and thus ascertain whether they can be used as a translational model for these illnesses.

The sensory gating response of the CYFIP1 mice was diminished, an effect that could only be observed in the HPC recording sites. The opposite effect was observed for the mismatch negativity effect, for which the CYFIP1 mice showed an increase response. Finally, while the WTs showed a strong gamma ASSR response it was completely abolished for the CYFIP1 mice.

MMN differences were also observed in both HPC and epidural electrodes. However, rather than a decrease, which is usually observed in SZ[234,256,257], the CYFIP1 mice exhibited an increase. This effect seems to be more similar, therefore, to FXS and mental retardation. Indeed, a stronger N1 response in children with FXS over the centre of the scalp was recorded in a response to a standard tone in a short train of four tones[258]. It was also found that while not exhibiting a change in amplitude, a delay in N1 latency, similarly to the one exhibited by the CYFIP1 mice, is observed in both autistic children and valproate-treated-mouse model of ASD[259]. Also, the MMN to a deviant tone was found to be significantly larger in autistic children with mental retardation[236]. The abolition of the gamma band steady state response that was found in the current study is also similar to that found in both ASD[229,239] and SZ[139,141,142].

The changes in the CYFIP1 animals seemed to suggest that these mice present auditory symptoms more akin to ASD rather than SZ. This fits the results of a recent study that showed that the CYFIP1 mice present key phenotypes similar to those of *fmr1-KO* mice, a model of an FXS phenotype[152]. It has also been shown that expression levels of CYFIP1, targeted in the haplo-insufficiency model, were reduced in people with FXS[260], which is the most common identifiable cause of ASD[261,262]. In addition, it has recently been shown that

CYFIP1 is one of a series of genes that are able to differentiate between FMR1-related autism and dup (15q) related autism[263].

The sensory-gating deficit was observed only in the CA3 HPC region and not in the fronto-auditory electrodes. Indeed, differences in sensory gating in rodents have been most often shown in the CA3 hippocampal region[244,254,256]. The MMN difference could be recorded from both sites which also seems in line with previous research where MMN responses were recorded both in cortical[264] and deep brain areas[256] of mice. In humans, it was shown that there are two generators involved in the MMN response in the frontal and auditory cortices[265]. Interestingly, single neurons in the medial prefrontal cortex (mPFC) have been reported to decrease their response to a second tone out of pair of consecutive tones, which might be a single-unit demonstration of sensory gating[266]. However, the response of single neurons is different to that recorded by the LFP, which is mainly ensemble responses. Yet, the T/C ratio of the mPFC neurons, was 0.74 which is very similar to the 0.67 T/C ratio that was found with the cortical electrodes in this study. Both these values are rather low compared to gating observed in the HPC area of around 0.4 that was found in the current study. This might suggest that the main deficit in the generation of the response is mainly hippocampal or at least related to the temporal, rather than frontal lobe.

These results suggest that future research should focus on the CYFIP1 mice's HPC. Indeed, previous research has already found enhanced LTD in CYFIP1 hippocampal slices[152]. Thus, behaviourally, these animals' performance should be tested in hippocampal-dependent tasks, such as spatial novel object exploration[267]. It will also be important to investigate which hippocampal neuronal populations are affected by the CYFIP1 mutation and how they are in the AEP deficits reported above.

In summary, this study used AEPs to test whether the CYFIP1 mice present symptoms that are observed in patients with either FXS or SZ. The results suggest that the mice exhibit auditory deficits that are more similar to these presented in FXS, rather than in SZ. To take these results further, more behavioural tests should be performed to fully characterise the CYFIP1 mice. Once a robust phenotype is present, the mechanisms underlying the phenotype

could be further explored, and hopefully will lead to a better understanding of some of the phenotypes present in ASD and SZ.

Chapter 6 Recognition memory and familiarity-related ERPs in the CYFIP1 mice

6.1 Introduction

One of the common symptoms of many mental illnesses is a memory deficit. For example, FXS patients have deficits in verbal memory[268]. The Fmr1 KO model of FXS also shows myriad memory deficits. A study looking at fear-trace memory found that the Fmr1 KO mice show less freezing in response to conditioned tone with a long ITI between the tone and a foot-shock[269]. Another study found that these mice did not have a deficit in spatial learning, but showed much less freezing in response to regular fear conditioning[270]. In a test looking at their novel object exploration, these mice explored both novel and familiar objects for equal amounts of time[168], thus showing a deficit in object recognition memory. The FMR1 protein in a downstream target of the CYFIP1 protein[271], the protein that is affected in the CYFIP1 mice. Since a KO of the FMR1 gene causes at least some memory-related deficits, it is reasonable to examine whether the CYFIP1 deficient mice show similar deficits.

Memory deficits are also present in SZ. In a test asking patients to recognise pairs of objects presented earlier, their recognition was at chance level[158]. SZ patients also show deficits in delayed object and face recognition[159]. Accordingly, it is not surprising that a large meta-analysis looking at 70 studies, found strong evidence for memory deficits in SZ. Specifically, the analysis showed that while being less impaired than other types of memory, recognition-memory was still significantly deficient in SZ patients[160].

Animal models of FXS and ASD also show deficits in recognition memory. As discussed in section 5.1, the FMR1 is a gene that codes for a protein downstream from the CYFIP1 protein. Memory tests on FMR1 KO mice revealed deficits in short term object recognition memory[168]. Another study showed that these mice exhibited a deficit in the long-term memory for both locations and objects[272]. Interestingly, in this experiment the mice did not show a short-term memory deficit. However, the difference might stem from the definition of short-term, which was a 3-minute retention interval in the first case[168], and a 90-minute retention interval in the second[272]. Together, it might mean that these mice have deficits in

very short retention interval and longer ones. Similarly BTBR T+tf/J (BTBR) mice, which model ASD, show a deficit in long term location recognition memory[273].

There are similar deficits in various SZ models. For example, mice treated with PCP show deficits in long-term object recognition memory[165,274]. Importantly, the administration of the anti-psychotics, aripiprazole[165], or clozapine[11], reversed these deficits. Sandy mice, a genetic model for SZ with a reduction in dysbindin-1 protein, show deficits in short-term[167] and long-term[275] object recognition compared to WT. Interestingly, ketamine-injected rats show deficits in both short-term object recognition and location recognition memory[276]. Ketamine-injected rats also show a deficit in long-term object recognition memory[277]. Also, rats exposed to viral mimetic during pregnancy, which models pregnancy infection that leads to mental illness, show a deficit in object-in-place association[278]. The social isolation rat model of SZ also shows deficits in short-term NOR[279,280]. MK-801-treated mice also show deficits in object-place association as assayed in a touch-screen paired-associate task[281].Thus, both FXS and SZ animal models show deficits in recognition memory that mimics to some extent the deficits shown in humans having these conditions.

Visual ERPs seem to also be impaired in SZ. Thus, SZ patients show a reduction in response to both chromatic and achromatic checkerboard patterns[282]. Another study found a similar result only for achromatic checkerboard pattern[283]. Also, SZ patients show a reduction in the visual ERP in response to light stimulation of different frequencies[284,285]. In contrast, in children suffering from FXS, the amplitude of the visual ERP is increased when they are compared to controls of the same chronological age, but not when they are compared to controls of the same developmental age[286]. The same is true for mentally retarded children when compared with same age controls[287]. No data exists for ERPs in higher-order areas in these disorders.

The previous study, described in chapter Chapter 5 showed that the CYFIP1 mice show at least some abnormalities in AEPs that are common in both FXS and SZ. Recognition memory in CYFIP1 mice has not been tested up to date. Since memory deficits are present in both FXS and SZ, it would be useful to know whether this model also exhibits some of the deficits shared by human patients and other animal models of these mental illnesses. Thus, these mice were exposed to a battery of object recognition tests, specifically, tests for memory for object novelty, object location novelty and object-in-place associations.

In chapter Chapter 3 , a visual ERP was observed in V1 and the PRH for both pictures and grating stimuli. Further, it was found that when using picture stimuli, under certain condition the ERP in V1 changes with novelty. Thus, along with their memory the CYFIP1 mice were implanted in both the PRH and V1 to test if they exhibit any changes in the ERP in these areas compared to their WT littermates. Interestingly, despite the presence of deficits in AEP, no such difference could be observed for recognition memory or in the ERPs of the CYFIP1-deficient mice.

6.2 Methods

All surgery, post-surgery, care and recording methods are described in chapter 2.

6.2.1 Behavioural Protocol

The animals were habituated by letting them explore the behavioural arena, which was a 50X50X30 Perspex box (**Figure 6.1**D), for 10 min on 2 consecutive days. Following habituation, the animals went through 6 different protocols based on spontaneous object exploration (SOE). Normal day-to-day objects were used for the animal to explore (**Figure 6.1**E). The procedures were novel object, object location, and object-in-place recognition tasks. All procedures included a learning and a recall phase, in which the animal actively explored objects in the testing area, separated by a retention phase in which the animal returned to its home cage for either 30 min or 24 hrs. Each of the 3 tasks took place with both the 30-min (short-term) and a 24-hr (long-term) retention intervals. For the 30-min retention interval the learning and recall phases had the animal exploring objects in the test box as described below. For the 24-hr retention interval version of the tasks, the animal repeated the learning phase twice with a 30-min interval between the 2 repetitions. The novel object task involved the animal exploring two identical objects placed in the centre of the box for 5 min in the learning phase (**Figure 6.1**A1), and exploring the arena for 5 min again with one of the objects replaced by a novel one, thus having one novel and one familiar object (**Figure 6.1**A2). The object-in-place task had the animal explore an arena with 4 different objects in the 4 corners of the box for 5 min, for the learning phase (**Figure 6.1**B1). Then, two diagonal objects were swapped with one another for the recall phase, thus having two objects in a familiar location and two objects in a novel one (**Figure 6.1**B2), and the animal was put in the arena to explore it for 5 min. For the object location tasks, the animal explored an arena with 2 identical objects for 5 min during the learning phase (**Figure 6.1**C1), and explored the arena for 5 min again with one of the objects moved to the end of the arena, thus having one object in a familiar location and one in a novel location (**Figure 6.1**C2). The arena and the objects used for testing were cleaned using medi-wipes after every exploration trial to prevent the animal from using any olfactory cues. Each object

was used only once for each mouse. Also, the order of the tests was counter-balanced between the mice.

6.2.2 Visual Evoked potentials

All the experimental procedures and analysis were as described in Section 3.2.

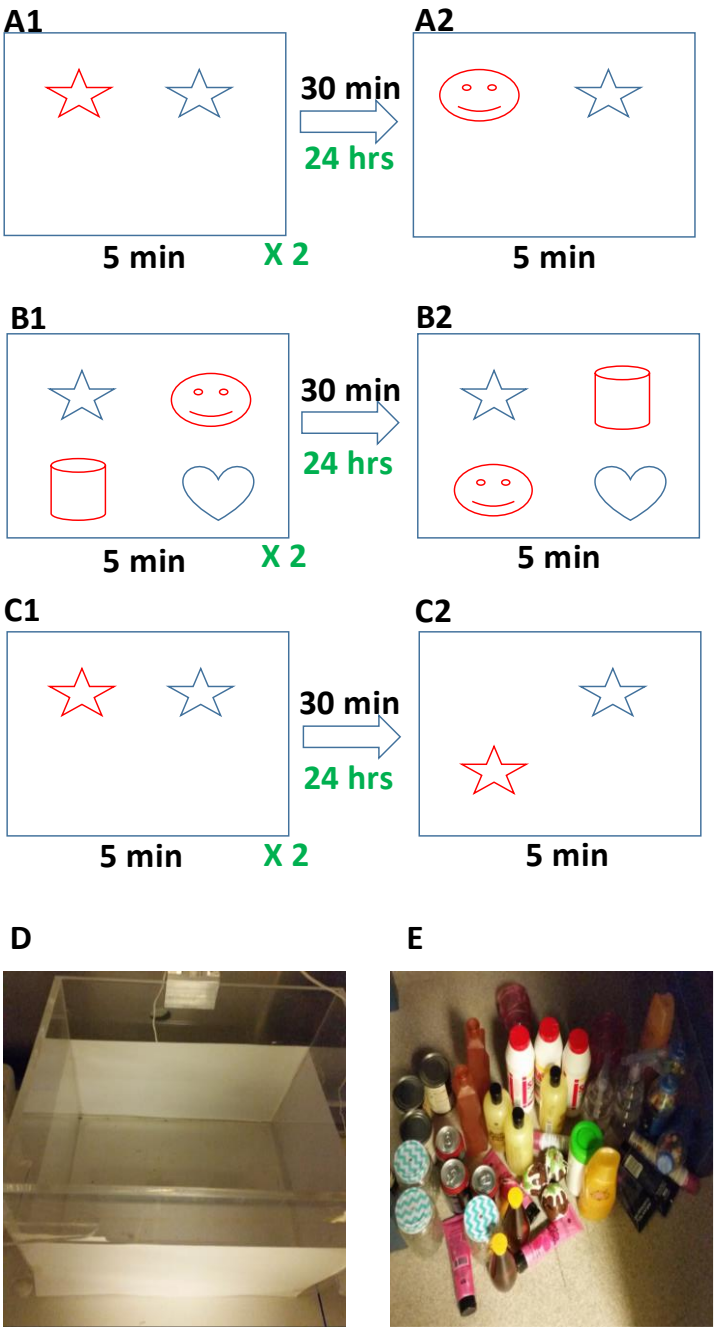

Figure 6.1. The SOE protocol. *A)* ***Object recognition protocol****. In this procedure, the animal was put in the exploration arena with two identical objects (A1) and left to explore it for 5 min. Then after a 30 min or 24 hrs retention interval they were put in the arena when one object was changed and left again to explore it for 5 min (A2). B)* ***Object-in-place recognition protocol****. In this procedure, the animal was left to explore an arena with 4 different objects for 5 min (B1). Then, after a 30 min or 24 hrs retention interval it was put in the same area with the same objects, with 2 of the objects switching location and left to explore it for 5 min (B2). C)* ***Location recognition protocol****. In this procedure, the animal was left to explore an arena with two identical objects for 5 mins (C1). Then, after 30 mins or 24 hrs it was left to explore the same arena for 5 min, with the same two identical objects, where one of them was moved forward (C2). In all the figures, the changed objects are marked in red. Also, when the 24 hrs procedure was used as marked in green, the first part was repeated twice.* ***D)*** *A picture of the experimental arena.* ***E)*** *an example for the objects that were used in the various tests.*

6.3 Results

6.3.1 Novel Object

First the animals' response to a novel object was examined. Looking at the total object exploration time, the WT and mutant mice showed no difference in total object exploration time between the learning and recall phases when they were separated by 30-minute retention interval($F(1,14)=0.51;p=0.48;n=8$; **Figure 6.2**A). A 2-way ANOVA found no interaction with genotype ($F(1,14)=0.20;p=0.66;n=8$; **Figure 6.2**A).

Next, the animals' preference for novel object exploration was examined. The WT mice showed a preference for the novel object, exploring it for 61.3%±7.3% of the total exploration time of the recall phase. The CYFIP1 mice showed a similar preference, and explored the novel object 61.9%±7.3% of the exploration time. A 2-way ANOVA revealed no interaction effect for genotype ($F(1,28) = 0.01;p=0.9;n=8$); **Figure 6.2**B), while showing a statistically significant effect of novelty ($F(1,28) = 20.3;p<0.0001;n=8$; **Figure 6.2**B).

Similarly, in the case of a 24-hour retention interval there was no difference in time spent in object exploration between the 3 time periods ($F(1,14)=1.41;p=0.26;n=8$; **Figure 6.2**C) nor was there any difference between the genotypes revealed by the interaction($F(1,14)=1.49;p=0.24;n=8$; **Figure 6.2**C).

Again, the WT mice showed a preference for the novel object ($F(1,28) = 27.8;p<0.0001;n=8$; **Figure 6.2**D), exploring it for 60.43%±0.07% of the exploration time during the recall phase. The CYFIP1 showed a similar preference exploring the novel object for 66.72%±0.07% of the total exploration time. An ANOVA, again, revealed a statistically significant effect for novelty ($F(1,28) = 27.8;p<0.0001;n=8$; **Figure 6.2**D), while showing no interaction effect for genotype ($F(1,28) = 1.43;p=0.24;n=8$; **Figure 6.2**D).

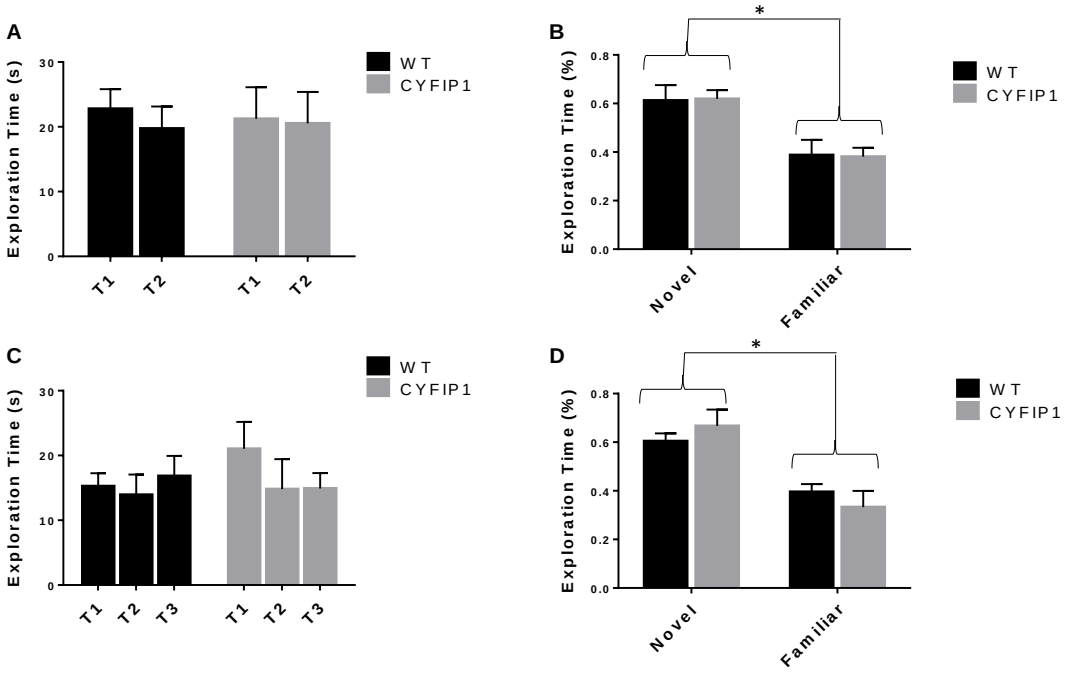

***Figure 6.2**. **Novel Object Exploration**. **A)** the exploration time in the short-term condition **B)** The difference in the exploration between the novel and familiar object. **C)** The exploration time the long-term condition. **D)** The difference in exploration between the novel and familiar object in the long-term condition. T1, T2, T3 – the first, second and third exploration trials. **Novel**, **Familiar** – the time spent exploring either a novel or a familiar object. N=8 mice per group. Data are expressed as mean ± SEM. *p < 0.05*

6.3.2 Object Location

Next, the animals' response to a change in an object's location was investigated. Looking at total object exploration time, the WT showed a trend towards more exploration on the recall phase, where one object was moved, when the retention and recall phases were separated by 30 minute retention interval ($F(1,14)=3.62;p=0.08;n=8$; **Figure 6.3**A), and there was no effect for genotype ($F(1,14)=0.45;p=0.51;n=8$; **Figure 6.3**A), according to a 2-way ANOVA.

After a 30-minute retention interval, the WT mice showed a preference for the object in the novel location, exploring it for 70%±0.8% of the total exploration time of the recall phase. The CYFIP1 mice showed a similar preference, and explored the novel object 63.1%±0.8% of the exploration time. A 2-way ANOVA revealed no interaction effect for the genotypes ($F(1,28) = 1.38;p=0.25;n=8$; **Figure 6.3**B), while showing a statistically significant difference for novelty ($F(1,28) = 32.44;p<0.0001;n=8$; **Figure 6.3**B).

After 24 hrs, the WT animals showed more exploration during the recall phase ($F(2,28) = 12.15;p<0.0005;n=8$; **Figure 6.3**C). This pattern was similar to one found in another study with spatial manipulations[288]. However, a 2-way ANOVA showed that there was no difference between them and the CYFIP1 animals as revealed by the interaction factor ($F(2,28) = 0.34;p=0.71;n=8$' **Figure 6.3**C).

Similarly, both genotypes showed a preference to the object in the novel location, with the WT exploring the object in the novel location 68.9%±0.8% of the time, and the CYFIP1 mice exploring it for 75%±0.9% of the time ($F(1,28) = 25.25;p<0.0001;n=8$; **Figure 6.3**D), with no statistically significant interaction difference between the genotypes ($F(1,28) = 0.48;p=0.49;n=8$; **Figure 6.3**D).

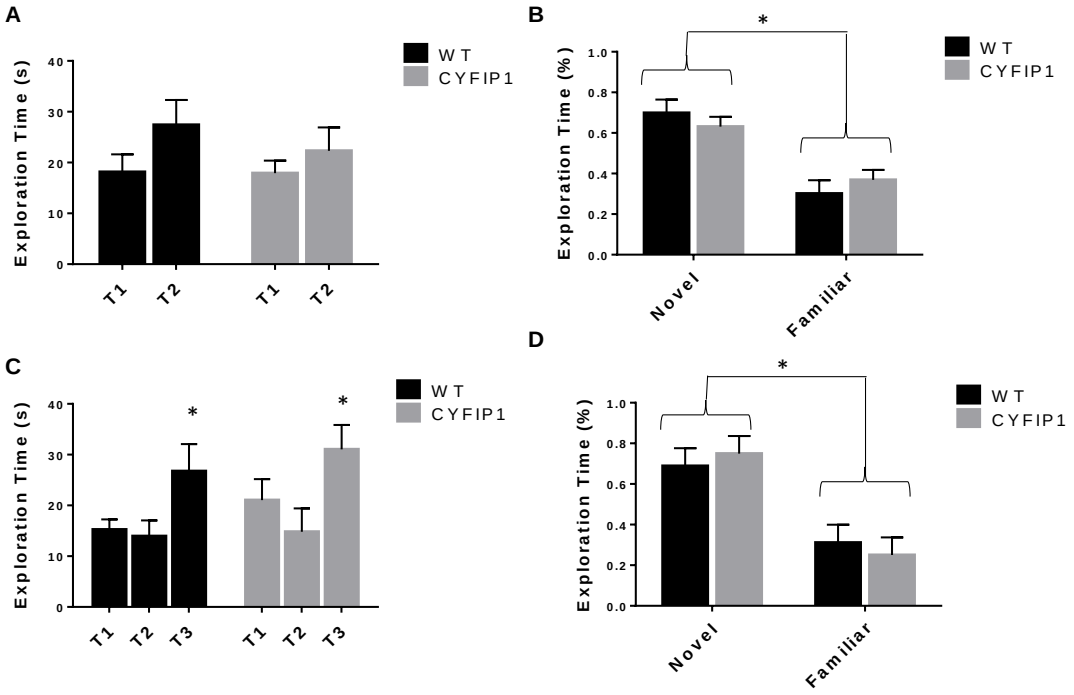

Figure 6.3*. *Novel Spatial Location*. *A) *the exploration time in the short-term condition* ***B)*** *The difference in the exploration between an object in a familiar location or a novel location in the arena.* ***C)*** *The exploration time the long-term condition.* ***D)*** *The difference in exploration between the novel and familiar object in the long-term condition.* ***T1, T2, T3*** *– the first, second and third exploration trials.* ***Novel***, ***Familiar*** *– the time spent exploring either the object in a novel or a familiar location. N=8 for both groups. Data are expressed as mean ± SEM. * p < 0.05.*

6.3.3 Object in Place

Next the changes in exploration when two objects switched location was examined. There was no difference in exploration between the learning and recall phase after 30 minute retention (F(1,14)=0.60;p=0.44;n=8;**Figure 6.4**A), and there was no difference between the genotypes as revealed by interaction (F(1,14)=2.21;p=0.16;n=8; **Figure 6.4**A) according the a 2-way ANOVA.

Looking at novelty preference, the WT mice explored the two objects in the novel position for 59%±0.6% of the time, and the CYFIP1 mice explored them for 69%%±0.5% of the time, thus showing an effect for novelty (F(1,28) = 22.54;p<0.0001;n=8; **Figure 6.4**B), but no interaction effect for genotype (F(1,28) = 2.94;p=0.09;n=8; **Figure 6.4**B).

In the 24 hrs retention interval, there was also no difference in exploration time between the different phases (F(2,28) = 0.22;p=0.81;n=8; **Figure 6.4**C), and there was no interaction effect for genotype (F(2,28) = 0.72;p=0.5;n=8; **Figure 6.4**C).

Again, there was a significant effect for novelty (F(1,28) = 12.8;p<0.005;n=8; **Figure 6.4**D), with the WT mice exploring the objects in the novel locations 58%±0.4% of the time, and the CYFIP1 mice exploring them for 63.5%±0.7% of the time. However, there was no interaction effect for genotype on the novelty preference (F(1,28) = 0.85;p=0.37;n=8; **Figure 6.4**D).

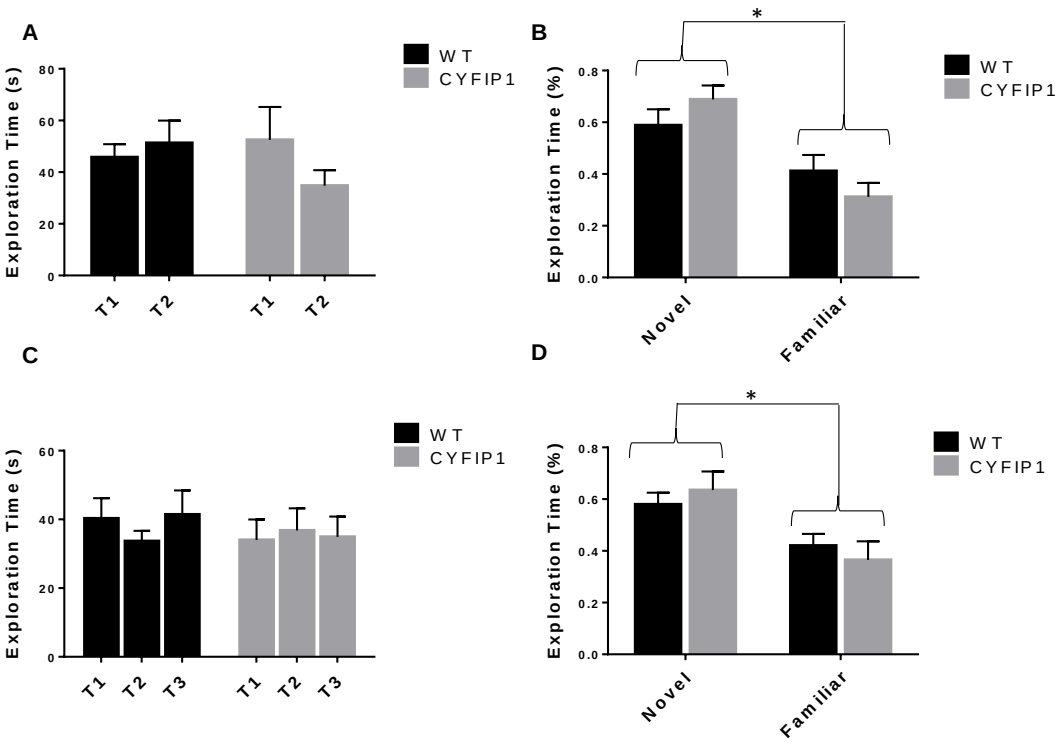

Figure 6.4. Object-In-Place exploration. ***A)*** *the exploration time in the short-term condition* ***B)*** *The difference in the exploration between an object in a familiar location or a novel location in the arena.* ***C)*** *The exploration time the long-term condition.* ***D****) The difference in exploration between the novel and familiar object-in-place in the long-term condition.* ***T1, T2, T3*** *– the first, second and third exploration trials.* ***Novel, Familiar*** *– the time spent exploring either the novel or familiar object-in-place association. N = 8 for each group. Data are expressed as mean ± SEM. * p < 0.05.*

6.3.4 Evoked potentials in the CYFIP1 mice

To examine the possibility of a genotype-related difference in the neuronal activity in the PRH and V1 a 2-way ANOVA was performed (Genotype X Novelty) on the amplitude and latency of the ERPs in those areas.

The response to stationary gratings was inspected first. In the PRH (**Figure 6.5**A), There was no detectable difference between genotypes in neither the amplitude (Genotype: $F(1,9)=1.48$, $p = 0.25$; Novelty: $F(3,27) = 1.097$, $p=0.36$; Interaction: $F(3,27)=0.05$, $p=0.98$; Cyfip1: N=6, WT: N = 5; **Figure 6.5**B) , nor the latency (Genotype: $F(1,9)= 0.22$, $p = 0.65$; Novelty: $F(3,27) = 0.98$, $p=0.41$; Interaction: $F(3,27)=0.44$, $p=0.72$; Cyfip1: N=6, WT: N = 5; **Figure 6.5**C) of the ERP . The same was true in V1 (**Figure 6.5**D), where no difference could be observed in both amplitude (Genotype: $F(1,8)=0.25$,$p=0.63$;Novelty: $F(3,24) = 0.83$, $p=0.49$; Interaction: $F(3,24)=0.08$, $p=0.97$; Cyfip1: N=5, WT: N = 5; **Figure 6.5**E) or latency (Genotype: $F(1,8)= 0.001$, $p = 0.97$; Novelty: $F(3,24) = 0.95$, $p=0.43$; Interaction: $F(3,24)=0.35$, $p=0.78$; Cyfip1: N=5, WT: N = 5; **Figure 6.5**F).

Then, the response to images of objects was tested. The PRH (**Figure 6.6**A) showed no detectable differences in either amplitude (Genotype: $F(1,9)=0.006$, $p = 0.94$; Novelty: $F(3,27) = 0.55$, $p=0.65$; Interaction: $F(3,27)=0.96$, $p=0.42$; Cyfip1: N=6, WT: N = 5; **Figure 6.6**B) or latency (Genotype: $F(1,9)=0.32$, $p = 0.59$; Novelty: $F(3,27) = 0.99$, $p=0.41$; Interaction: $F(3,27)=1.68$, $p=0.19$; Cyfip1: N=6, WT: N = 5; **Figure 6.6**C). In V1 (**Figure 6.6**D), too, no differences in amplitude (Genotype: $F(1,8)=0.58$, $p = 0.47$; Novelty: $F(3,24) = 0.53$, $p=0.66$; Interaction: $F(3,24)=0.49$, $p=0.69$; Cyfip1: N=5, WT: N = 5; **Figure 6.6**E) or latency (Genotype: $F(1,8)=0.07$, $p = 0.79$; Novelty: $F(3,24) = 0.75$, $p=0.53$; Interaction: $F(3,24)=0.63$, $p=0.59$; Cyfip1: N=5, WT: N = 5; **Figure 6.6**E) could be detected.

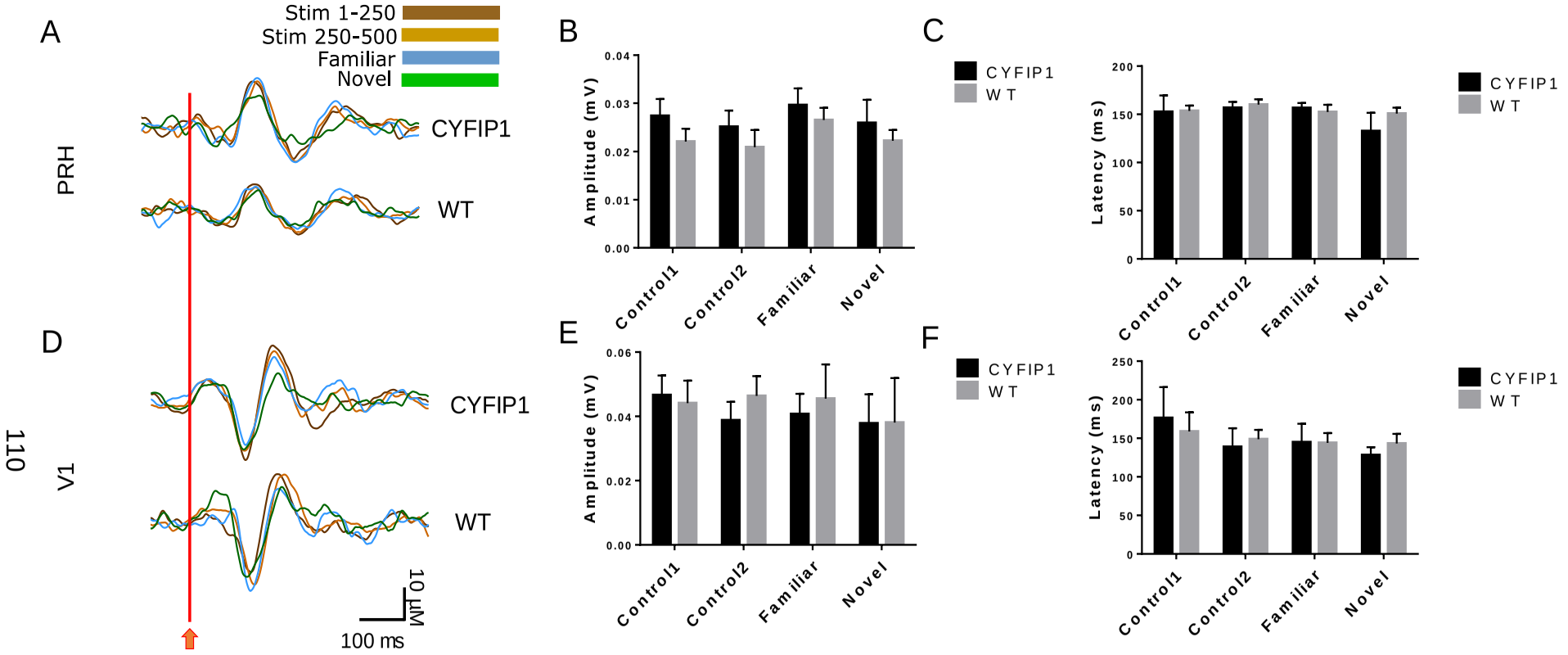

Figure 6.5. ERPs in the PRH and V1 in response to grating stimuli. ***A)*** *The ERP in the PRH.* ***B)*** *The average PRH amplitude of the ERP response in both the CYFIP1 and WT mice in first 250 presentations of a stimulus (Control1), the second 250 presentations of a stimulus (Control2), 250 presentations of the control stimulus after a 2-min retention interval (Familiar) and 250 of a stimulus in a different orientation (Novel).* ***C)*** *The latency of the PRH ERP for Control1, Control2, Familiar and Novel conditions.* ***D)*** *The ERP in V1.* ***E)*** *The average amplitude of the V1 ERP for Control1, Control2, Familiar and Novel conditions.* ***F)*** *The latency of the V1 ERP for Control1, Control2, Familiar and Novel conditions.*

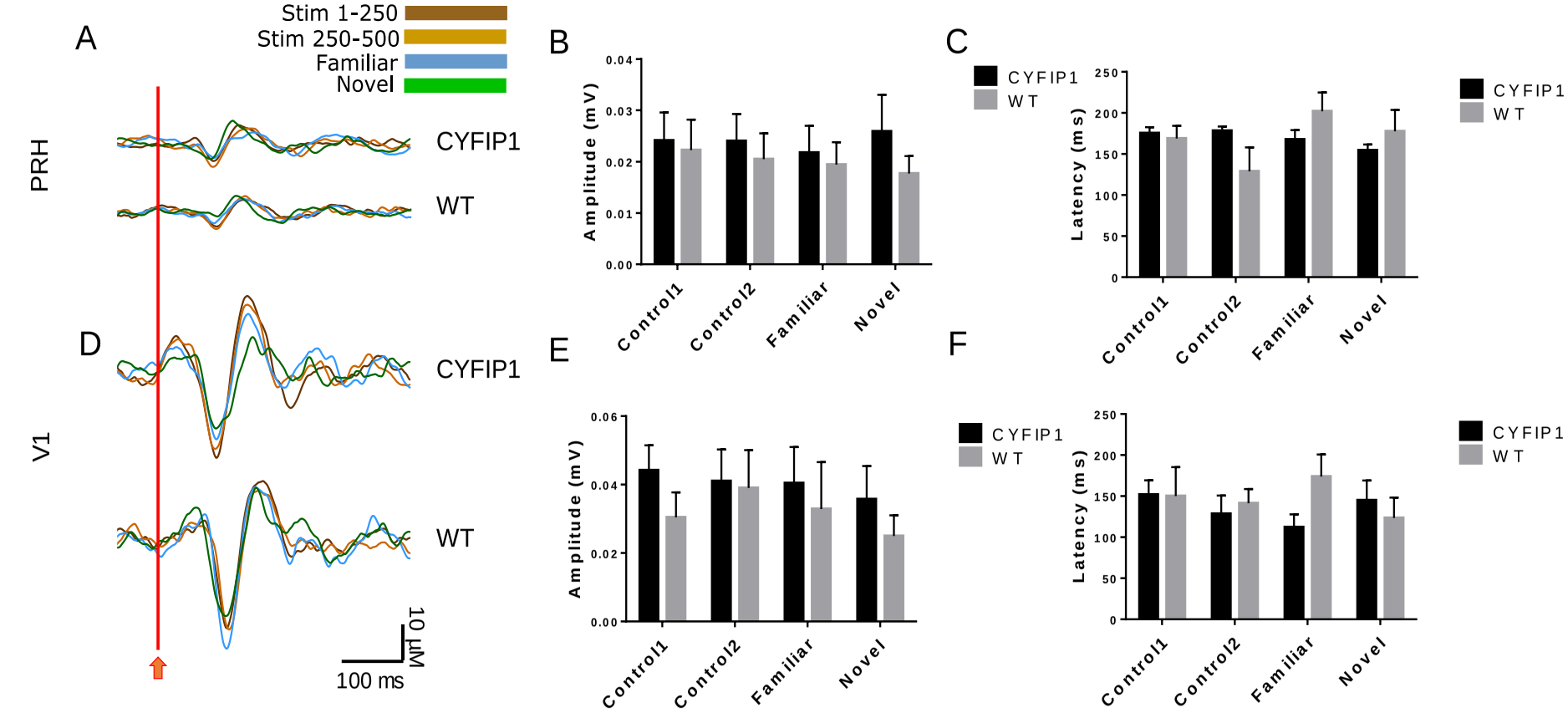

Figure 6.6. ERPs in the PRH and V1 in response pictures of objects. A) *The ERP in the PRH.* ***B)*** *The average PRH amplitude of the ERP response in both the CYFIP1 and WT mice in first 250 presentations of a stimulus (Control1), the second 250 presentations of a stimulus (Control2), 250 presentations of the control stimulus after a 2-min retention interval (Familiar) and 250 of a stimulus in a different orientation (Novel).* ***C)*** *The latency of the PRH ERP for Control1, Control2, Familiar and Novel conditions.* ***D)*** *The ERP in V1.* ***E)*** *The average amplitude of the V1 ERP for Control1, Control2, Familiar and Novel conditions.* ***F)*** *The latency of the V1 ERP for Control1, Control2, Familiar and Novel conditions.*

6.4 Discussion

The current experiments examined the CYFIP1 mice's recognition memory system both behaviourally and electrophysiologically. The short and long-term recognition memory of these mice was tested for objects, locations and object-location associations. Then, two parts of the visual recognition memory system were examined by recording from V1 and the PRH of these mice while they were performing some of the tasks described in chapter Chapter 3 . According to these experiments these animals perform as well as their WT littermates in all the forms of recognition memory under examination. Further, the ERPs recorded from both V1 and the PRH recorded in these mice was not different to those recorded in their WT littermates.

The fact that a change that causes haplo-insufficiency in a single gene does not fully replicate complex polygenetic conditions such as SZ or ASD is not surprising. As was discussed in chapter 5, the CYFIP1 haplo-insufficiency replicates, to some extent, the auditory phenomena seen in these diseases. However, they do not exhibit the memory conditions that usually accompany ASD and SZ. Further, they do not seem to show sensory deficits in V1 or the PRH. Together, these results seem to suggest that the deficits in these animals are limited to the auditory modality.

The FMR protein (FMRP) is downstream target of the CYFIP1 protein, such that an increase in CYFIP1 suppresses the translation of genes downstream from FMRP[271]. Importantly, CYFIP1 does not directly control the translation of the FMR1 gene, the gene that codes for the FMRP[271]. As discussed in section 5.1, FMR1 KO mice show auditory phenomena similar to the CYFIP1 heterozygous mice[251]. However, they also show recognition memory deficits, at least when tested on NOR[168], unlike the CYFIP1 mice that showed no deficits in three different types of recognition memory. Similar to the CYFIP1 mice the FMR1 KO mice show no difference in the visual ERP amplitude in V1 compared to WT[289]. However, the FMR1 KO mice do show some changes in plasticity in V1. These mice show increased plasticity after monocular deprivation, revealed by a reduction in the visual ERP of the ipsilateral eye[289]. In the experiments reported here, the CYFIP1 showed to difference in latency or amplitude of the visual ERPs in either V1 or the PRH. Moreover, in the case where a form of familiarity-related

plasticity could be observed in V1 (section 3.3.1), no difference could be observed between the CYFIP1 mice and their WT littermates.

These results might suggest that maybe haplo-insufficiency alone while sufficient to cause the auditory deficits observed in mental illness, is not sufficient to cause cognitive deficits, for example in memory, or deficits in visual responses. Support to this idea is given by the fact that a complete KO of the FMR1 gene causes auditory deficits[251] together with recognition memory[168] and visual ERP deficits[289]. To test this, since CYFIP1 KO mice are not viable[290], conditional CYFIP1 KO mice should be used for future experiments.

One caveat of the current results is that only 5-6 animals were tested per group due to availability issues with the CYFIP1 mice. Thus, it might be that the reason no differences were picked up is due to lack of statistical power, rather than due to lack in difference. One Indication of that is the lack of a significant novelty effect similar to the one observed in pure WT. Looking at the graphs it seems that indeed the amplitude of the V1 response tends to be lower than the familiar one, however it is was not statistically significant under these conditions. Still, the current results at least mean that there is no = general difference in ERP in V1 and the PRH between CYFIP1 haplo-insufficient mice and their WT littermates.

Yet, taken together these results show that, at least in the modalities tested, the deficits the CYFIP1 mice show are limited to the auditory domain. These experiments help delineate the profile of deficits exhibited by this model, and aid our understanding of the genetic pathways leading to mental illnesses.

Chapter 7 General Discussion

7.1 Summary of main findings

This work set out to examine the neuronal responses of the PRH in response to visual stimuli in general and to familiarity and novelty in particular. A major finding was that the PRH, while visually responsive, did not change its response with familiarity, at least under the experimental condition used. A second part of the work, examined the CYFIP1 animal model, which is a putative model for mental illness. AEP deficits are prevalent in many mental illnesses, and therefore they were examined in these mice. These experiments revealed that the CYFIP1 haplo-insufficient mice exhibited AEP deficits akin to those found in people suffering from and animal models related to FXS. When memory processes were assessed in CYFIP1 mice, however, there was no evidence of impairment in any aspect of recognition memory. Furthermore, ERP recording from V1 and the PRH revealed no differences between CYFIP1 and WT control mice.

7.2 The CYFIP1 model

In Chapter 5 and Chapter 6 the CYFIP1 mice were examined. These mice exhibited several deficits in their AEP, which mimic those that can be seen in SZ and FXS. However, when their recognition memory was tested they exhibited recognition memory performance comparable to their WT littermates, at least when tested using the SOE paradigm.

These tests add to other deficits and changes observed in these mice compared to WT[153,290]. The fact that these deficits are limited to only some modalities is not surprising, and one might say, even encouraging. Knowing exactly what deficits a disruption of a specific gene causes will help to eventually understand the part that each gene plays in the onset of mental illnesses and the deficits they cause.

7.3 Evoked Potentials

The technique used in a large part of this work was ERP recordings. This technique is an important part of the electrophysiological toolbox as it can under some conditions be directly translated from animals to humans and vice versa. One clear example is the AEPs reported in chapter Chapter 5 , which are clearly exhibited in both humans and animals[132,147,218,291,292]. The ERPs reported in chapter 1 were recorded in mice for the first time. Like the AEPs, they are very similar to the ones reported in humans[177,178]. Both AEPs, and ERPs from other areas, such as the ones recorded in V1 and the PRH, can be used to assess mouse models for various mental illnesses, as was done in chapters 5 and 6. The results obtained from these experiments can, then, be readily compared to humans. And, of course, using this technique enables to gain basic knowledge on the neuronal responses in various areas, and thus contribute to our basic understanding of brain function, as was done in chapter 3.

7.4 Head-Restrained NOR

In all of the behavioural memory experiments, the method used had the mouse freely-moving on a wheel with its head restrained, similarly to previous research into V1, with the slight difference of having the mouse freely run on a wheel[197]. While, of course, an NOR arena is more natural, in it one loses the ability to control the visual stimulus properties. Thus, in the NOR arena the mouse encounters objects from different angles, with slightly different lighting conditions and so on, and this might affect the neuronal response in the various visual area and subsequently the higher-order areas too. In comparison, in the head-restrained condition, one has fuller control on the time, duration and shape of the stimulus. This greater control over the stimulus comes at a price of less freedom of movement. However, as discussed below that can be compensated for by using 3D technologies to get as close to a freely-moving paradigm as possible. Thus, in my opinion, this paradigm, with the adjustments discussed below, should continue to be used in future research in this area.

7.5 Implications for recognition memory

One important finding was that there was no modulation of neuronal activity in the PRH as a product of stimulus familiarity. This was even in cases when changes could be observed in V1, when a highly familiar object picture had a stronger ERP than less familiar novel object pictures. These results contrast with current theoretical opinion that views the PRH as the area of the brain that underlies familiarity[27].

However, as discussed in chapter Chapter 3 , the apparent contradiction might help refine the current knowledge of PRH function. The experiments that established the PRH as an area of familiarity were mainly lesion studies[52,53,58,65,68,293] (section 1.3.1), which have their own set of problems, the least of which is the destruction of connections to other areas. Furthermore, even when using lesion studies, a PRH lesion was shown not to actually impair recognition memory, when visual interference during the retention interval was low[51]. In this experiment animals that were put in a dark room right after an NOR session showed normal recognition memory even after PRH lesion, thus suggesting that lesions to the PRH is necessary for familiarity memory only in high-interference conditions.

Studies recording single neurons in the PRH showed changes in neuronal response to familiar visual stimuli[54,55,103,104,109,111,114]. However, when these studies are examined more closely one can learn two crucial bits of information. First, previous research was done under rewarded condition (see discussion in section 3.4). Second, the familiar stimulus was presented over several days. Presentation of a stimulus over days was shown to change the response in V1[197], and there is no reason to believe it does not affect areas downstream from this area. More importantly, one should remember that this neuronal activity should explain ‘immediate familiarity’, for example as the one exhibited in the NOR task, or the human experience of recognising that a person seen once in the past had already been encountered, and not a prolonged habituation to a stimulus or an event.

Thus, alongside other experiments using the NOR task[112,113], the current results show that despite the PRH responding to novelty under certain conditions, it is

not the main area responsible for the type of incidental familiarity experienced in day-to-day life and operationalised in the NOR task in animals. This means that instead of one holistic mental phenomenon of familiarity, I would argue that two different types of familiarity have been actually studied. One is ‘ultra-familiarity’ or habituation, which is what has been mainly studied in the literature. The PRH might indeed underlie this type of familiarity, as studies have repeatedly identified neuronal populations in the area that respond differently to familiar cues[54,55,103,104,109,111]. The other type, operationalised mainly by the NOR task in animals, seems not to rely on the PRH, as no familiarity-responsive neurons could be observed using the NOR task[112,113], or in the current experiments. Also, under certain conditions, rats were shown to perform well on the NOR task even after complete PRH ablation[51], which further strengthens the evidence that the PRH is not necessarily crucial for this ‘incidental familiarity’.

7.6 Future work

7.6.1 The CYFIP1 Model

This mouse model is relatively new. If one wants to fully study the effects of this gene, much more work should be done to characterise the deficits that these mice exhibit. Being heterozygous mice, there is always the possibility that they will not present the entire extent of a mutation in the gene. Also, there might be some compensatory mechanisms in play, since the deficit is present since inception. In order to truly get the full picture of the involvement of this gene in mental illness, more sophisticated mouse lines, employing conditional KO should be used.

Since the CYFIP1 mice show AEP deficits, they might also show other deficits as well. In the current set of experiments, only MTL-related recognition memory was tested. The memory deficits of this model might be specific to a certain type of memory. For example, the FMR1-KO mice show normal spatial memory, while showing deficits in fear learning[270]. Thus, more memory tests should be done in the future, to have the complete behavioural deficit profile of these mice.

For example, deficits in fear memory seem to be a robust feature of the FMR1 KO model[270,294]. Similarly, the CYFIP1 mice, showed enhanced extinction[152]. Thus, the next step in the study of the CYFIP1 model should be looking into the fear-related circuitry. This can be done by first finding out the extent of the deficits in fear learning they exhibit, and then by recording the neuronal activity in the amygdala, the centre of the fear-learning circuitry, and determine how neuronal changes there may help explain these behavioural differences.

7.6.2 Familiarity Memory

My work mainly suggests that the PRH does not underlie familiarity memory. That means that the ultimate question of what areas support familiarity is still open. It is known that there are familiarity responses as early as V1 as shown in this thesis and in previous research[197]. It might be the areas further down the 'what' stream support familiarity of more complex feature. This was suggested previously in a model that postulates that the more rostral the area is in the visual stream the more complex are the features it processes[295]. It might be that the PRH exhibits familiarity response but to complex combination of features, possibly even multi-modal. This hypothesis can be readily tested.

To begin with, the experimental paradigm presented here is ideal, since it enables full control of the visual stimuli presented. Using this behavioural method, large-scale recordings from various areas in the visual stream, informed by connectivity, should be done to investigate how their response changes with familiarity. Thus, future experiments should combine the method used in this thesis to examine familiarity responses, but have stimulus complexity as another factor. Thus, an example experiment can include recording from V1, PHC, HPC and PRH, while one familiarity session includes a square as a familiar stimulus and a triangle as a novel one. Then, a second more 'complex' session might have a triangle on top of a square as the familiar stimulus, and a square on top of a triangle as the familiar one. It might be the case both the shapes will not cause any difference in response in V1, since both are similarly familiar, they might cause a difference in response in higher order areas such as the PHC, and maybe different spatial dimensions might cause a response in the HPC as well (See **Figure 7.1** fore suggested stimuli and areas).

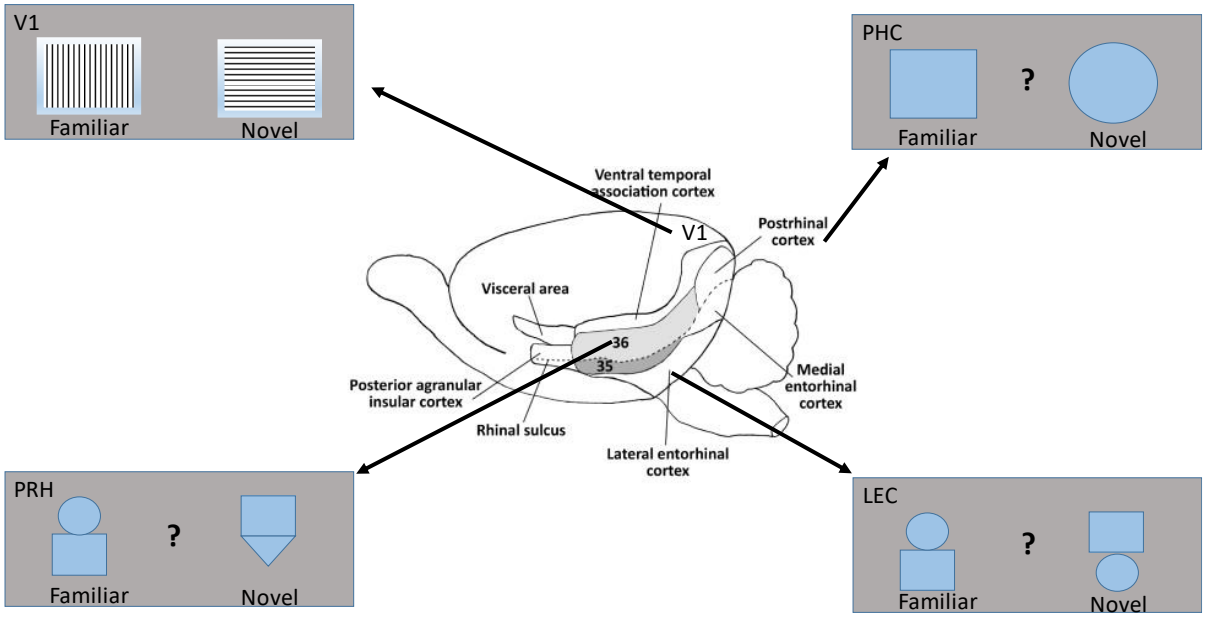

Figure 7.1. A possible model for visual-stream familiarity. *A proposed model for what level of stimulus familiarity areas of the 'what' visual stream support. Areas for which no clear evidence for the neuronal response in relation to familiarity are marked '?'. Main image adapted from*[171]*.*

7.6.3 Neuronal populations

To fully understand the activity of the PRH, one needs to know how the different neuronal types in this brain region respond to visual stimuli and how they interact. Previous research, done in vitro, found many different neuronal populations in the PRH (see section 1.3.3). To be able to know how the isolated units in extra-cellular recordings relate to a specific neuronal type one has to start with a simultaneous intracellular and extra-cellular recording of different cell types in the PRH and the HPC. This would help in building a 'dictionary' of the correspondence between extra and intra-cellular spikes. Having this 'dictionary' will, in turn, stablish which cell population is being passively recorded using silicon probes.

The experiments described in this thesis examined neuronal response while looking at all recorded neurons at the electrode. However, it might be that different neuronal sub-populations react differently to visual stimuli. Knowing how extra-cellular spikes relate to intracellular spiking activity will enable studying how the different cell-types in the PRH and HPC and any other area of interest, react to visual stimuli. Once that is established, one can then move on to manipulating the different cell types using opto-[296] or chemo-genetic[297] approaches to establish their necessity for visual perception and memory.

Further, it is known that different cell-types appear in different proportion in the layers of the PRH. Might it be that some layers are more sensitive to familiarity due to the cell-types they are populates with? This can be further explored by designing specific probes that enable recording over the entire stretch of the PRH, and with the addition of optogenetic approach, one can identify specific cells within these layers and examine their response to visual stimuli in general and to the novelty-valence of the stimuli.

7.6.4 Behavioural Paradigm

As discussed above, the behavioural paradigm used in the current experiments was meant to emulate what the mouse experiences while freely moving in an arena as closely as possible, while maintaining control of the visual stimulus it receives. In this thesis, only one 'type' of recognition memory – visual object recognition was used. However, the same method could be used to test any type of memory studied in the normal SOE environment. For example, to study the neuronal response to spatial memory an object could be presented on the right or left or the visual field for a number of trials, and then it can be presented on either the 'familiar' side or a different 'novel' side. Many more such manipulations can be used. Further, with 3D technology[298,299] one might be able to fully mimic a freely-moving arena, while controlling for the exact point-of-view of the mouse.

In this thesis, an attempt was made to study familiarity in its most basic form in a way that does not involve a reward or other motivational cues. However, one drawback of the approach is that no indication exists of whether an item was actually recognised as familiar or not by the animal. Thus, the distinction between trials in which the mouse recognises the item and those in which he fails to do so is missed. At the cost of making it into a motivated learned task, one can train a mouse to respond in a specific way to familiar stimuli. This will allow to distinguish between trials in which the familiar object was indeed recognised and those where it was not.

7.7 Conclusion

In the first part of the thesis, the long-standing theory that views the PRH as the centre of familiarity memory was challenged. No familiarity-related changes in response were observed at both the ERP level, and the single-unit level of analysis. This raises the question as to where familiarity actually occurs in the brain, and, indeed, whether a single familiarity region exists at all. Future research should, therefore, focus on finding out what areas of the brain contribute to familiarity and on computational theories explaining how this process occurs. The second part of the thesis focused on the CYFIP1 mice. It was found that these mice exhibit AEP deficits similar to ones observed in FXS patients, while not showing any recognition memory deficits, or changes in the V1 and PRH ERP. These results add to the current knowledge base built on this mouse model, and also show that their deficits are fairly specific, and maybe limited to the auditory domain. The important question is then what mechanism leads from CYFIP1 haplo-insufficiency to auditory deficits. This, in turn, might help explain why auditory deficits are such a pervasive feature of SZ and ASD.

Chapter 8 References

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