

1 **Heterozygous deletion of SYNGAP enzymatic domains in rats causes selective**  
2 **learning, social and seizure phenotypes**

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## 1 **Abstract**

2 Pathogenic variants in *SYNGAP1* are one of the most common genetic causes of  
3 nonsyndromic intellectual disability (ID) and are considered a risk for autism spectrum disorder  
4 (ASD). *SYNGAP1* encodes a synaptic GTPase activating protein that modulates the intrinsic  
5 GTPase activity of several small G-proteins and is implicated in regulating the composition of  
6 the postsynaptic density. By targeting the deletion of exons encoding the calcium/lipid binding  
7 (C2) and GTPase activating protein (GAP) domains, we generated a novel rat model to study  
8 SYNGAP related pathophysiology. We find that rats heterozygous for the C2/GAP domain  
9 deletion (*Syngap*<sup>+/ $\Delta$ -GAP</sup>) exhibit reduced exploration and fear extinction, altered social  
10 behaviour, and spontaneous seizures, while homozygous mutants die within days after birth.  
11 This new rat model reveals that the enzymatic domains of SYNGAP are essential for normal  
12 brain function and provide an important new model system in the study of both ID/ASD and  
13 epilepsy.

## 14 **Introduction**

15 Pathogenic mutations in genes expressed early in development contribute significantly to  
16 neurodevelopmental disorders that manifest during childhood and persist through adulthood  
17 (Parikshak et al., 2013). Such disorders often result in global developmental delay,  
18 compromised cognition and other impaired behaviours including delayed motor function,  
19 delayed or absent language acquisition and communication, as well as limited adaptive skills.  
20 Large-scale exome sequencing studies indicate that *SYNGAP1* is one of the most prevalent  
21 recurring genes accounting for as many as 0.5-1% of individuals with neurodevelopmental  
22 disorders (Deciphering Developmental Disorders, 2015, 2017; Satterstrom et al., 2020).  
23 Individuals with *de novo* pathogenic mutations in *SYNGAP1* present with moderate-to-severe  
24 intellectual disability (ID) and autism spectrum disorder (ASD) (Hamdan et al., 2011; Hamdan  
25 et al., 2009). Mutations in *SYNGAP1* are also a risk factor for epileptic encephalopathies and  
26 almost all individuals with such mutations have co-occurring childhood epilepsy (Berryer et  
27 al., 2013; Carvill et al., 2013; Mignot et al., 2016; Parker et al., 2015; Vlaskamp et al., 2019;  
28 von Stulpnagel et al., 2015).

29 *SYNGAP1* encodes multiple isoforms of a multifunctional, synaptically enriched protein,  
30 SYNGAP, that is essential for development and survival (Chen et al., 1998; Kim et al., 2003;  
31 Kim et al., 1998; Knuesel et al., 2005; Komiyama et al., 2002). *Syngap* heterozygosity  
32 (*Syngap*<sup>+/-</sup>) in mice is associated with behavioural and neurological phenotypes including  
33 deficits in learning and memory, pronounced hyperactivity, as well as reduced threshold for  
34 induced seizures and spontaneous epileptiform activity (Berryer et al., 2016; Clement et al.,

1 2013; Creson et al., 2019; Guo et al., 2009; Muhia et al., 2009; Nakajima et al., 2019; Ozkan  
2 et al., 2014; Sullivan et al., 2020).

3 SYNGAP isoform identity regulates its function and subcellular distribution (Araki et al., 2020;  
4 Gou et al., 2020; Li et al., 2001; McMahon et al., 2012). However, all isoforms share a central  
5 region comprised of a calcium/lipid binding domain (C2) and a GTPase activating protein  
6 (GAP) domain that function together to regulate the intrinsic GTPase activity of the small G  
7 proteins Ras and Rap (Krapivinsky et al., 2004; Pena et al., 2008; Walkup et al., 2015). In  
8 addition to its GAP activity, SYNGAP also regulates synaptic strength and size through its role  
9 as a scaffolding molecule by restricting access to PSD95 PDZ domains (Walkup et al., 2016);  
10 its binding to PSD-95 also appears to regulate the phase transition of the postsynaptic density  
11 (PSD) (Zeng et al., 2016).

12 While SYNGAP has both enzymatic and scaffolding functions, it is not known how the  
13 alteration of these individual functions contribute to *SYNGAP1* related pathophysiology.  
14 Interestingly, although most pathogenic *SYNGAP1* variants identified to date result in  
15 premature termination or complete loss of protein, missense or in-frame mutations within  
16 exons encoding the C2 or GAP domain have been identified in at least 14 individuals with ID  
17 (Berryer et al., 2013; Deciphering Developmental Disorders, 2017; Mignot et al., 2016;  
18 Vlaskamp et al., 2019). This raises interesting questions about the extent to which the  
19 enzymatic function of SYNGAP is responsible for behavioural and physiological phenotypes  
20 associated with *SYNGAP1* haploinsufficiency. For example, is the C2/GAP domain necessary  
21 for survival? And do these domains regulate a wide-range of behavioural traits, indicating that  
22 loss of its enzymatic function is the main feature of *SYNGAP1* haploinsufficiency? Answers to  
23 these questions will be important for understanding mechanisms underlying clinical traits  
24 associated with pathogenic *SYNGAP1* variants and related rasopathies as well as for  
25 developing targeted treatments for these disorders. To test the role of the C2/GAP domains  
26 in behaviour and physiology independent of its scaffolding role, we generated a rat model in  
27 which *Syngap* C2 and GAP domains were deleted.

## 28 **Results**

### 29 **A novel rat model of *SYNGAP1* haploinsufficiency**

30 To address whether loss of the C2/GAP domain recapitulates traits associated with *SYNGAP1*  
31 haploinsufficiency, rats were generated with specific ablation of exons encoding these  
32 domains. To delete these regions selectively, zinc finger nucleases designed to target *Syngap*  
33 exons 8 to 12 (Figure 1A) were microinjected into the pronucleus of fertilized, one-cell  
34 embryos, and then bred onto a Long-Evans (LE) background. A 3584bp selective deletion and

1 3bp insertion in one rat line were confirmed by sequencing, which resulted in a mutant protein  
2 that is 377 amino acids smaller than the original (Figure 1B). Mutant protein expression was  
3 confirmed by immunoblotting of hippocampal homogenates and found to be located at  
4 synapses by immunoblotting of hippocampal synaptosome (SNS) fractions (Figure 1C and  
5 **Supplementary Figure 1**); several mutant bands can be observed as would be predicted due  
6 to the presence of multiple SYNGAP isoforms (McMahon et al., 2012). Full-length SYNGAP  
7 protein levels in homogenates and SNS were reduced in heterozygous mutant (*Syngap*<sup>+/ $\Delta$ -GAP</sup>)  
8 rats relative to wild-type (*Syngap*<sup>+/+</sup>; WT) (+/+<sub>hom</sub>: 1  $\pm$  0.076; +/ $\Delta$ -GAP<sub>hom</sub>: 0.415  $\pm$  0.04;  
9  $t_{\text{hom}(6)}$ =6.846,  $p$ =0.0005; Figure 1D; +/+<sub>syn</sub>: 1  $\pm$  0.006; +/ $\Delta$ -GAP<sub>syn</sub>: 0.5906  $\pm$  0.082;  $t_{\text{syn}(4)}$ =4.441,  
10  $p$ =0.0113; Figure 1E), while total SYNGAP (full length + mutant) was comparable between  
11 genotypes (**Supplementary Table 1**). While *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats appeared healthy, fertile and  
12 indistinguishable from WT littermates, homozygous rats (*Syngap* <sup>$\Delta$ -GAP/ $\Delta$ -GAP</sup>) did not survive  
13 beyond P10 (Figure 1F), suggesting that the C2/GAP domains are essential for postnatal  
14 viability.

15 Since SYNGAP plays a key role in synaptic modulation (McMahon et al., 2012; Walkup et al.,  
16 2016) we asked whether heterozygous C2/GAP domain deletion results in alterations in the  
17 molecular composition of synapses. Because SYNGAP is thought to regulate incorporation of  
18 glutamate receptors in the PSD (Rumbaugh et al., 2006; Vazquez et al., 2004; Walkup et al.,  
19 2016), we first compared the expression level of several proteins associated with post-  
20 synaptic function in purified SNS fractions from *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats and wild-type hippocampus,  
21 prefrontal cortex, and amygdala. Western blot analysis revealed no statistical differences in  
22 levels of post-synaptic proteins PSD95, AMPA receptor subunits GluA1 and GluA2, and  
23 NMDA receptor subunits GluN2A and GluN2B or of pre-synaptic protein synaptophysin (Synp)  
24 between genotypes in SNS from P60 animals (Figure 1G, **Supplementary Figure 1** and  
25 **Supplementary Table 1**). This suggests that the SYNGAP scaffolding function is maintained  
26 in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats.

### 27 ***Syngap*<sup>+/ $\Delta$ -GAP</sup> rats display impaired extinction in a cued-fear conditioning paradigm**

28 Pathogenic SYNGAP1 mutations are associated with significantly limited cognitive ability and  
29 consequent deficits in adaptive functioning, with anecdotal caregiver reports of behavioural  
30 inflexibility. To investigate the effect of heterozygous loss of the SYNGAP C2/GAP domain on  
31 cognition and adaptive behaviour, we used a cued fear conditioning task (flashing light CS,  
32 footshock US) to examine acquisition, recall and extinction of fear memory (Figure 2). Neither  
33 *Syngap*<sup>+/ $\Delta$ -GAP</sup> nor WT littermates expressed freezing in the conditioning chamber before  
34 experiencing the first US. Both genotypes showed comparable freezing over 6 paired CS-US  
35 presentations during conditioning (2-way RM ANOVA, effect of CS presentation  $F_{(5,105)}$ =54.87,

1  $p < 0.0001$ ; genotype  $F_{(1,21)} = 0.1912$ ,  $p = 0.6664$ ; CS x genotype  $F_{(5,105)} = 1.368$ ,  $p = 0.2425$ ; Figure  
2 **2A**).

3 24 hours after training, rats were placed in a different testing context to assess retention and  
4 extinction of the conditioned response to unreinforced CS presentations. Both WT and  
5 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats showed low freezing responses in the testing context prior to the first CS  
6 presentation, suggesting little fear generalization to the testing context (2-way ANOVA effect  
7 of genotype  $F_{(1,33)} = 3.667$ ,  $p = 0.0642$ ; protocol  $F_{(1,33)} = 7.702$ ,  $p = 0.009$ ; genotype x protocol  
8  $F_{(1,33)} = 2.407$ ,  $p = 0.1303$ ; Figure **2B**). Both groups showed similar high levels of freezing to the  
9 first presentation of the unreinforced CS during recall testing, suggesting comparable fear  
10 memory retention and expression. However, *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats showed more overall freezing  
11 during the recall test compared to WT rats which was due to greater freezing to subsequent  
12 unreinforced CS presentations (2-way RM ANOVA, effect of genotype  $F_{(1,396)} = 91.16$ ,  
13  $p < 0.0001$ ; also see **Supplementary Table 1**; Figure **2B**). Moreover, while WT rats decreased  
14 their freezing over repeated unreinforced CS presentations, *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats showed no  
15 apparent extinction learning (2-way ANOVA, effect of genotype  $F_{(1,33)} = 5.653$ ,  $p = 0.0234$ ; CS  
16  $F_{(1,33)} = 40.93$ ,  $p < 0.0001$ ; genotype x CS  $F_{(1,33)} = 2.198$ ,  $p = 0.1477$ ; Figure **2C**), suggesting  
17 reduced behavioural flexibility in this associative learning task.

18 Control rats receiving unreinforced CS during training (CS-only) showed significantly less  
19 freezing than rats trained with the paired CS-US protocol (3-way ANOVA, effect of protocol  
20  $F_{(1,198)} = 203.3$ ,  $p < 0.0001$ ; CS x protocol  $F_{(5,198)} = 14.27$ ,  $p < 0.0001$ ; Figure **2A**), implying that  
21 the flashing light of the CS is not aversive on its own. Rats trained with the paired CS-US  
22 protocol froze more during the CS presentations than between CS presentations regardless  
23 of genotype, whereas the CS did not phasically modulate freezing behaviour in control rats  
24 that received unreinforced CS during training (Figure **2D**); this is confirmed by calculation of a  
25 modulation index whereby a positive value indicates more freezing during the CS than in its  
26 absence (2-way ANOVA, effect of protocol  $F_{(1,33)} = 29.26$ ,  $p < 0.0001$ ; Figure **2E**). Although  
27 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats show less modulation of freezing by the CS overall (2-way ANOVA, effect  
28 of genotype x protocol  $F_{(1,33)} = 5.551$ ,  $p = 0.025$ ), the modulation index of rats trained with the  
29 paired CS-US protocol was significantly greater than zero (one sample *t*-test,  $t_{WT(11)} = 14.545$ ,  
30  $p < 0.001$ ;  $t_{\Delta\text{-GAP}(10)} = 4.340$ ,  $p = 0.001$ ), indicating that greater freezing of *Syngap*<sup>+/ $\Delta$ -GAP</sup> across the  
31 extinction trial was not due to generalised fear. Consistent with this, of rats trained with the  
32 paired CS-US protocol, *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats exhibited less modulation of freezing by the CS than  
33 WT early in the recall test, but modulation was comparable between genotypes later in the  
34 test (**Supplementary Table 1**).

1 Several reports indicate that mouse models of *SYNGAP1* haploinsufficiency exhibit  
2 hyperactivity and abnormal measures of anxiety (Berryer et al., 2016; Guo et al., 2009; Muhia  
3 et al., 2009; Nakajima et al., 2019; Ozkan et al., 2014) which can affect performance in, and  
4 confound the analysis of, tasks designed to study animal cognition (Crawley et al., 1997).  
5 Therefore, we determined whether deletion of the C2/GAP domain leads to anxiety,  
6 hyperactivity or locomotor abnormalities in our *Syngap*<sup>+Δ-GAP</sup> rats, assessing their behaviour  
7 in the open field and elevated plus maze. Overall distance travelled in the open field was  
8 significantly greater in *Syngap*<sup>+Δ-GAP</sup> rats compared to WT controls, but both groups showed  
9 a similar decrease in locomotion over the 20 min session (2-way RM ANOVA effect of  
10 genotype  $F_{(1,16)}=5.660$ ,  $p=0.0301$ ; effect of time  $F_{(9,144)}=60.04$ ,  $p<0.0001$ ; genotype x time  
11  $F_{(9,144)}=1.235$ ,  $p<0.2782$ ; Figure **2F**). Both groups also decreased their locomotor activity  
12 between the first and second day of exposure and distance travelled on day 2 was comparable  
13 between genotypes (2-way RM ANOVA, effect of day  $F_{(1,16)}=16.34$ ,  $p=0.0009$ ; genotype  
14  $F_{(1,16)}=3.579$ ,  $p=0.0768$ ; interaction day x genotype  $F_{(1,16)}=1.653$ ,  $p=0.2169$ ; Figure **2G**). These  
15 data suggest that, although *Syngap*<sup>+Δ-GAP</sup> rats may be initially hyperactive in an open field, this  
16 rapidly normalises as they habituate to the environment. *Syngap*<sup>+Δ-GAP</sup> and WT rats spent  
17 equivalent amounts of time in the centre of the open field, suggesting comparable anxiety  
18 levels in both groups (2-way RM ANOVA, effect of genotype  $F_{(1,16)}=2.633$ ,  $p=0.1242$ ; day  
19  $F_{(1,16)}=0.1767$ ,  $p=0.6798$ ; interaction day x genotype  $F_{(4,88)}=0.2019$ ,  $p=0.6592$ ; Figure **2H**).  
20 Similarly, spontaneous activity in the elevated plus maze as indicated by the distance travelled  
21 was comparable between *Syngap*<sup>+Δ-GAP</sup> and WT littermates (unpaired *t*-test;  $t_{(16)}=0.1149$ ,  
22  $p=0.9099$ ; Figure **2I**). Both groups also presented with similar levels of anxiety as indicated by  
23 entries into open arms (unpaired *t*-test;  $t_{(16)}=0.6892$ ,  $p=0.5006$ ; Figure **2K**) and the percentage  
24 of time spent in the open arms (unpaired *t*-test;  $t_{(16)}=1.273$ ,  $p=0.2212$ ; Figure **2J**).

25 To test whether heterozygous C2/GAP deletion affects motor coordination or learning, we  
26 measured latency to fall from the rotating cylinder on both the fixed speed and accelerating  
27 versions of the rotarod test. Performance was indistinguishable between *Syngap*<sup>+Δ-GAP</sup> and  
28 WT littermates (baseline Rotarod: 2-way RM ANOVA, effect of day  $F_{(4,88)}=12.43$ ,  $p<0.0001$ ;  
29 genotype  $F_{(1,22)}=1.606$ ,  $p=0.2183$ ; interaction day x genotype  $F_{(4,88)}=0.1084$ ,  $p=0.9793$ ;  
30 accelerating Rotarod: 2-way RM ANOVA, effect of day  $F_{(4,88)}=4.757$ ,  $p=0.0016$ ; genotype  
31  $F_{(1,22)}=2.528$ ,  $p=0.1261$ ; interaction day x genotype  $F_{(4,88)}=0.0724$ ,  $p=0.9903$ ; Figure **2L, M**).  
32 Overall, these data indicate that both groups had similar balance, coordination and motor  
33 learning. Taken together, our findings from these different behavioural tasks suggest that  
34 heterozygous deletion of the C2/GAP domains does not affect basal levels of anxiety, activity,  
35 or motor coordination.

## 1 ***Syngap*<sup>+Δ-GAP</sup> rats display normal spatial reference memory and reversal learning**

2 To further investigate the effect of the loss of C2/GAP domain of SYNGAP on cognitive  
3 function and adaptive behaviour, we tested allocentric spatial learning in a hippocampus-  
4 dependent reference memory task in the water maze ((Morris et al., 1982); Figure **3A**). The  
5 task assesses the ability to use distal cues in order to navigate to a hidden escape platform in  
6 a circular pool. During acquisition, both WT and *Syngap*<sup>+Δ-GAP</sup> rats showed similar learning of  
7 the hidden platform location over six days of training as indicated by the decrease in mean  
8 path length taken to reach the platform location (2-way RM ANOVA, effect of day  $F_{(5,75)}=16.49$ ,  
9  $p<0.0001$ ; genotype  $F_{(1,15)}=2.845$ ,  $p=0.1123$ ; interaction day x genotype  $F_{(5,75)}=1.849$ ,  
10  $p=0.1136$ ; Figure **3B**). During the two probe trials (i.e. the first trials of days 3 and 6,  
11 respectively), the percentage of time spent in the platform zone increased for both genotypes  
12 (2-way RM ANOVA, effect of probe trial  $F_{(1,15)}=7.246$ ,  $p=0.0167$ ; genotype  $F_{(1,15)}=0.4692$ ,  
13  $p=0.5038$ ; interaction probe trial x genotype  $F_{(1,15)}=0.0002$ ,  $p=0.9888$ ; Figure **3C**), indicating  
14 that spatial learning and recall is intact in *Syngap*<sup>+Δ-GAP</sup> rats.

15 We assessed reversal learning in this task as a measure of behavioural flexibility, by moving  
16 the platform to the opposite quadrant of the pool. Thus, in order to find the platform at the new  
17 location, animals need to stop visiting the old location of the platform while learning to swim  
18 to its new place. Reversal learning was equivalent between genotypes, as the path-length to  
19 reach the new escape location decreased similarly over days in both groups (2-way RM  
20 ANOVA, effect of day  $F_{(5,75)}=29.48$ ,  $p<0.0001$ ; genotype  $F_{(1,15)}=1.159$ ,  $p=0.2987$ ; interaction  
21 day x genotype  $F_{(5,75)}=0.56$ ,  $p=0.7303$ ; Figure **3D**). Furthermore, in the reversal probe trials,  
22 both WT and *Syngap*<sup>+Δ-GAP</sup> rats spent a higher percentage of time in the new target zone than  
23 in the previous platform location ('old') (Sidak's multiple comparison test: for P4  $p_{WT}=0.0018$ ,  
24  $p_{HET}<0.0001$ ; see **Supplementary Table 1**; Figure **3E**), suggesting that behavioural flexibility  
25 was comparable between both groups in this spatial reversal task. While path length to escape  
26 was indistinguishable between genotypes during training and reversal learning, swim speed  
27 in the *Syngap*<sup>+Δ-GAP</sup> rats was significantly higher than WT (2-way RM ANOVA, effect of  
28 genotype  $F_{\text{training}(1,15)}=4.945$ ,  $p=0.0419$ ; day  $F_{\text{training}(5,75)}=3.580$ ,  $p=0.0059$ ; interaction day x  
29 genotype  $F_{\text{training}(5,75)}=0.7567$ ,  $p=0.5838$ ; effect of genotype  $F_{\text{reversal}(1,15)}=6.041$ ,  $p=0.0266$ ; day  
30  $F_{\text{reversal}(5,75)}=4.714$ ,  $p=0.0008$ ; interaction day x genotype  $F_{\text{reversal}(5,75)}=1.885$ ,  $p=0.1070$ ; Figure  
31 **3F, G**). Similar to locomotion in the open field, this difference was only apparent at the start of  
32 training on each task (reference memory and reversal), so after day 3 of each task, the swim  
33 speed of *Syngap*<sup>+Δ-GAP</sup> rats had decreased to the levels of WT rats. Together, these  
34 experiments indicate that *Syngap*<sup>+Δ-GAP</sup> rats exhibit normal learning, recall, and behavioural  
35 flexibility in this spatial reference memory task.

36

## 1 **Altered social behaviour in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats**

2 Since impairments in social interactions are prevalent among ASD individuals, we used an  
3 adjusted three-chamber social interaction paradigm to assess social interaction and social  
4 preference ((Yang et al., 2011); Figure **4A**). After habituating the rats to the apparatus, we first  
5 assessed interaction with a caged, same-sex non-familiar WT conspecific compared to  
6 interaction with an empty cage. Sociability in this assay is typically defined as more time spent  
7 in the chamber with the non-familiar rat rather than in the other chamber, and more time spent  
8 sniffing the social than the non-social cage. Although *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats showed a decrease  
9 in overall exploratory behaviour (effect of genotype<sub>chamber time</sub>  $F_{(1,24)}=4.647$ ,  $p=0.0414$ ; Figure  
10 **4B**; and effect of genotype<sub>sniffing time</sub>  $F_{(1,24)}=24.55$ ,  $p<0.0001$ ; Figure **4D**), indices calculated  
11 using these measures indicate that both WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats prefer to explore the social  
12 cage significantly more than would be expected by chance alone (see Supplementary Table  
13 1; Figure **4C** and **4E**).

14 As the empty cages were present during habituation and therefore rats were familiar with them  
15 prior to the test, we aimed to determine whether this preference was due to preference for a  
16 social stimulus per se or to a more general novelty preference. A separate cohort of rats was  
17 run in a modified configuration of the task to assess whether there was a preference for  
18 interacting with an unfamiliar rat over a novel inanimate object. In this task configuration, WT  
19 and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats showed similar exploration of the social cage (time in chamber:  
20 genotype x stimulus  $F_{(1,18)}=0.3826$ ,  $p=0.5440$ , Figure **4F**; and time sniffing: genotype x  
21 stimulus  $F_{(1,18)}=0.4159$ ,  $p=0.5271$ ; Figure **4H**). Both WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats preferred to  
22 spend time in the chamber containing the social stimulus over the novel object, as indicated  
23 by a discrimination index significantly greater than zero (**Supplementary Table 1**; Figure **4G**).  
24 However, by the same measure, the *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats did not show a significant preference  
25 for actively exploring (sniffing) the social stimulus over the novel object (**Supplementary**  
26 **Table 1**; Figure **4I**). This was true for the entirety of the experiment (10 min) but also for the  
27 novelty phase, i.e the first 3 min (**Supplementary Figure 2A-H**). Together, these findings  
28 suggest that *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats lack preference for active interaction with social over non-  
29 social novel stimuli, but do prefer to be in the vicinity of social over non-social stimuli.

30 To determine whether an inability to detect object novelty prevents *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats from  
31 preferentially interacting with social versus non-social stimuli, we tested performance on a  
32 series of spontaneous recognition memory tasks (Kwon et al., 2006; Till et al., 2015) which  
33 assess the ability to discriminate novel from familiar objects (OR), changes in pairings of  
34 objects with context (OCR), object with place (OPR) and object with place and context (OPCR)  
35 over a short (2 min) retention interval (see schema in **Supplementary Figure 3A**). First, we

1 assessed engagement with novel stimuli by examining the average time rats spend exploring  
2 objects during the first sample phase of each discrimination task. Compared to WT littermates,  
3 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats tended to spend less time exploring novel objects, but this difference was  
4 not statistically significant (2-way RM ANOVA, effect of genotype  $F_{(1,11)}=4.752$ ,  $p=0.0519$ ; task  
5  $F_{(3.587, 39.46)}=1.185$ ,  $p=0.3309$ ; interaction task x genotype  $F_{(7,77)}=0.6130$ ,  $p=0.7436$ ;  
6 **Supplementary Figure 3B**). Consistent with this trend, *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats also show  
7 decreased exploration in another task, the marble interaction task, traditionally used to assess  
8 repetitive behaviours in rodent models of autism (Silverman et al., 2010). *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats  
9 display significantly decreased duration (unpaired *t*-test;  $t_{(22)}=2.161$ ,  $p=0.0419$ ;  
10 **Supplementary Figure 3D**) and frequency of interaction with the marbles (unpaired *t*-test;  
11  $t_{(22)}=2.634$ ,  $p=0.0152$ ; **Supplementary Figure 3E**). Therefore, to eliminate the possibility that  
12 reduced exploration affected performance in the discrimination tasks, we imposed an object  
13 exploration criterion (see Methods) during the sampling/testing phase(s). When we only  
14 considered WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats that had reached this criterion, their discrimination index  
15 (which is a measure of preference to explore the novel stimulus configuration over the familiar  
16 configuration) of both groups was significantly greater than zero (which would reflect equal  
17 exploration of novel and familiar) in all four recognition memory tasks (i.e. OR, OCR, OPR,  
18 OPCR; **Supplementary Figure 3C, Supplementary Table 1**). Moreover, the discrimination  
19 index did not differ between genotypes for any task (**Supplementary Table 1**), suggesting  
20 that even complex associative recognition processes remain intact in SYNGAP mutant rats.

21 To control for the possibility that olfactory impairments prevent *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats from  
22 discriminating non-social and social odours, we tested both groups in a modified odour  
23 habituation-dishabituation task (Yang & Crawley, 2009). Although *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats explored  
24 most odours significantly less than their WT littermates on the first exposure (two-tailed  
25 unpaired *t*-tests;  $t_{\text{banana}(19)}=5.568$ ,  $p<0.0001$ ;  $t_{\text{almond}(18)}=5.213$ ,  $p<0.0001$ ;  $t_{\text{social1}(12)}=2.427$ ,  
26  $p=0.0319$ ;  $t_{\text{social2}(11)}=0.5930$ ,  $p=0.5652$ ; **Supplementary Figure 4C and F**) both genotypes  
27 showed a progressive decrease in sniffing over repeated presentations of the same odour and  
28 increased sniffing levels when a novel odour was presented (**Supplementary Figure 4A, B,**  
29 **D, and E**). These data indicate normal function of the main olfactory system and vomeronasal  
30 organ and suggest that the altered social behaviour in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats is not driven by an  
31 inability to discriminate among social and non-social odours.

### 32 **Behavioural and network analysis reveals the presence of seizure-like events in** 33 ***Syngap*<sup>+/ $\Delta$ -GAP</sup> rats that can be suppressed by ETX**

34 In addition to cognitive and behavioural symptoms, more than 85% of individuals with  
35 pathogenic SYNGAP1 mutations exhibit epilepsy (Berryer et al., 2013; Hamdan et al., 2009;

1 Klitten et al., 2011; Mignot et al., 2016; Pinto et al., 2010; Vlaskamp et al., 2019). Moreover,  
2 several patients with epileptic encephalopathy, a debilitating form of epilepsy with poor  
3 diagnosis due to refractory seizures and cognitive arrest, were found to carry *de novo*  
4 truncating mutations in *SYNGAP1* (Carvill et al., 2013). We noted home-cage behaviours  
5 associated with absence seizures among *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats, including head bobbing and  
6 occasional forelimb clonus and loss of balance (**Supplemental video 1**), which map directly  
7 onto low-level Racine stages used to rate seizure intensity (Racine, 1972). To verify that these  
8 behaviours represent seizures we recorded from chronically implanted 32-channel skull  
9 surface grid EEG probes (Figure **5A**) coupled with video and accelerometer recordings.

10 Off-line visual and automated scoring identified prominent spike and wave discharges (SWDs)  
11 that generalized across all channels (Figure **5B**, **Supplementary Figure 5** for seizure  
12 detection). The incidence of SWDs in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats was significantly higher (75%, 9/12  
13 rats) than in WT littermates (16%, 2/12 rats), or in WT LE rats of the same age from an  
14 unrelated colony in the lab (0/6 rats) (Fisher's exact test,  $p=0.014$ ; Figure **5C**). In both  
15 *Syngap*<sup>+/ $\Delta$ -GAP</sup> and WT littermates, SWDs co-occurred with an absence of locomotion and head  
16 bobbing related to absence seizures (**Supplemental video 2**). Spectral analysis of SWDs  
17 showed a prominent peak power in the theta band and a robust second harmonic (Figure **5D**)  
18 with no differences in power at any frequency bands between genotypes (Figure **5E**). The  
19 majority of SWDs occurred during quiet wakefulness (98.5%  $\pm$  0.6% *Syngap*<sup>+/ $\Delta$ -GAP</sup> and 99.2%  
20  $\pm$  0.8% WT) although the spectral properties of wakefulness were not significantly different  
21 between genotypes (**Supplementary Figure 5A-B**). Both the total number of SWDs (Figure  
22 **5F**) and number of SWDs per time awake were significantly higher in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats  
23 compared to WT littermates (Mann-Whitney U test,  $p=0.002$  and unpaired *t*-test;  $t_{(22)}=3.794$ ,  
24  $p<0.001$ ; **Supplementary Figure 5B and C** respectively). Moreover, cumulative frequency  
25 distribution profiles of SWD durations reveal that *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats had significantly longer  
26 SWDs than the WT littermates that exhibited SWDs (Kolmogorov-Smirnov test  $D_{(130)}=0.862$ ,  
27  $p<0.001$ ; Figure **5G**).

28 Since SWDs are often associated with behavioural immobility in humans and rodents  
29 (Blumenfeld, 2005; Coenen & Van Luijtelaar, 2003), we tested whether the flashing lights used  
30 as a CS in the fear conditioning paradigm induced photosensitive SWDs that presented as  
31 enhanced behavioural immobility in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats potentially confounding measures of  
32 freezing during fear recall and extinction (Figure **2A**). We recorded EEG from motor/parietal  
33 cortex and olfactory bulb while rats were introduced to flashing visual stimuli with the same  
34 properties as the CS previously used during the cued fear conditioning experiments  
35 (**Supplementary Figure 7A**). Flashing light exposure did not cause a change in the number

1 of SWD events observed in either *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats or WT littermates (**Supplementary**  
2 **Figure 7B, C**) indicating that the observed increases in freezing during fear recall/extinction  
3 are not driven by CS induced seizures.

4 To assess whether the SWDs we observed are related to absence-like seizures, we evaluated  
5 whether they could be suppressed by ethosuximide (ETX), an T-type voltage-gated calcium  
6 channel antagonist commonly used to treat absence epilepsy in humans (Zimmerman &  
7 Burgemeister, 1958) and which blocks SWD in rodents (Terzioglu et al., 2006). EEG  
8 recordings over 5 consecutive days (Figure **6A**) revealed that a single dose of ETX  
9 significantly reduced SWD event number over a 2 hour period compared to no treatment or  
10 injection of saline alone (one-way RM ANOVA, effect of treatment  $F_{(2,12)}=9.25$ ,  $p=0.004$ ; post-  
11 hoc paired t-tests - Holm-Sidak correction saline vs. ETX  $t_{(6)}=4.25$ ,  $p=0.003$ ; untreated vs ETX  
12  $t_{(6)}=2.69$ ,  $p=0.04$ ; untreated vs saline  $t_{(6)}=1.56$ ,  $p=0.146$ ; Figure **6B**). Seizure suppression by  
13 ETX was confirmed by calculation of a seizure index, whereby a negative value indicates  
14 fewer seizures than on the previous untreated day (one-way RM ANOVA, effect of treatment  
15  $F_{(3,18)}=18.24$ ,  $p<0.001$ ; post-hoc paired *t*-test - Holm-Sidak correction: ETX v pre-ETX vs post-  
16 ETX v pre-ETX  $p=0.004$ , ETX v pre-ETX vs sal v pre-sal  $p=0.006$ , ETX v pre-ETX vs post-sal  
17 v pre-sal  $p=0.005$ ; Figure **6C**). The pharmacosensitivity of SWDs to ETX suggests the seizure-  
18 like events observed in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats are related to absence epilepsy.

## 19 **Discussion**

20 To test whether reduction in the GAP enzymatic activity of SYNGAP is key to clinical traits  
21 associated with *SYNGAP1* haploinsufficiency, we generated a rat model with a heterozygous  
22 deletion of the C2 and GAP domains of *Syngap*. Although overall total levels of SYNGAP  
23 expression were not affected, endogenous, full length SYNGAP was reduced to ~60% of WT  
24 levels in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats. Importantly, the mutant protein localised to synapses and key  
25 post-synaptic proteins are present at normal levels in SNS from *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats enabling  
26 us to selectively test the role of the C2/GAP domains in behaviour and cognition independent  
27 of its scaffolding role. *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats demonstrate reduced exploration and fear extinction,  
28 altered social behaviour, and spontaneous seizures, indicating that many of the features of  
29 *SYNGAP1* haploinsufficiency result from a reduction in the regulation of the small G-proteins,  
30 Ras and Rap. Furthermore, the seizures and accompanying SWD are blocked by ETX, a drug  
31 commonly used to treat absence epilepsy suggesting a potential route to clinical benefit.

32 Using a range of behavioural tasks involving objects, social stimuli, and odours, we identified  
33 reduced exploration as a prevalent feature of *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats that is unlikely to have resulted  
34 from altered motor abilities or hyperactivity. Reduction in exploration is also unlikely to have

1 resulted directly from absence seizures, since we did not observe behaviours characteristic of  
2 absence seizures during any of our behavioural tasks and seizures have previously been  
3 shown to be suppressed by mild sensory stimuli, for example those present during behavioural  
4 tasks (Pearce et al., 2014; Rodgers et al., 2015; Vergnes et al., 1982; Wiest & Nicoletis, 2003).  
5 Instead, the decrease in exploration may reflect an inability to maintain attention or a relative  
6 lack of interest and motivation to explore novel stimuli. Of note, mice with a genetic deletion  
7 of RICH2, a synaptic Rho-GAP that binds SHANK3, show a significant fear response to novel  
8 objects but above chance performance in object recognition (Sarowar et al., 2016). This  
9 suggests a more general involvement of modulators of small GTPases in behavioural  
10 responses to novelty.

11 Despite the decrease in exploration, we found that associative learning and fear learning were  
12 unaffected in *Syngap*<sup>+Δ-GAP</sup> rats. However, they did exhibit a marked reduction in extinction of  
13 conditioned fear. This deficit in extinction learning did not result from a generalised increase  
14 in anxiety since we found no change in the open field or elevated plus maze. Furthermore,  
15 *Syngap*<sup>+Δ-GAP</sup> rats exhibited modulation of their freezing to the CS. Together, this suggests  
16 heterozygous deletion of the GAP and C2 domains compromised the animals ability to learn  
17 that the conditioned stimulus no longer predicts the footshock. However, behavioural flexibility  
18 is not globally impaired in *Syngap*<sup>+Δ-GAP</sup> rats since reversal learning in the watermaze was  
19 unaffected; this may indicate differential roles of SYNGAP across brain regions with circuits  
20 underlying emotional responses being particularly affected. Importantly, while discrimination  
21 of non-social objects and contexts was unaffected, we found that *Syngap*<sup>+Δ-GAP</sup> rats display a  
22 significant impairment in exploring social over non-social stimuli. This may suggest that  
23 deletion of the C2/GAP domain of SYNGAP also results in other social impairments. Yet, we  
24 found that, like their WT littermates, *Syngap*<sup>+Δ-GAP</sup> rats do prefer to interact with other rats  
25 rather than remaining alone. Because overall associative learning and memory also appears  
26 unaffected, it is possible that social preference impairments in *Syngap*<sup>+Δ-GAP</sup> rats arise from  
27 altered sensory processing. In fact, recent studies in *Syngap*<sup>+/-</sup> mice highlighted dysfunction  
28 in the primary somatosensory cortex that is accompanied with abnormal tactile processing  
29 (Michaelson et al., 2018) and reports from clinicians indicate that sensory processing  
30 impairments are prevalent in individuals with pathogenic variants of *SYNGAP1* (Weldon et al.,  
31 2018). Alternatively, as both fear extinction and social behaviours involve the mPFC and  
32 amygdala in humans and rodents (Ko, 2017; Shin & Liberzon, 2010), alterations in these  
33 circuits could also contribute to these deficits. In support of this possibility, altered mPFC  
34 function has been described in *Syngap*<sup>+/-</sup> mice (Clement et al., 2013; Ozkan et al., 2014).

1 SWDs are a key electrophysiological feature in genetic rat models of absence seizures  
2 (Coenen & Van Luijckelaar, 2003; Shaw, 2004). SWDs were significantly more prevalent and  
3 had longer durations in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats when compared to age-matched WT littermates,  
4 and similar to previous reports, these SWDs do not occur during our behavioural tasks and  
5 were suppressed by ethosuximide (Shaw, 2004, 2007; Terzioglu et al., 2006). Although SWDs  
6 were found in our WT animals, these were at a frequency similar to previous reports (Taylor  
7 et al., 2019). Moreover, the higher incidence of SWDs in mutants suggests that the deletion  
8 of the C2/GAP domain in rats drives cortical networks to a state of hyperexcitability leading to  
9 absence-like electrophysiological phenomena.

10 SYNGAP is a large, highly abundant synaptic protein with numerous isoforms arising from  
11 alternative promoter use and mRNA splicing (Chen et al., 1998; Kim et al., 1998; Li et al.,  
12 2001; McMahon et al., 2012). While a key role of SYNGAP is to regulate small G protein  
13 signalling, it also plays a key role as a scaffolding protein, anchoring AMPA receptors to the  
14 PSD through the regulation of transmembrane AMPA receptor-associated proteins (TARPs)  
15 and LRRTM2 (Walkup et al., 2016). Our finding that the  $\Delta$ -GAP mutant protein, which  
16 maintains its PDZ binding domain, localises to synapses would appear to rule out the  
17 possibility that disruption to the scaffolding function contributes to the phenotypes observed in  
18 our animals. However, it should be noted that SYNGAP regulation of TARPs is a process that  
19 appears selectively in neurons from female rats (Mastro et al., 2020), while the majority of rats  
20 used in this study were males. Furthermore, SYNGAP has recently been demonstrated to  
21 form a homotrimer that binds PSD-95 to cause liquid phase separation of the PSD95-  
22 SYNGAP complex (Zeng et al., 2016). It has been proposed that this process mediates the  
23 association of SYNGAP with the PSD. Activity-dependent release from the PSD during stimuli  
24 that induce LTP cause the dispersal of SYNGAP, allowing AMPA receptor recruitment (Araki  
25 et al., 2015). However, whether this process happens *in vivo* or is important for the expression  
26 of clinical features of *SYNGAP1* haploinsufficiency is not known. SYNGAP dispersal from the  
27 PSD occurs even in the presence of RAS/RAP inhibitors (Araki et al., 2015) suggesting it is  
28 independent of the GAP domain function. Hence, the phenotypes presented here would be  
29 predicted to be independent of the role of SYNGAP in phase transition of the PSD. Ultimately,  
30 this would need to be directly tested with a mutation that prevents SYNGAP dispersal following  
31 LTP-induction while maintaining the ability of SYNGAP to regulate small G protein signalling.

32 Individuals with deleterious missense mutations in the C2 and GAP domains exhibit similar  
33 behavioural and neurological profiles to individuals with mutations predicted to lead to loss or  
34 truncation of the full length protein (Vlaskamp et al., 2019). This suggests that many of the  
35 clinical features of SYNGAP haploinsufficiency result from the decrease in the enzymatic

1 function of SYNGAP. Our study supports an important role for the GAP and C2 domains,  
2 however a precise role for the enzymatic functions of SYNGAP in mediating behavioural and  
3 neurological phenotypes will require a direct comparison of the *Syngap*<sup>+Δ-GAP</sup> rats with rats  
4 heterozygous for a null mutation in *Syngap*. While the behavioural domains that are affected  
5 in mice heterozygous for a null allele of *Syngap* and *Syngap*<sup>+Δ-GAP</sup> rats are similar, the direction  
6 and quantitative nature of those changes appears to be quite different. However it is  
7 impossible to determine whether these reflect specific roles for the enzymatic domain or  
8 species specific differences in the expression of behaviours. Of note, *Syngap*<sup>+/-</sup> mice exhibit  
9 hyperactivity on a much greater scale (Berryer et al., 2016; Guo et al., 2009; Muhia et al.,  
10 2010; Nakajima et al., 2019; Ozkan et al., 2014) compared to that identified in *Syngap*<sup>+Δ-GAP</sup>  
11 rats. Hyperactivity is a potential confounding factor in measuring performance in tasks  
12 designed to study animal cognition, including expression of defensive behaviours used in fear  
13 conditioning and other behavioural and cognitive phenotypes reported in *Syngap*<sup>+/-</sup> mice. What  
14 is clear is that the enzymatic domain is essential for survival since homozygous deletion of the  
15 C2/GAP domains results in perinatal lethality, similar to *Syngap* homozygous null mice (Kim  
16 et al., 2003; Knuesel et al., 2005; Komiyama et al., 2002) and rats (Mastro et al., 2020).

17 *SYNGAP1* haploinsufficiency is a complex disorder and further research will be necessary to  
18 identify how the other functions of SYNGAP may contribute to human pathophysiology. Our  
19 findings from a new rat model provide valuable insight into the phenotypic spectrum  
20 associated with mutations in the *SYNGAP1* gene in human patients of ID and further  
21 reinforces the need for more animal models in the field of neurodevelopmental disorders.  
22 Using a novel rat model, we demonstrate that disruption of the enzymatic domain of SYNGAP  
23 is a major contributor to the pathophysiology associated with *SYNGAP1* haploinsufficiency,  
24 providing key insight into potential therapeutic strategies. Further studies into the pathology  
25 associated with mutations that affect the scaffolding functions of SYNGAP will be required to  
26 further dissect the contribution of the structural properties of SYNGAP to the varied features  
27 of this disorder.

## 1 **Methods**

### 2 **Animals**

3 Subjects were Long Evans-*SG<sup>em2/PWC</sup>*, hereafter referred to as *Syngap<sup>+/-GAP</sup>* bred in-house  
4 and kept in a 12h/12h light dark cycle with ad libitum access to water and food. Colony  
5 founders were produced by Sigma Advanced Genetic Engineering (SAGE) Labs (St. Louis,  
6 MO, US) using zinc finger nuclease (ZFN)-mediated deletion (Geurts et al., 2009) of the GAP  
7 domain of *Syngap*. Pups were weaned from their dams at postnatal-day 22 (P22) and housed  
8 in mixed genotype cages with littermates, 2-4 animals per cage. Animals were genotyped by  
9 PCR. 3-6 month old male/female animals were subsequently used for all experiments.

### 10 **RNA isolation and RT-PCR**

11 Total hippocampus RNA was isolated from 4-month olds rats using RNeasy Lipid Tissue Kit  
12 (Qiagen) as per manufacturer's instructions. 2 µg total RNA was used for cDNA synthesis  
13 using SuperScriptIII (Invitrogen) with oligo(dT) and random hexamers. PCR was performed  
14 using GC-RICH PCR System (Roche). *SYNGAP* primers (rSG\_F: ATG ACC GGG CCC GGC  
15 TG and rSG\_R: CTT CAG GAG GGC TTC CTT GCT GAG CT) spanning exons 5/6 and 12/13  
16 boundaries, respectively with endogenous amplicon ~1630bp and mutant amplicon ~363 bp.  
17 Samples were run on a 1.0% agarose gel and gel purified prior to Sanger sequencing. Protein  
18 sequences were aligned using ClustalW; location of functional domains predicted using  
19 SMART (Letunic & Bork, 2018).

### 20 **Tissue preparation and immunoblotting**

21 Hippocampi were dissected in ice cold ACSF from P60 *Syngap<sup>+/-GAP</sup>* and WT littermates, snap  
22 frozen and stored at -80C until SNS preparation. Total tissue lysate was prepared in ice cold  
23 1XSucrose/EDTA buffer (0.32M Sucrose, 1mM EDTA, 5mM Tris, PH 7.4) using 5-6 up-and-  
24 down strokes of a pre-chilled motorized Teflon glass homogenizer, followed by centrifugation  
25 at 1075 g for 10 minutes at 4°C. Pure synaptosomes (SNS; pinched off nerve terminals) were  
26 prepared by layering supernatant gently on top of a discontinuous Percoll-density gradient  
27 (3% uppermost, 10% middle, and 23% bottom; Percoll, P1644, Sigma-Aldrich, UK) and  
28 centrifuged at 47,807 g for 8 min at 4°C. The fraction between 23% and 10% was collected  
29 and re-suspended in HEPES-Buffered-Krebs (HBK- 118.5mM NaCl, 4.7mM KCl, 1.18mM  
30 MgSO<sub>4</sub>, 10mM Glucose, 1mM Na<sub>2</sub>HPO<sub>4</sub>, 20mM HEPES, PH 7.4 balanced with Trizma) and  
31 SNS were pelleted out by centrifugation at 20,198 g for 15 min at 4°C. Homogenates were  
32 prepared from total tissue lysate by centrifugation at 25,128 g for 30 min. SNS pellets and  
33 homogenates were dissolved in RIPA buffer containing protease inhibitors (Roche complete  
34 mini EDTA- free protease inhibitor cocktail 4693159001, Sigma-Aldrich, UK) and phosphatase  
35 inhibitors (cocktail II P5726, Cocktail III P0044, Sigma-Aldrich, UK); proteins were estimated  
36 by MicroBCA Assay (Pierce BCA protein estimation kit, 23225, Thermofisher, UK).

37 Approximately 10µg of each protein extract was separated on a precast gradient gel (NuPAGE  
38 4-12% Bis-Tris Protein Gels, NP0336BOX, Thermofisher) and transferred to PVDF membrane  
39 (GE10600022, Thermofisher, UK). The membrane was then blocked with 5% milk (Blotting  
40 grade blocker, 1706404, Bio-Rad) in TBST 1X at RT for 1 hour followed by incubation at 4°C  
41 overnight with primary antibodies (SYNGAP- 1:2K, PA1-046, Thermofisher; b-Actin- 1:5K,  
42 A2228, Sigma Aldrich). Membranes were washed thrice with TBST (0.1% Tween 20) followed  
43 by 1-hour incubation with HRP conjugated secondary antibodies (1:10K dilution) at RT. After  
44 washing the membranes three times with TBST, ECL (ECL-Prime western blotting system,  
45 GERPN2232, Sigma-Adrich, UK) was applied and digitally scanned using ImageQuant  
46 (ImageQuant LAS4000 scanner, GE healthcare and life Sciences). The density of individual  
47 bands was calculated using ImageJ (Version: 2.0.0). For SYNGAP levels, each value was  
48 normalized to β-actin and then to their control littermates. For pre/post-synaptic protein levels,  
49 each value was normalized to total protein and then to the average WT value.

## 1 **Open field**

2 8 WT and 10 *Syngap*<sup>+/ $\Delta$ -GAP</sup> male rats were acclimated to a holding room for at least 30 min  
3 before being individually placed in the corner of an empty grey painted wooden open arena  
4 (dimensions 100 x 100 x 50 cm, no bedding) evenly lit (avg. 40 lux on the floor). Spontaneous  
5 exploration was recorded for 20min/day on two consecutive days and activity measured using  
6 ANY-maze tracking and analysis software (Stoelting Co., IL, USA).

## 7 **Elevated plus maze**

8 The elevated plus maze apparatus was raised 80 cm above the floor, made of dark plexiglass,  
9 and comprised of four arms (two open and two enclosed by 17 cm high walls). Arms were 70  
10 cm long and 12 cm wide connected by a central square (dimensions 10 cm  $\times$  10 cm). 8 WT  
11 and 10 *Syngap*<sup>+/ $\Delta$ -GAP</sup> male rats were acclimated to a holding room for at least 30 min before  
12 the start of the experiment. Rats were then placed individually at the central square of the  
13 apparatus facing an open arm and their spontaneous behaviour was recorded and tracked  
14 with ANY-maze tracking and analysis software (Stoelting Co., IL, USA) for 10 min .

## 15 **Rotarod**

16 Rats were acclimated to the testing room for 60 min before being placed on individual lanes  
17 of a rotarod (Rotamex, Columbus Instruments, OH, USA) facing a white wall. Two trials of 90  
18 sec each were performed at a constant speed of 4 rotations per minute (rpm) for baseline  
19 assessment of motor coordination. Rats were then left to rest for 30 min in their homecage.  
20 To assess motor learning, four trials of 90 sec each were performed, during which the rotarod  
21 speed started at 4 rpm and accelerated every 8 sec, until it reached 40 rpm. The above  
22 protocol was repeated for a total of 5 consecutive days. Latency to fall from the rotating drum  
23 was quantified through the Rotamex software and averaged across trials for analyses.

## 24 **Cued Fear Conditioning**

25 12 WT and 11 *Syngap*<sup>+/ $\Delta$ -GAP</sup> male rats were acclimated to a holding room and handled there  
26 for 5 min/day for two days before habituation to the testing context (a modified Coulbourn  
27 Instruments rat Habitest box dimensions 30 cm  $\times$  25 cm  $\times$  32 cm, containing a curved plastic  
28 black and white striped wall insert, smooth plastic grey floor, no electrified grid, scented with  
29 70% ethanol by cleaning between trials) for two 5 min sessions on non-consecutive days (2  
30 or 3 days apart). Conditioning followed on the day after the second habituation to the test  
31 context and was performed in a standard, unmodified Habitest rat box with aluminium wall  
32 inserts and electrified shock floor (Coulbourn H10-11R-TC-SF) cleaned with Distel<sup>TM</sup>  
33 disinfectant wipes between trials. Conditioning occurred over a 21 min period and consisted  
34 of a 3 min period to allow for exploration of the chamber followed by 6 pairings of a conditioned  
35 stimulus (CS) co-terminating with the unconditioned stimulus (US). The CS was a 10 sec blue  
36 flashing light (5 Hz 110 lux flashes, 50 / 50 duty cycle); the US was a 1 sec, 0.8 mA scrambled  
37 foot shock delivered through the bars of the floor; CS presentations started at 180, 360, 490,  
38 770, 980, and 1280 sec into the training period. A separate control group (7 WT and 7  
39 *Syngap*<sup>+/ $\Delta$ -GAP</sup> males; CS-only) was exposed to 6 presentations of the CS alone in the same  
40 context. Before and after each session, rats rested in the holding room for at least 20 minutes.  
41 A video camera mounted above each context recorded the sessions. Percent time freezing  
42 was calculated for the first 9 sec of CS presentation during conditioning (when the footshock  
43 was absent). 24 hr after conditioning, retention of the conditioned response was tested. After  
44 rats were placed into the testing context, a 2 min period followed to allow for exploration, then  
45 twelve 30 sec long presentations of the CS, separated by 30 sec of no CS were given. An  
46 extinction index was calculated as the average freezing to CS 1-4 and CS 9-12/ total time  
47 freezing to CS 1-4 and 9-12; a modulation index was calculated as (time freezing<sub>CS</sub> - time  
48 freezing<sub>postCS</sub>)/(time freezing<sub>CS</sub> + time freezing<sub>postCS</sub>) for average freezing to CS 1-3 (early) and  
49 CS 9-11 (late).

### 1 **Spatial reference memory water maze**

2 8 WT and 9 *Syngap*<sup>+/ $\Delta$ -GAP</sup> male rats were trained in three stages in a 2 m diameter water maze  
3 containing a 10 cm escape platform. Water was made opaque using liquid latex (Palace  
4 chemicals, Liverpool, UK) and kept at a temperature of at 18-20 °C. First, rats were trained for  
5 2 days on the visible platform version of the water maze (4 trials / day, 15 min ITI, extra-maze  
6 cues obscured by a white curtain). In the second stage, the curtain was removed and wall-  
7 mounted extra-maze cues approx 1 m from the edge of the pool were visible. Rats received  
8 one daily hidden-platform training session (4 trials / day, 15 min ITI) for 6 consecutive days.  
9 Reinforced probe trials were given on the first trial on the 3<sup>rd</sup> and 6<sup>th</sup> day of training, followed  
10 by three standard training trials separated by 15 min ITI. During reinforced probe trials, an  
11 'Atlantis' platform (Spooner *et al.*, 1994) was used, which is submerged to a depth the animals  
12 cannot reach during the 1 min of the probe trial, but then automatically raises to the same  
13 depth the platform has during the training trials, i.e., 4 cm below the water surface. Each trial  
14 lasted a maximum of 2 min; rats failing to escape were guided to the platform. All rats remained  
15 on the platform for 15 sec before removal from the pool. The final (reversal) stage of the  
16 protocol started the following day and was identical to the second, with the exception that the  
17 platform was relocated to the opposite side of the pool. Platform locations were  
18 counterbalanced across genotypes. Release location was pseudorandomised for each trial  
19 and counterbalanced for genotypes across all days. During the ITI rats were dried with a towel  
20 and were returned to a holding cage (identical to their homecage), which was placed on a  
21 heating pad with monitored temperature. A video camera mounted above the pool recorded  
22 the sessions through WaterMaze software to obtain swim paths, path lengths, and swim  
23 speed. Data was averaged across trials for analyses, with the exception of data recorded  
24 during probe trials.

### 25 **Spontaneous object exploration tasks**

26 5 WT and 8 *Syngap*<sup>+/ $\Delta$ -GAP</sup> male rats underwent object recognition (OR), object-place  
27 recognition (OPR), object-context recognition (OCR) and object-place-context recognition  
28 (OPCR) testing as previously described (Langston & Wood, 2010; Till *et al.*, 2015). Briefly,  
29 rats were tested in a rectangular testing box (dimensions 60 cm x 40 cm x 50 cm) with  
30 removable walls and floor inserts that could change into two context configurations. In context  
31 1, white textured wallpaper and laminate floor were used. Blue wood laminate walls and a  
32 black rubber floor were used for context 2. After 5 consecutive days of habituation to the boxes  
33 (5 min / day), rats received 2 trials (one/day) on each of the four tasks, consisting of a 3 min  
34 sampling phase(s), a 2 min ITI, and a 3 min test phase. A video camera above the box  
35 recorded the sessions for subsequent scoring of time exploring, by quantifying time rats spent  
36 sniffing the objects. If rats did not reach a 5 sec minimum of exploration for both objects or a  
37 15 sec minimum of total object exploration during the sample phase, or did not reach a  
38 minimum of 15 sec of total object exploration in the test phase, their measures on that task  
39 were excluded from analysis as it cannot be confirmed they spent enough time exploring to  
40 learn/discriminate. For each test phase, a discrimination index  $d$  was calculated as follows:  
41 [(time exploring novelty—time exploring familiarity)/(sum time exploring)]. To determine  
42 whether animals prefer the novelty, observed index  $d$  was compared against chance  
43 performance (score of  $d = 0.0$ ) using a two-tailed one-sample  $t$ -test. Values significantly above  
44  $d = 0.0$  indicate preference for novelty. During ITIs, rats were placed in a covered plastic  
45 holding bucket containing sawdust. All objects, locations, and/or contexts were  
46 counterbalanced for trial and genotypes.

### 47 **Three chamber task**

48 Rats were habituated for 3 consecutive days to the testing apparatus: a plexiglass rectangular  
49 box (dimensions 150 cm x 50 cm x 30 cm), divided into three chambers; left and right  
50 chambers (60 cm x 50 cm each) communicated to the centre chamber (30 cm x 50 cm) via  
51 removable doors. After 2 min exploration of the central chamber, doors were opened to enable  
52 the test rat to explore the entire arena for 10 minutes. For the first habituation session (day1,  
53 H<sub>1</sub>), all three chambers were empty, whereas for consecutive habituations days 2-4 (H<sub>2</sub>-H<sub>4</sub>)

1 each outer chamber contained one wire cage. After the last habituation session, the test rat  
2 was removed from the apparatus and placed in a covered plastic holding bucket containing  
3 sawdust for 5 min before phase 1 began. Rats were either tested in a social interaction or a  
4 social preference task. 12 WT (8 male, 4 female) and 14 *Syngap*<sup>+Δ-GAP</sup> (7 male, 7 female) rats  
5 were used to assess social interaction. After exploring the central chamber for 2 min, the doors  
6 were raised, and the test rat was free to explore the entire arena for 10 min; one wire cage  
7 was left empty and the other contained a non-familiar wild-type Long-Evans rat of the same-  
8 sex and similar age. Data from males and females were pooled for this analysis as both sexes  
9 showed the same level of preference for the rat over the empty (males: effect of stimulus  
10  $F_{(1,13)}=36.39$ ,  $p<0.0001$ ; females: effect of stimulus  $F_{(1,9)}=51.55$ ,  $p<0.0001$ ). A separate cohort  
11 of rats (10 WT and 10 *Syngap*<sup>+Δ-GAP</sup> males) were used to assess social preference. In this  
12 task configuration, one wire cage contained a non-familiar rat while the other cage contained  
13 a novel object. Rats used as social stimuli were habituated to being restrained in the wire  
14 cages for at least 3 days prior to the start of the experiment, by simulating the entire procedure  
15 with WT Long-Evans rats (not used as testing animals). A video camera above the apparatus  
16 recorded the sessions for subsequent scoring of time in close interaction, by quantifying time  
17 rats spent actively sniffing, and time in chamber through ANY-maze tracking and analysis  
18 software (Stoelting Co., IL, USA). Sociability index was calculated as follows [(time exploring  
19 rat—time exploring empty)/(sum time exploring)], social preference index was calculated as  
20 follows [(time exploring rat—time exploring object)/(sum time exploring)]. To determine  
21 whether animals prefer the social stimulus, the observed index was compared against chance  
22 performance (theoretical  $u = 0.0$ ) using a two-tailed one-sample t-test. Values significantly  
23 above 0.0 indicate preference for social over non-social. The apparatus was thoroughly  
24 cleaned with baby wipes and 70% ethanol between trials. During ITIs, rats were placed in a  
25 covered plastic holding bucket containing sawdust. All experiments were counterbalanced  
26 across conditions: location, ID of stimulus rat, genotype and sex.

### 27 **EEG with 32-channel skull-surface grid probe**

28 12 WT and 12 *Syngap*<sup>+Δ-GAP</sup> male rats were anaesthetised and prepared for stereotaxic  
29 surgery. Two craniotomies were drilled for bilateral anchor screw placement (+4.0 mm AP, ±  
30 0.5 mm ML) and one for ground screw implantation (-11.5 mm AP, 0.5 mm ML), according to  
31 the frontal and caudal edges of the EEG array probe (H32-EEG – NeuroNexus, MI, USA). The  
32 EEG probe was placed on the skull with its cross symbol aligned over bregma. The ground  
33 electrode and screw were connected, the implant was covered with dental cement, and  
34 animals were allowed to recover for a minimum of 1 week post-surgery. Prior to recording,  
35 rats were habituated to the room. On recording days, up to 4 rats, in their individual home  
36 cages, were placed concurrently inside a 1 x 1 m faraday cage. 6 hour EEG recordings were  
37 acquired with an Open Ephys (Siegle et al., 2017) acquisition system (OEPS, Portugal),  
38 through a 32-channel recording headstage amplifier linked to an accelerometer (RHD2132  
39 Intantech, USA), at a sampling rate of 1 KHz.

### 40 **Manual detection of SWD**

41 For off-line visual seizure scoring, 6 EEG channels were selected from each recording and  
42 analysed on a custom-designed interphase using Igor Pro V6.3 (Wavemetrics, OR, USA).  
43 After identifying the presence of SWD events, as well as wake and sleep epochs, the data  
44 was visually scored in successive 0.2 sec epochs by an observer blinded to genotypes SWDs  
45 with an inter-SWD interval shorter than 1 sec were considered as one, while individual SWDs  
46 shorter than 0.8 sec were discarded.

### 47 **Automatic detection of SWD**

48 Spectral analysis revealed that visually scored SWDs behave as a high energy echo of a  
49 fundamental frequency ( $f_0$ ) located on the 5-10 Hz theta band ( $7.7 \pm 0.1$  on both genotypes),  
50 that resonates in several periodic harmonics across the frequency spectrum.  
51 **(Supplementary Figure 5A)**. This oscillating spectral structure of SWDs resembles a periodic

1 waveform, allowing their automatic identification through a cepstral analysis approach  
2 (Childers et al., 1977) by searching for a high amplitude peak located on a frequency band of  
3 interest. We applied an automated SWD seizure detection algorithm to voltage traces from  
4 the EEG grid electrode lead overlaid approximately on S1 (right hemisphere, AP -3.0 mm and  
5 ML 2.8 mm from bregma), as, by visual assessment, was the channel most frequently  
6 associated with high amplitude SWDs across animals. After deconvolving the raw signal using  
7 a Fast Fourier Transform (number of tapers =5), a logarithm was applied to obtain the  
8 magnitude. The signal could then be treated as semi-periodic so that the inverse Fast Fourier  
9 Transform could be applied to obtain the cepstrum and reveal the period of the fundamental  
10 frequency ( $f_0$ ) as a spike in a pseudo-time domain frequency (**Supplementary Figure 5B**).  
11 After obtaining the cepstrum for the entire EEG recording (in sliding windows of 0.2 sec), peak  
12 power cepstrum values within the relevant frequency range (5-10 Hz) were identified and  
13 normalized by their absolute maximum. The resulting vector was transformed into z-scores to  
14 homogenise possible power differences between recordings that could distort seizure  
15 threshold identification. A threshold of  $\geq 2.2 \times 10^{-5}$  standard deviations was set by comparing  
16 the values of visually scored seizures against other high magnitude noise that resulted in false  
17 positives (**Supplementary Figure 5C**). 0.2 sec time windows were time-stamped as seizures  
18 when z-scored peak cepstral power in the theta band was greater than or equal to the  
19 established standard deviation threshold (**Supplementary Figure 5D**). As in visual analysis,  
20 time-stamped SWDs with an inter-SWD interval shorter than 1 sec were considered as one,  
21 while individual SWDs shorter than 0.8 sec were discarded. For validation, the results from  
22 the automated method were compared against the visual analysis and show that over a  
23 recording period of 6 hrs 100% of the visually counted SWDs were accurately detected, as  
24 confirmed by a non-significant difference between the two methods in the number of SWDs  
25 detected (paired *t*-test;  $t_{(10)} = 1.624$ ,  $p = 0.135$ ; **Supplementary Figure 5E**). Automatically  
26 detected SWDs were also compared between genotypes, obtaining a significant statistical  
27 difference equivalent to that found by visual scoring (Mann-Whitney U test, visual detection:  
28  $U = 23$ ,  $p = 0.002$ ; automatic detection:  $U = 25$ ,  $p = 0.003$ , **Supplementary Figure 5F and 5G**  
29 respectively). The code used for analysis of this section in the study is freely available via  
30 GitHub repository (<https://github.com/Gonzalez-Sulser-Team/SWD-Automatic-Identification>).

### 31 **Pharmacological suppression of SWD**

32 2-hour EEG recordings were performed daily over 5 consecutive days (see Figure 7A for  
33 treatment timeline). Briefly, animals received no treatment on days 1, 3 or 5. On days 2 and  
34 4, recordings were made starting 1 hour after animals received a single treatment of either  
35 ETX (100 mg/mL, Sigma-Aldrich) or 0.9% saline (vehicle) with a volume dose of 1mL/kg  
36 delivered by intraperitoneal injection. Animals were counterbalanced for whether drug  
37 treatment was received before saline or *vice versa*. SWDs were quantified using the automatic  
38 seizure detection method described above by a researcher blinded to genotype and drug  
39 treatment used on each day. A seizure suppression index was calculated over the two hours  
40 of recording as follows:  $((\text{SWD}_{\text{treated}} - \text{SWD}_{\text{pre-treated}}) / (\text{SWD}_{\text{treated}} + \text{SWD}_{\text{pre-treated}}))$ . A negative  
41 value indicates fewer seizures than pre-treatment, whereas a positive value indicates more  
42 seizures than pre-treatment.

### 43 **Statistical analysis**

44 Unless otherwise stated, error bars in all graphs indicate standard error of the mean (sem)  
45 and all statistical tests were two-tailed. Unless otherwise stated, mean, median, standard error  
46 and statistics were calculated across animals. Where 3-way ANOVAs were performed (i.e  
47 figure 2), we used a mixed-effects restricted maximum likelihood (REML) model with genotype  
48 as a matching factor. This was because group sizes were different for *CSonly* and *CS-US*  
49 paired. Exact *p* values are reported within the text. All the statistical tests performed can be  
50 found in **Supplementary Table 1**. In the figures, asterisks denote significant results for alpha  
51 set at 0.05. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Diamonds illustrate above chance  
52 performance with  $p < 0.05$ .

1 **Acknowledgements**

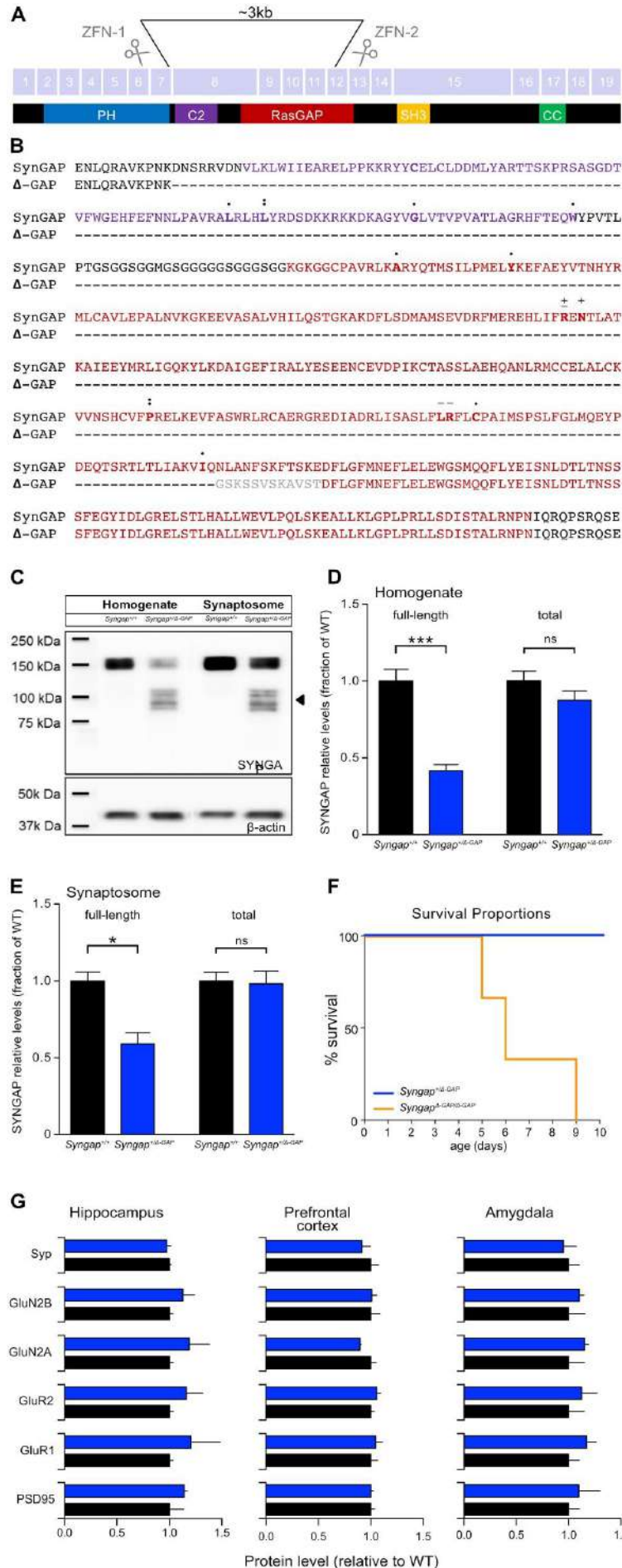
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5 Biotechnology India, Wadhvani Foundation, the Shirley Foundation, The Wellcome Trust  
6 (204954) and RS Macdonald Trust.

7 **Author contributions**

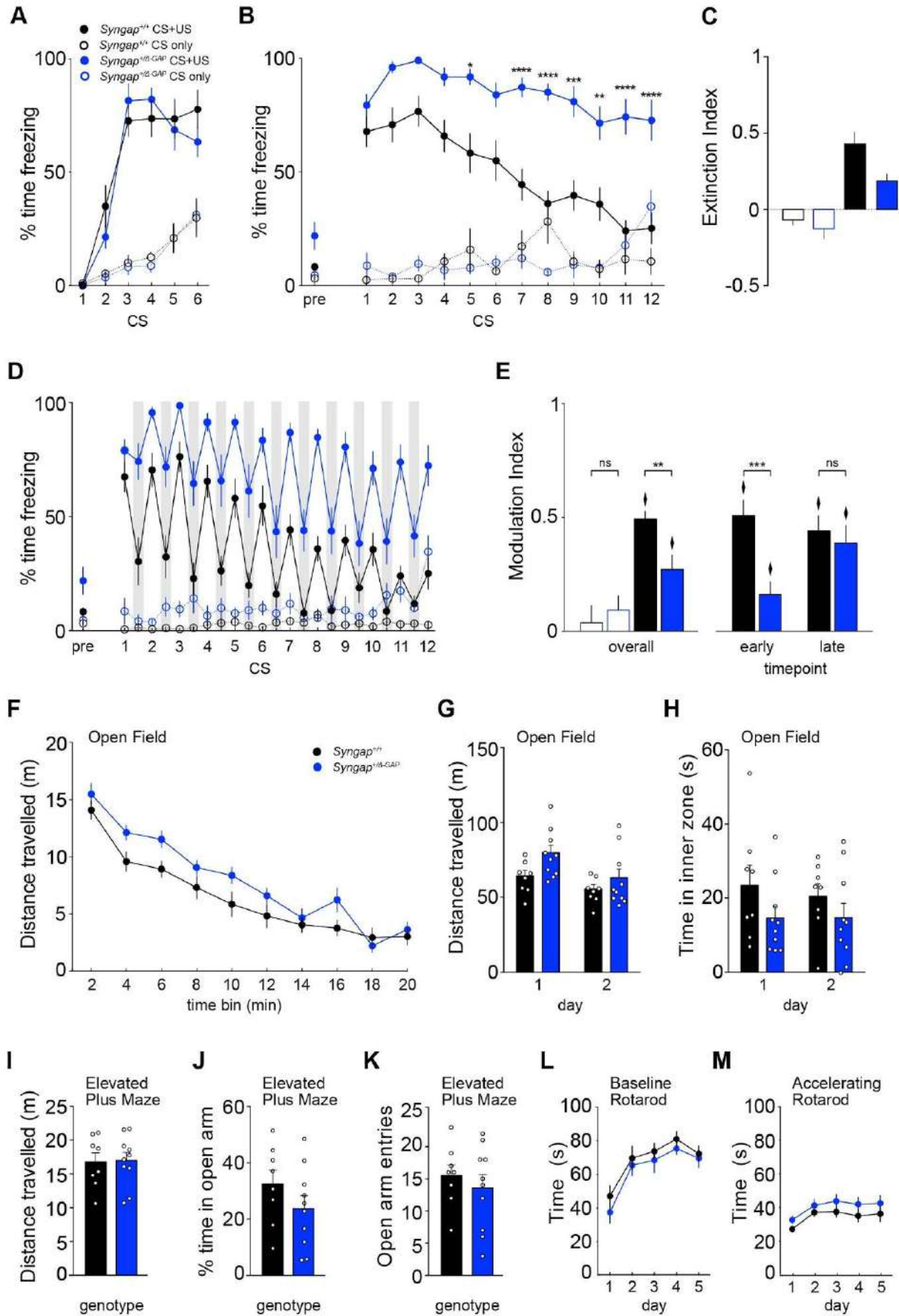
8 D.K., S.M.T., M.J., M.A.C., S.C., A.G.S., E.R.W., O.H., P.C.K. designed the experiments. D.K.,  
9 S.M.T., I.B.P., T.C.W., M.N., D.A., S.T., V.K., J.S., N.A., L.M. performed the experiments.  
10 D.K., S.M.T., I.B.P., T.C.W., S.N., S.T., V.K., N.P. analysed the data. D.K., S.M.T., and P.C.K.  
11 wrote the manuscript with input from all authors.

12 **Competing Interests**

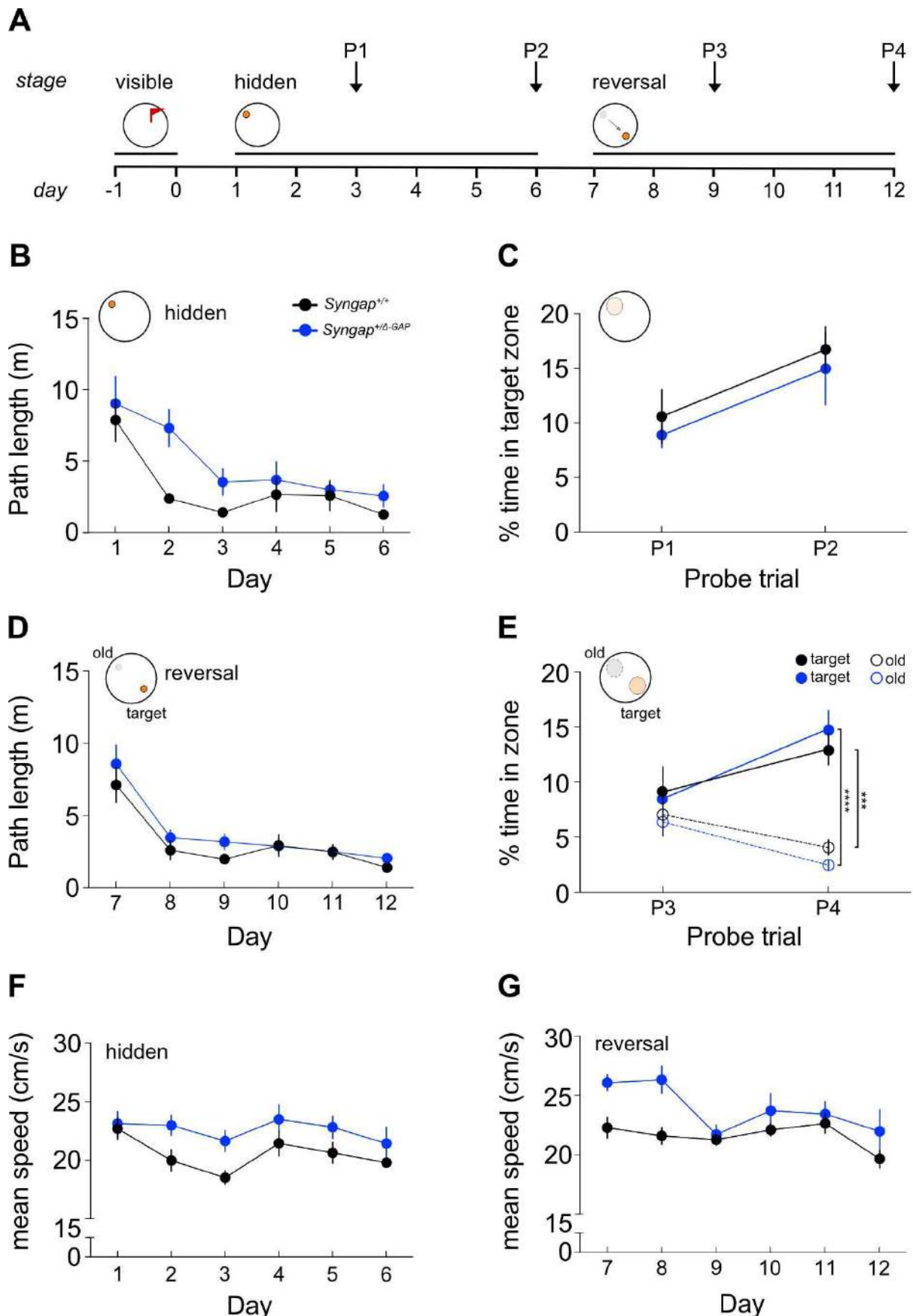
13 Authors declare no competing interests.



1 **Figure 1. SYNGAP C2/GAP domain deletion strategy in rats results in reduction of**  
2 **endogenous SYNGAP expression and reduced viability.** (A) Targeting strategy for ZFN-  
3 mediated selective deletion of *Syngap* exons 8-11 encoding C2/GAP domains. (B) Alignment  
4 of amino acids (aa) encoded by exons 8-13 of full-length and mutant SYNGAP proteins  
5 indicating the 377aa targeted deletion and unique aa resulting from 3bp insertion during  
6 targeting (grey) in relation to C2 (purple) and GAP (red) functional domains. + and - denote  
7 residues mutated to disrupt GAP function *in vitro* (Pena et al, 2008 and Vasquez et al, 2004,  
8 respectively); ± aa maps onto catalytic residue in p120GAP and NF1 (Ahmadian et al., 1997;  
9 Klose et al., 1998); dots indicate the number of instances and location of aa affected by  
10 missense mutations identified in these regions in individuals with MRD5. (C) Representative  
11 Western blot of extracts from rat hippocampal brain homogenates and synaptosomes. Bands  
12 in the molecular weight range expected for full length SYNGAP isoforms (~150kDa) were  
13 detected in homogenates and synaptosomes (SNS) from WT animals (Lanes 2 and 4,  
14 respectively). Additional bands (arrow) corresponding to the molecular weight range predicted  
15 for mutant SYNGAP isoforms are detected in homogenates and SNS from *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats.  
16 (D) Quantitation of full-length SYNGAP protein from homogenates reveals a significant  
17 decrease in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats relative to WT while total SYNGAP (full length and mutant) is  
18 comparable to between genotypes ; n<sub>+/+</sub> = 4, n<sub>+/ $\Delta$ -GAP</sub> = 4. (E) As in homogenates, full-length  
19 SYNGAP protein is reduced in SNS from *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats but total protein levels are  
20 comparable to WT ; n<sub>+/+</sub> = 3, n<sub>+/ $\Delta$ -GAP</sub> = 3. mean ± SE is noted. (F) Juvenile *Syngap* <sup>$\Delta$ -GAP/ $\Delta$ -GAP</sup>  
21 rats die by postnatal day 10 (n <sub>$\Delta$ -GAP/ $\Delta$ -GAP</sub> = 10). (G) Quantitation of pre- and post-synaptic  
22 proteins in SNS from hippocampus, prefrontal cortex and amygdala normalised to total protein  
23 and wild-type littermate controls.



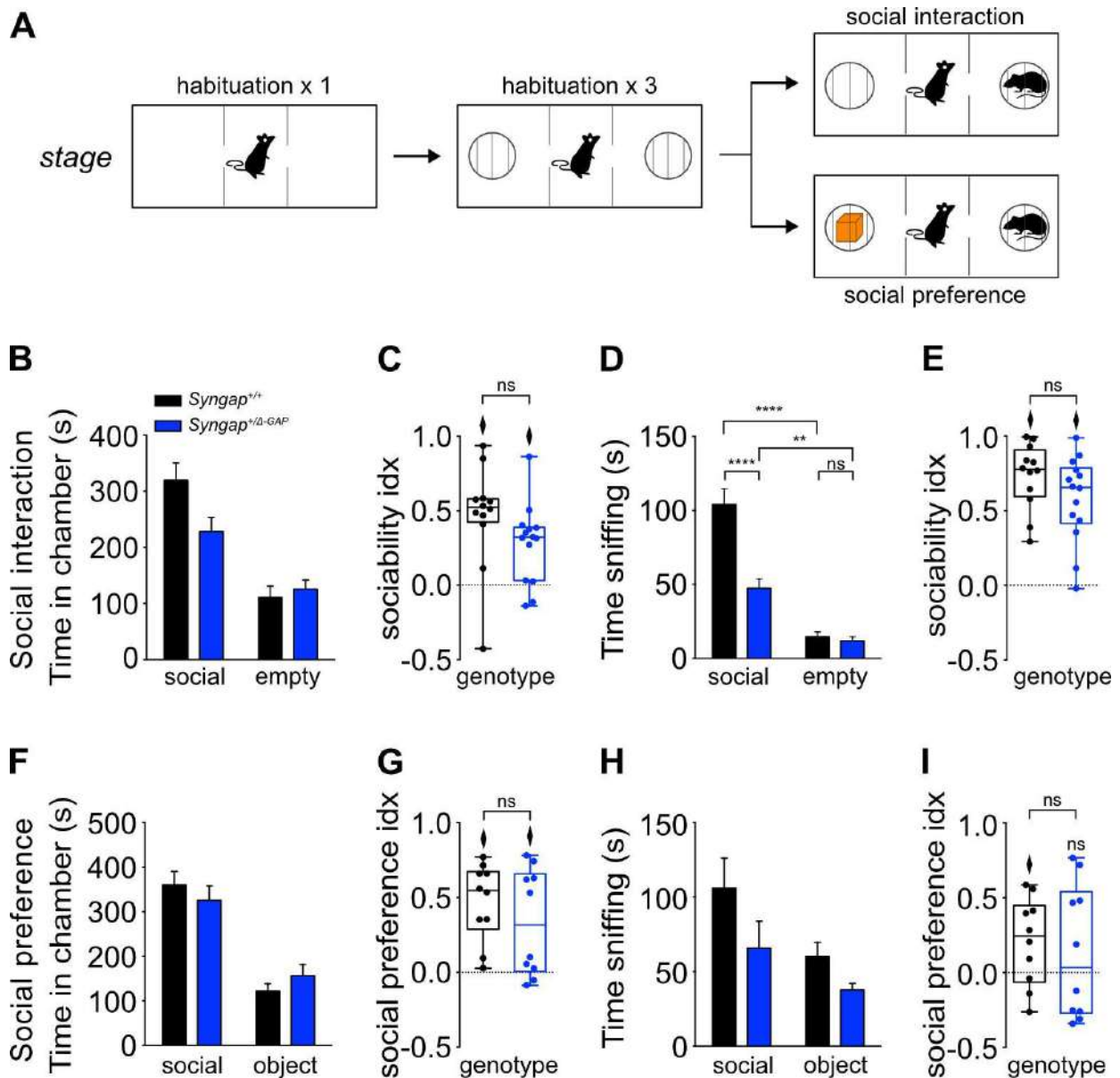
1 **Figure 2. *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats display impaired extinction of fear association in a cued-**  
2 **fear conditioning paradigm.** (A) During training, both WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats display  
3 comparable levels of freezing to the flashing light that was paired with a mild foot shock (CS-  
4 US) ( $n_{+/+ \text{ CS-US}} = 12$ ,  $n_{+/\Delta\text{-GAP CS-US}} = 11$ ). (B) 24 hours after conditioning, *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats show  
5 increased fear responses to the neutral context but recall of fear memory to the first CS is  
6 comparable to WT. However, freezing to subsequent unreinforced CS presentations is  
7 significantly higher for *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats with the difference becoming more pronounced over  
8 consecutive presentations. In contrast, WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> CS-only controls ( $n_{+/+ \text{ CS-only}} = 7$ ,  
9  $n_{+/\Delta\text{-GAP CS-only}} = 7$ ) do not exhibit robust freezing to the CS during training (A) or recall testing  
10 (B). (C) Extinction index calculated as the change in % time freezing to the CS at the beginning  
11 and end of recall testing was significantly greater in conditioned WT than conditioned  
12 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats or CS-only controls ( $n_{+/+ \text{ CS-only}} = 7$ ,  $n_{+/\Delta\text{-GAP CS-only}} = 7$ ,  $n_{+/+ \text{ CS-US}} = 12$ ,  $n_{+/\Delta\text{-GAP CS-}}$   
13  $_{\text{US}} = 11$ ). (D, E) Comparison of the % time freezing *during* and *between* (shaded columns) CS  
14 presentations shows the CS specifically modulates freezing in conditioned WT and *Syngap*<sup>+/ $\Delta$ -</sup>  
15 <sup>GAP</sup> rats ( $n_{+/+ \text{ CS-only}} = 7$ ,  $n_{+/\Delta\text{-GAP CS-only}} = 7$ ,  $n_{+/+ \text{ CS-US}} = 12$ ,  $n_{+/\Delta\text{-GAP CS-US}} = 11$ ). (F, G) *Syngap*<sup>+/ $\Delta$ -</sup>  
16 <sup>GAP</sup> rats show an initial increase in locomotion during the first 20 min in the open field which  
17 habituates by day 2. (H) Time spent in the middle of the OF is comparable between genotypes  
18 ( $n_{+/+ \text{ OF}} = 8$ ,  $n_{+/\Delta\text{-GAP OF}} = 10$ ). (I-K) Behaviour of *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats was also indistinguishable  
19 from WTs during elevated plus maze testing, as indicated by locomotion, percentage of time  
20 spent in open arms ( $n_{+/+ \text{ EPM}} = 8$ ,  $n_{+/\Delta\text{-GAP EPM}} = 10$ ). (L, M) Motor coordination and learning is  
21 unaffected in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats ( $n_{+/+ \text{ RTR}} = 12$ ,  $n_{+/\Delta\text{-GAP RTR}} = 12$ ). ITI: Inter-trial-Interval. *mean*  $\pm$   
22 SE is noted.



1  
2 **Figure 3. *Syngap*<sup>+/Δ-GAP</sup> rats have normal spatial reference memory acquisition and**  
3 **reversal learning.** (A) Timeline of experimental protocol for spatial memory reference and  
4 reversal training in the water maze. (B) *Syngap*<sup>+/Δ-GAP</sup> rats learn the hidden-platform version of

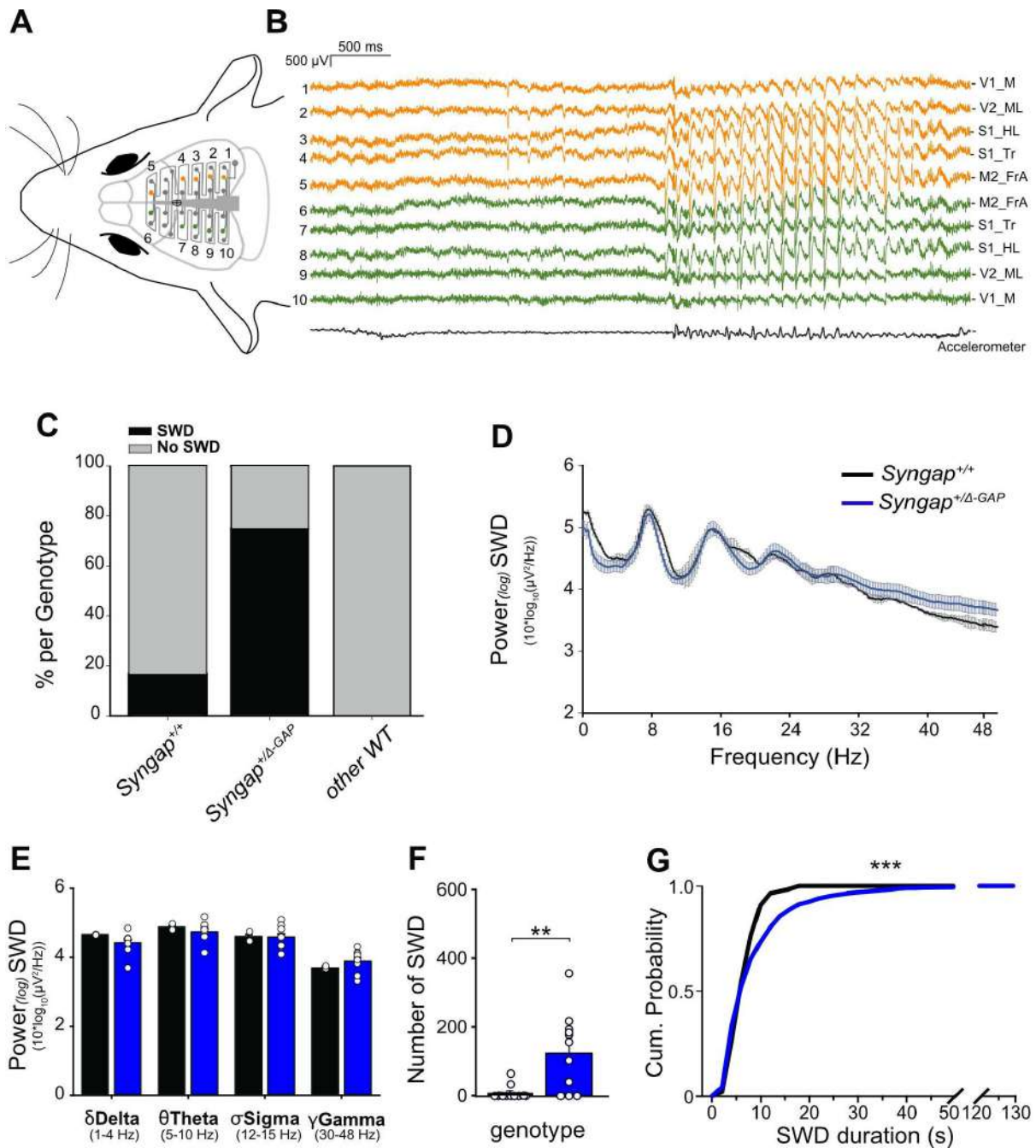
1 the water maze similarly to WT<sub>s</sub> as measured by a decrease over days in the path taken to  
2 escape and (C) the percent time in the platform location on daily probe trials. Performance  
3 during reversal learning was comparable between genotypes as measured by path to escape  
4 (D) and the percent time in the old and new platform locations during probe trials (E). (F, G)  
5 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats swim faster than WT littermates overall ( $n_{+/+} = 8$ ,  $n_{+/\Delta-GAP} = 9$ ). *mean*  $\pm$  SE is  
6 noted.

1



2

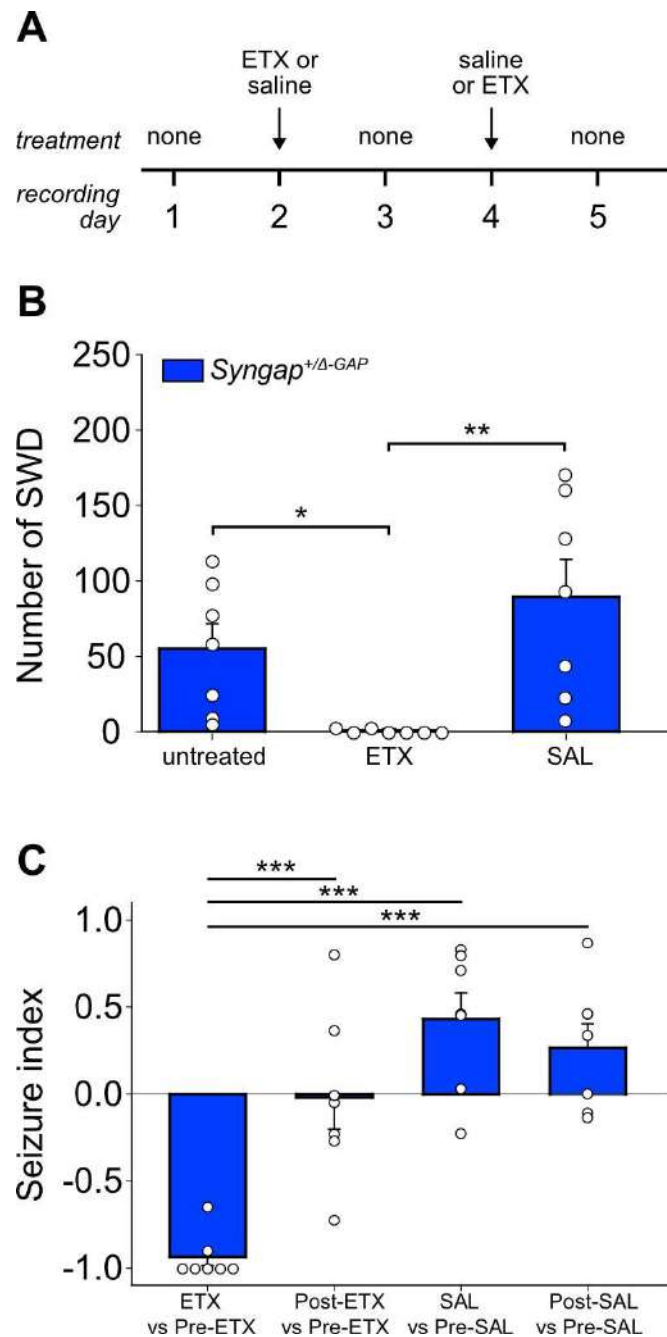
3 **Figure 4. Altered social behaviour in *Syngap*<sup>+Δ-GAP</sup> rats.** (A) Schematic of the 3 chamber  
4 tasks. In the social interaction task, time in chamber (B) and sociability index (C) indicate WT  
5 and *Syngap*<sup>+Δ-GAP</sup> rats show a preference for spending time in the chamber containing a caged  
6 social stimulus compared to the chamber containing an empty wire cage. (D) Time actively  
7 exploring (sniffing) and (E) sociability index for active exploration suggest that both WT and  
8 *Syngap*<sup>+Δ-GAP</sup> littermates explore the social stimulus more ( $n_{+/+} = 12$ ,  $n_{+Δ-GAP} = 14$ ). In the social  
9 preference task, time in chamber (F) and social preference index (G) indicate WT and  
10 *Syngap*<sup>+Δ-GAP</sup> rats spend significantly more time in the chamber containing a caged social  
11 stimulus compared to the chamber containing a novel object. However, *Syngap*<sup>+Δ-GAP</sup> rats do  
12 not show preference for actively exploring the social stimulus over the object (H-I) ( $n_{+/+} = 10$ ,  
13  $n_{+Δ-GAP} = 10$ ). Diamonds illustrate above chance performance ( $p < 0.05$ ). See also  
14 Supplementary Figure 3 for results during the novelty phase of the tasks, i.e the first 3 min.



1

2 **Figure 5. EEG analysis reveals the presence of SWDs in *Syngap*<sup>+/-GAP</sup> rats.** (A) Schematic  
3 of a 32-channel skull-surface EEG implant illustrating approximate location of electrodes  
4 relative to the brain and (B) representative traces from selected electrodes over both  
5 hemispheres (orange and green) reveal bilaterally occurring SWDs. (C) A significantly higher  
6 proportion of *Syngap*<sup>+/-GAP</sup> rats exhibit SWDs than their WT littermates controls or WT rats  
7 from unrelated colonies ( $n_{+/+} = 12$ ,  $n_{+/-GAP} = 12$ ,  $n_{+/+other} = 6$ ). (D) SWD power from electrode  
8 overlaying S1 is comparable between *Syngap*<sup>+/-GAP</sup> and WT littermates ( $n_{+/+} = 2$ ,  $n_{+/-GAP} = 9$ ).  
9 (E) Averaged spectral power by EEG band for *Syngap*<sup>+/-GAP</sup> and WT during SWDs ( $n_{+/+} = 2$ ,  
10  $n_{+/-GAP} = 9$ ). (F) The number of SWD events found in *Syngap*<sup>+/-GAP</sup> and WT rats was  
11 significantly higher compared to WT littermates ( $n_{+/+} = 12$ ,  $n_{+/-GAP} = 12$ ). (G) Average  
12 cumulative probability of SWDs, by event duration in seconds, for *Syngap*<sup>+/-GAP</sup> and WT rats  
13 suggests that SWD events detected in *Syngap*<sup>+/-GAP</sup> rats were longer than those from WTs  
14 (from  $n_{+/+} = 2$ ,  $n_{+/-GAP} = 9$ ). *mean* ± SE is noted. V1\_M: primary visual cortex, medial  
15 component; V2\_ML: secondary visual cortex, lateral component; S1\_HL: primary

- 1 somatosensory cortex, hindlimb region; S1\_Tr: primary somatosensory cortex, trunk region;
- 2 M2\_FrA: secondary motor cortex, frontal association area.



- 3
- 4 **Figure 6. Ethosuximide reduces the number of SWDs in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats.** (A)
- 5 Treatment timeline. (B) Number of SWD events identified in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats after no
- 6 treatment or following injection with ETX or saline (SAL) alone. (C) Seizure index compared
- 7 to the previous untreated day shows greater suppression of SWD by ETX compared to other
- 8 conditions ( $n_{+/\Delta-GAP} = 7$ ). *mean*  $\pm$  SE is noted.

## 1 **Supplementary Methods and Figures**

### 2 **EEG with implanted screw electrodes**

3 Rats were anaesthetised and prepared for stereotaxic surgery. Craniotomies were drilled and  
4 a single recording screw positioned at each of the following coordinates relative to bregma:  
5 +7.56 mm AP, 1 mm ML (olfactory bulb), +2.16 mm AP, 3 mm ML (motor cortex), -3.24 mm  
6 AP, 2.5 mm ML (parietal association cortex). A screw positioned over the cerebellum served  
7 as reference/ground (-12 mm AP, 0 mm ML). Recording screws were implanted unilaterally  
8 and then connected to an electronic interface board (EIB 16, Neuralynx). The incision was  
9 then closed using surgical sutures (Ethicon, Henry Schein, UK) and rats were left to recover  
10 for a minimum of 1 week post-surgery. Recordings were made via a 16 channel digitising  
11 headstage (C3334, Intan Technologies, USA) in the same system as described in main  
12 Methods. LFP signals were bandpass-filtered from 0.1 - 600 Hz and sampled at 2 kHz in  
13 OpenEphys software. Video recordings were made using Freeze Frame software (15 frame  
14 per sec; Actimetrics) synchronised with electrophysiological signals via TTL pulses.

### 15 **Visual stimulation during EEG recordings**

16 EEG recordings were made from implanted 6 WT and 6 *Syngap*<sup>+/ $\Delta$ -GAP</sup> male rats within a 35 x  
17 20 x 40 cm plastic cage positioned within a sound attenuating chamber. Rats were given 2  
18 min to explore the context prior to presentation of a 10 s visual stimulus (5 Hz 110 lux flashes,  
19 50 / 50 duty cycle). This was followed by a post-stimulus time of >1 min with recordings  
20 maintained throughout. SWD events were manually identified. Their total number was  
21 quantified and their timing relative to the onset of the visual stimulation was calculated in 10  
22 sec bins. Neuroexplorer software (Nex Technologies, CO, USA) was used to generate  
23 spectrograms (0.4 sec shifting window, 50% shift overlap, time-bandwidth product = 3, number  
24 of tapers = 5).

### 25 **Olfactory habituation-dishabituation task**

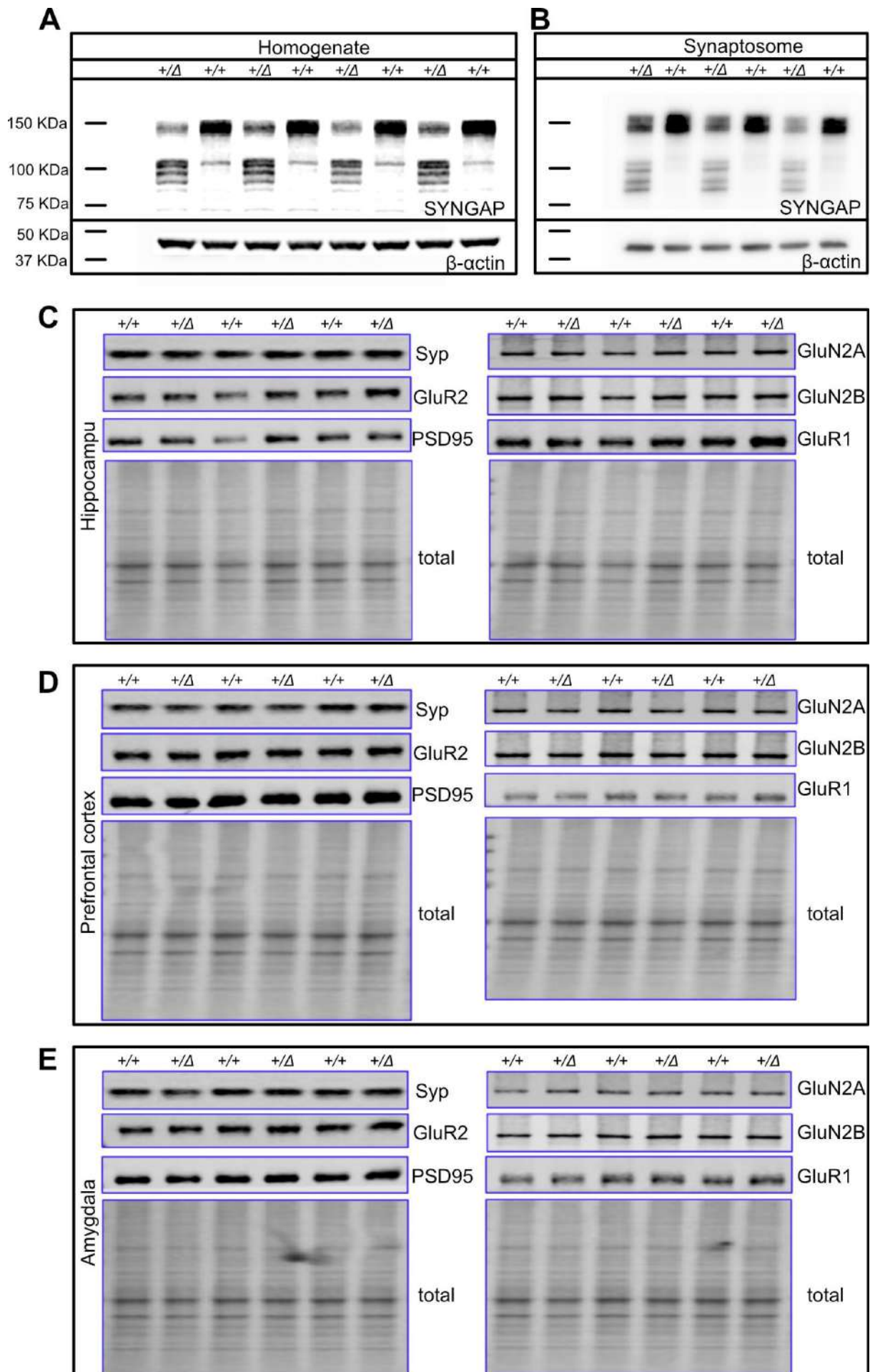
26 Rats are transferred to an empty cage, similar to their home cage, and a series of odour-  
27 infused cotton swabs are presented for 2 min each, with a 1 min ITI (adapted from (Yang &  
28 Crawley, 2009)). Rats are acclimatised to the testing environment a day prior to the  
29 experiment, by being placed in an empty cage with a cotton swab infused with ddH<sub>2</sub>O. The  
30 odour order is as follows:

31 *ddH<sub>2</sub>O, ddH<sub>2</sub>O, non-social odour 1.1, non-social odour 1.2, non-social odour 1.3, non-*  
32 *social odour 2.1, non-social odour 2.2, non-social odour 2.3, social odour 1.1, social odour*  
33 *1.2, social odour 1.3, social odour 2.1, social odour 2.2, social odour 2.3, ddH<sub>2</sub>O*

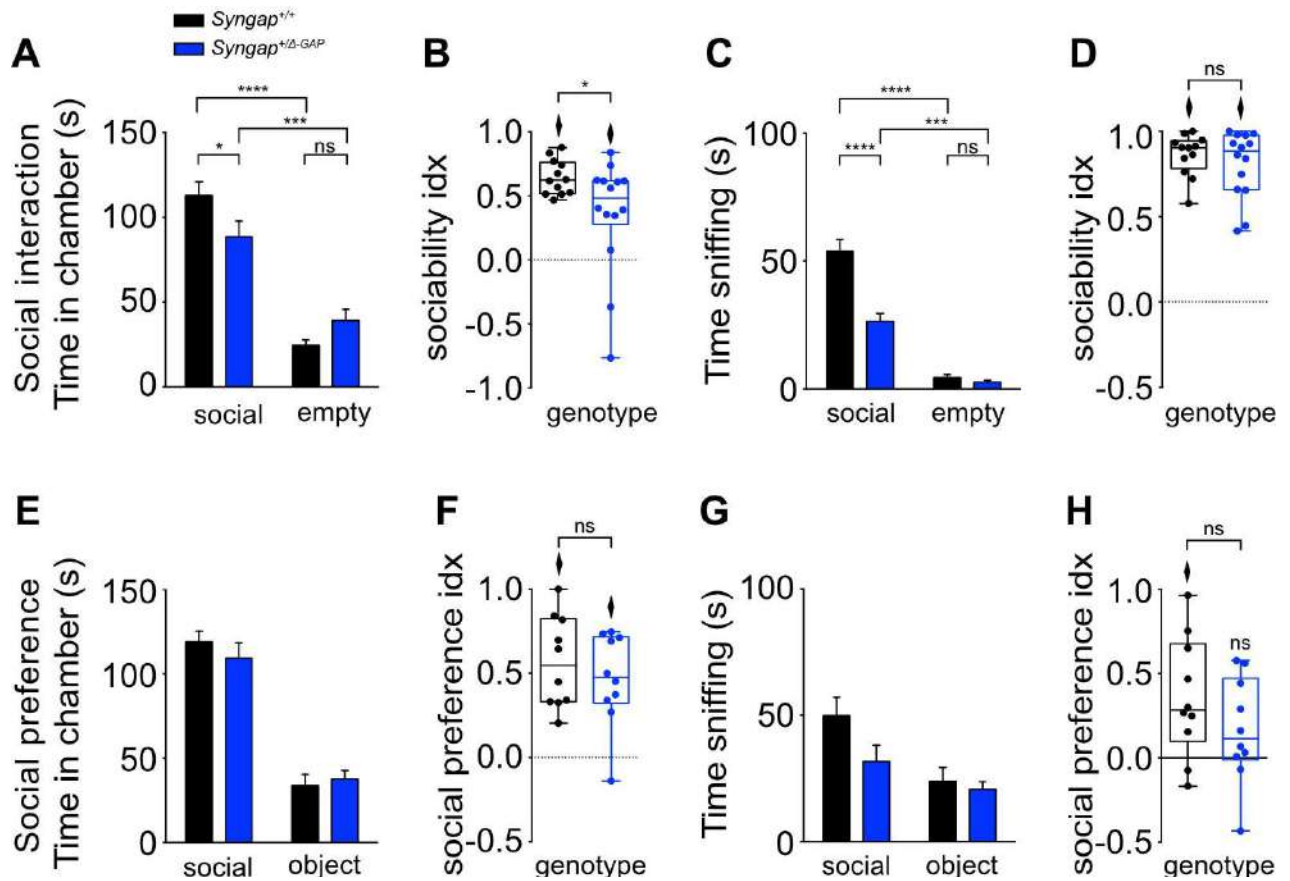
34 Banana extract (1:1000 diluted in ddH<sub>2</sub>O; Foodie Flavors™) and almond extract (1:1000;  
35 Foodie Flavors™) were used as non-social odours. Swabs of the bedding surface of home  
36 cages of 4 group-housed adult rats (sex-matched) were used as social odours. Odours were  
37 counterbalanced for order of exposure.

### 38 **Supplementary Table 1. Summary of data and exact p-values related to Figures.**

39 Applicable figure panels are listed followed by the relevant measure (task) for which data is  
40 reported. Data values are given in mean  $\pm$  SE. Statistical tests (t-tests, ANOVAs) are then  
41 followed by descriptive statistics and exact *p*-values for results and comparisons made for  
42 each relevant measure. All one-sample t-tests are compared against chance level (theoretical  
43 mean of 0.0). +/+ for *Syngap*<sup>+/+</sup>, +/ $\Delta$ -GAP for *Syngap*<sup>+/ $\Delta$ -GAP</sup>. See Methods.

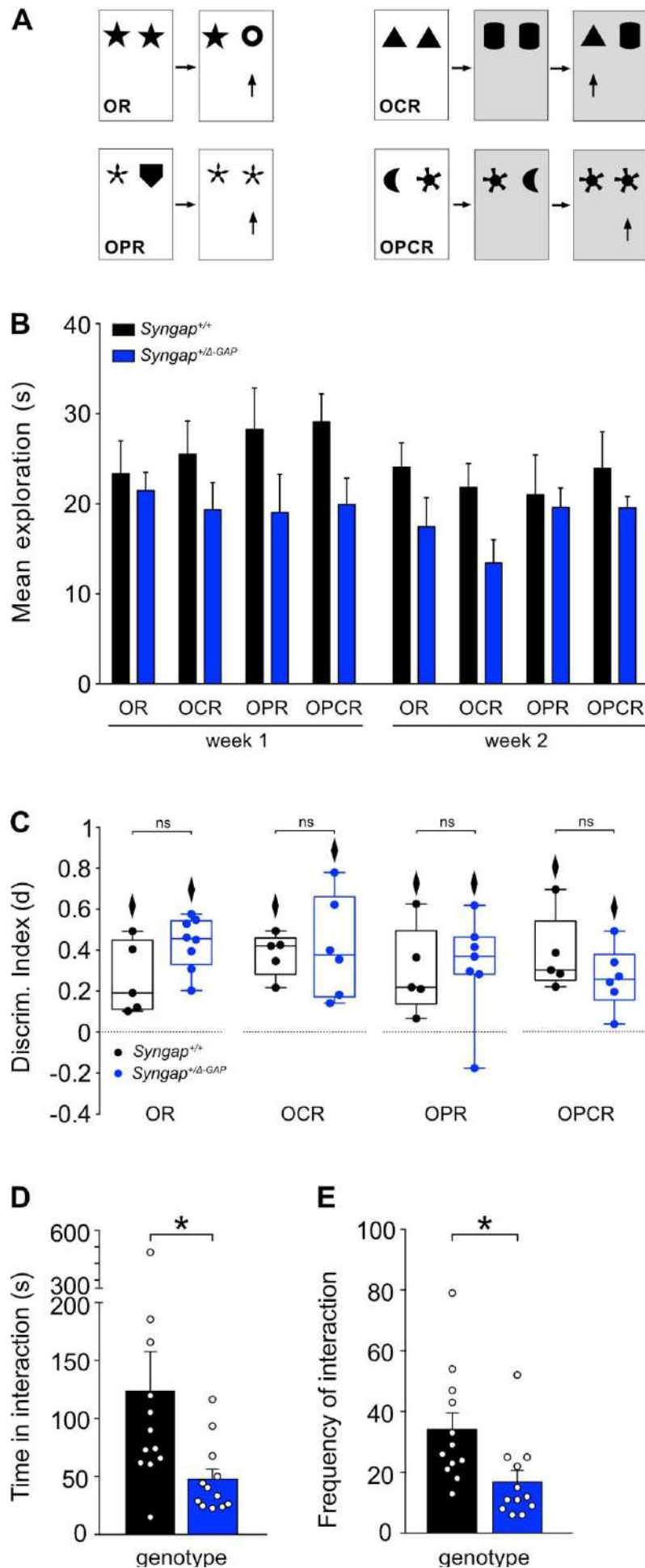


1 **Supplementary Figure 1. Full-length SYNGAP levels are reduced in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats.**  
2 Western blots of (A) homogenates ( $n_{+/+} = 4$ ,  $n_{+/ $\Delta$ -GAP} = 4$ ) and (B) synaptosomes ( $n_{+/+} = 3$ ,  $n_{+/ $\Delta$ -$   
3  $GAP} = 3$ ) from adult rat hippocampus confirm that full-length endogenous SYNGAP protein  
4 (~150kDa) is located in synapses; additional bands in the molecular weight range predicted  
5 for mutant SYNGAP isoforms are detected in both homogenates and synaptosomes from  
6 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats. Western blots of purified synaptosomes ( $n_{+/+} = 3$ ,  $n_{+/ $\Delta$ -GAP} = 3$ ) from adult  
7 rat hippocampus (C), prefrontal cortex (D), and amygdala (E) to quantify the level of several  
8 proteins associated with pre- and post-synaptic function. Syp; synaptophysin, GluR1;  
9 Glutamate receptor 1, GluR2; Glutamate receptor 2, PSD95; Postsynaptic density protein 95,  
10 GluN2A; NMDA receptor subtype 2A, GluN2B; NMDA receptor subtype 2B, +/ $\Delta$ ; +/ $\Delta$ -GAP.  
11 Tissue from 3 animals was pooled for each sample.



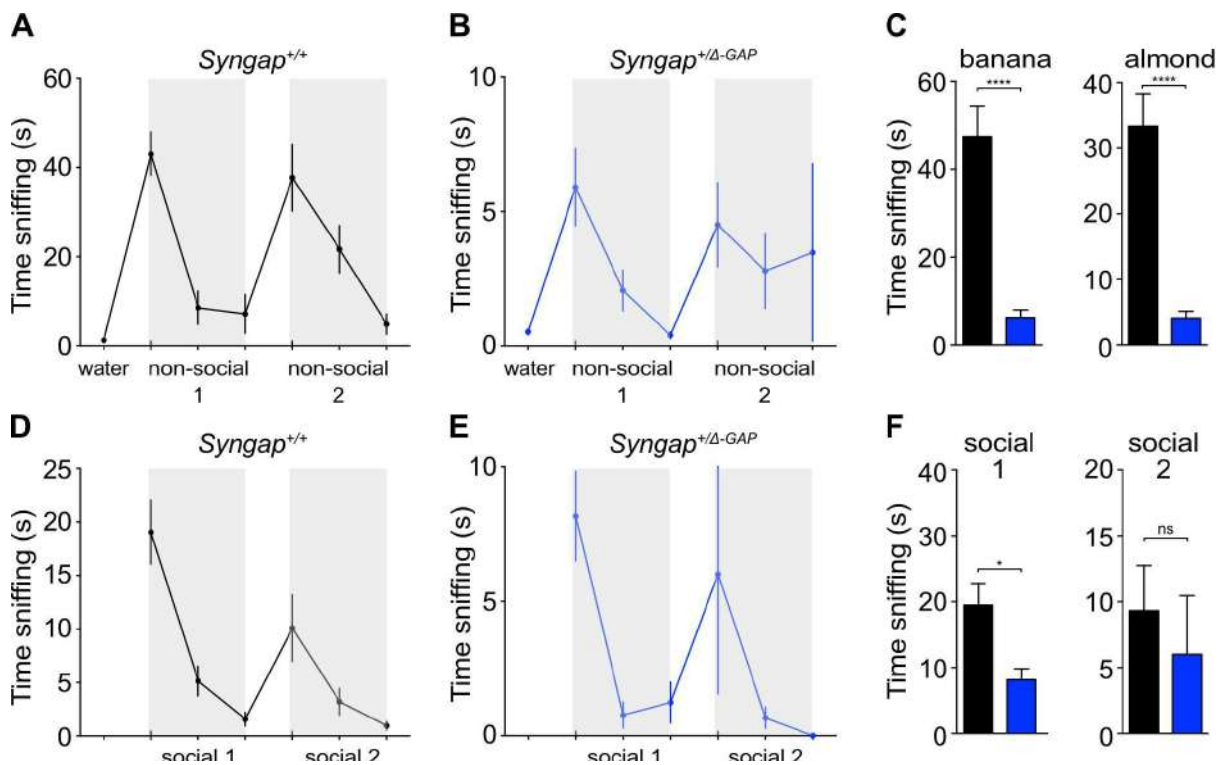
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**Supplementary Figure 2. Social behaviour data for 0-180 seconds.** In the social interaction task, time in chamber (A) and sociability index (B) indicate WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats show a preference for spending time in the chamber containing a caged social stimulus compared to the chamber containing an empty wire cage. WT rats were more reliably spending time in the social chamber than *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats. (C) Time actively exploring (sniffing) and (D) sociability index for active exploration suggest that both genotypes explore the social stimulus more ( $n_{+/+} = 12$ ,  $n_{+/ $\Delta$ -GAP} = 14$ ). In the social preference task, time in chamber (E) and social preference index (F) indicate WT and *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats spend significantly more time in the chamber containing a caged social stimulus compared to the chamber containing a novel object. (G, H) *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats do not show preference for actively exploring the social stimulus over the object ( $n_{+/+} = 10$ ,  $n_{+/ $\Delta$ -GAP} = 10$ ). Diamonds illustrate above chance performance ( $p < 0.05$ ).



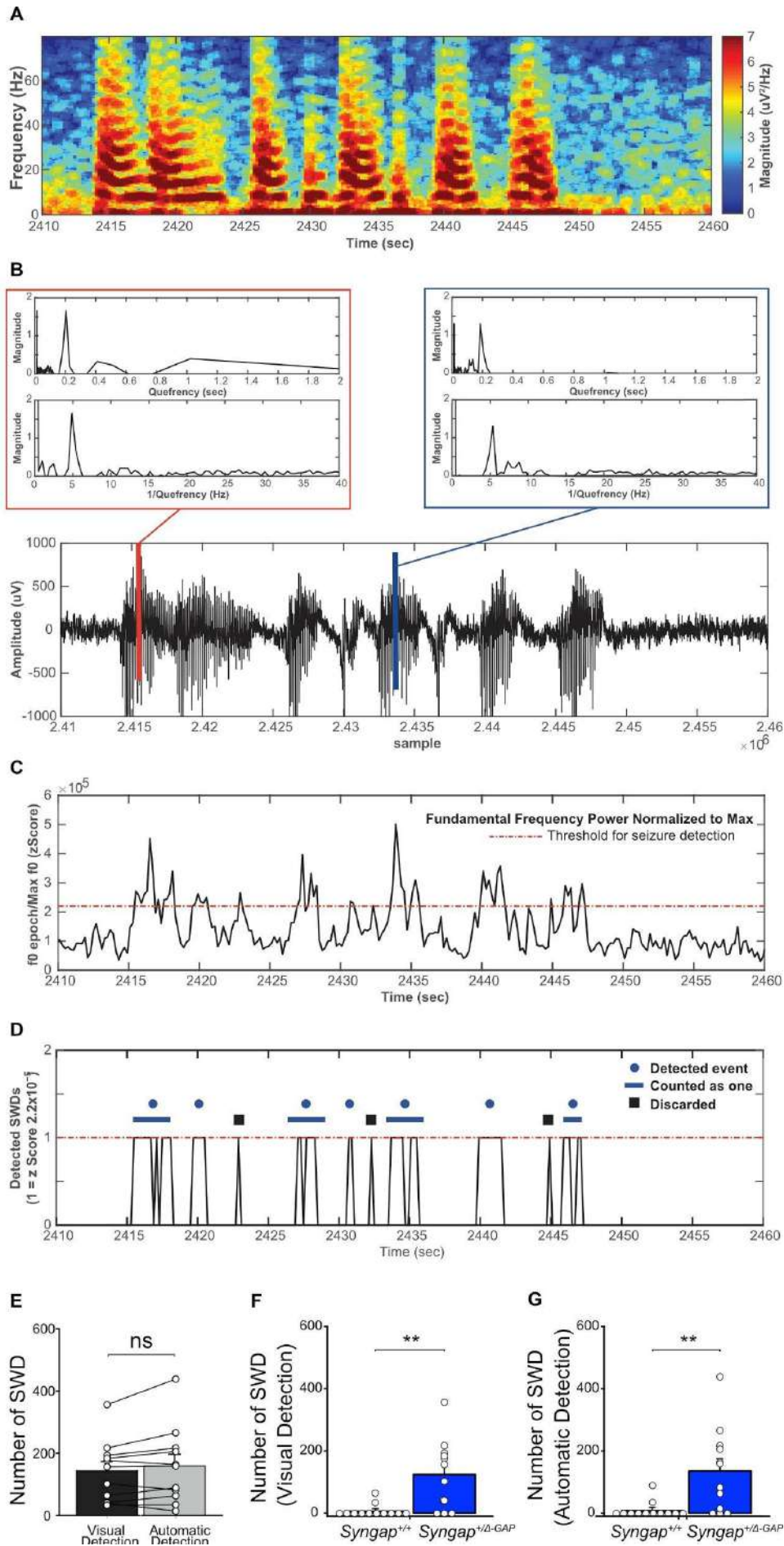
1 **Supplementary Figure 3. Associative recognition memory remains unaffected after**  
2 **heterozygous deletion of the C2/GAP domain in SYNGAP.** (A) Schematic illustration of  
3 spontaneous exploration tasks for object (OR), object-context (OCR), object-place (OPR) and  
4 object-place-context (OPCR) novelty recognition. (B) *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats spend less time  
5 exploring during the first exposure of the objects in different configurations. (C) Both WT and  
6 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats that reach exploration criterion (see methods) exhibit short term memory  
7 for all four tasks, as measured by above chance performance (illustrated by diamonds for  
8  $p < 0.05$ ). In the marble burying task, *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats (D) spent significantly less time  
9 interacting with marbles and (E) their interactions were less frequent compared to WT  
10 littermates. *mean*  $\pm$  SE is noted.

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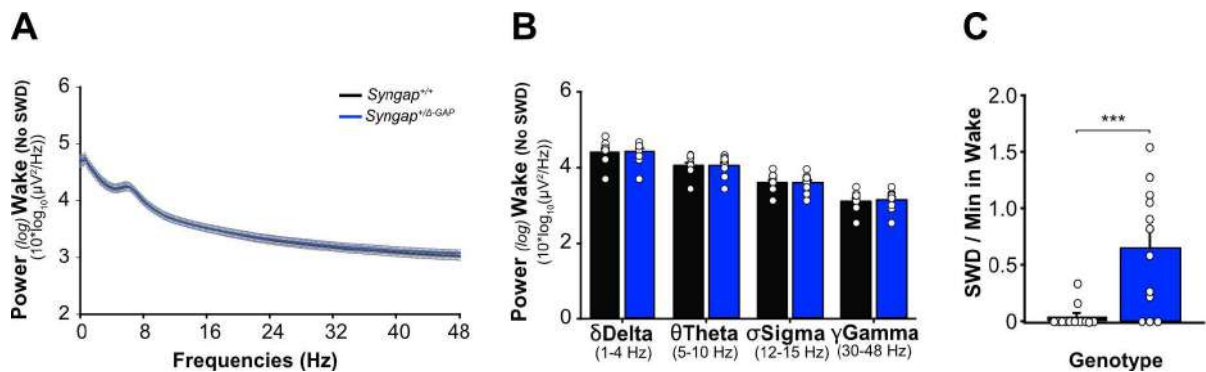


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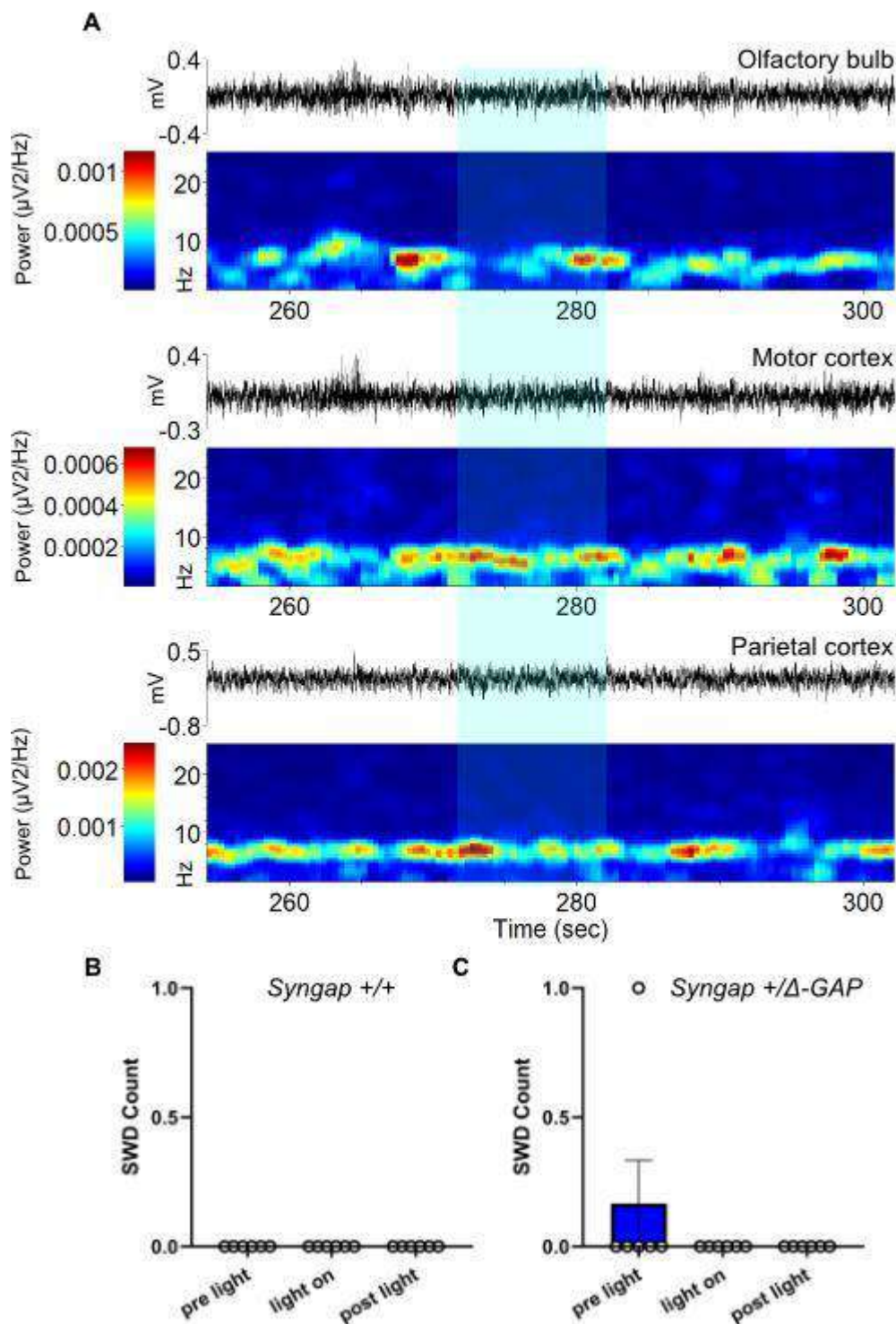
13 **Supplementary Figure 4. *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats can detect and discriminate odours.** The  
14 average time WT (A) and *Syngap*<sup>+/ $\Delta$ -GAP</sup> (B) rats spent investigating non-social odours  
15 habituates over three consecutive presentations of an individual non-social odour; a relative  
16 increase in time spent investigating a new non-social odour indicates olfactory discrimination.  
17 (C) *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats spent less time overall investigating each non-social odour than WT  
18 rats. WT (D) and *Syngap*<sup>+/ $\Delta$ -GAP</sup> (E) rats reduce the amount of time spent investigating social  
19 odours over three consecutive presentations; a relative increase in time spent investigating a  
20 new social odour indicates olfactory discrimination. (F) *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats spent less time  
21 overall investigating each social odour than WT rats. Colour change indicates odour change.  
22 *mean*  $\pm$  SE is noted.



1 **Supplementary Figure 5. Automatic Detection of SWD.** (A) Example spectrogram of SWD.  
2 (B) Raw EEG trace corresponding to time interval of the spectrogram in A (bottom), with two  
3 selected SWD epochs (0.2 sec each) marked with blue and red vertical lines. Inserts (top)  
4 show respective cepstral power analysis showing the fundamental frequency ( $f_0$ ) peak on the  
5 pseudo-time domain (top inset) and pseudo-frequency domain (bottom inset). (C) Peak  
6 cepstral power on theta band range (5-10 Hz) calculated in 0.2 sec epochs, normalized to its  
7 absolute maximum value, and transformed into z-scores. Threshold for detecting SWD events  
8 marked with dashed red line. (D) Detected SWDs transformed into zeros (below threshold) or  
9 ones (threshold or over) on the time interval shown in A and B. Events counted as one (< 1 s  
10 between events) are marked by blue lines, whereas black squares designate discarded events  
11 (length < 0.8 s). (E) Total number of SWD detected by visual (black bar) and automatic  
12 counting (gray bar) is comparable. (F, G) Genotype comparison of visually detected and  
13 automatically detected SWDs shows a significantly increased number of SWD events in  
14 *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats with both methods. ( $n_{+/+}$  = 12,  $n_{+/\Delta-GAP}$  = 12). *mean*  $\pm$  SE is noted.



16 **Supplementary Figure 6. EEG analysis of wakefulness and associated SWD events.**  
17 Power spectral profile (A) and bands (B) of wake states (excluding SWDs) during wakefulness  
18 are comparable between *Syngap*<sup>+/ $\Delta$ -GAP</sup> and WT littermates. (C) Ratio of SWD events per  
19 minute of wakefulness is significantly greater in *Syngap*<sup>+/ $\Delta$ -GAP</sup> rats compared to WT. ( $n_{+/+}$  =  
20 12,  $n_{+/\Delta-GAP}$  = 12).



1  
2 **Supplementary Figure 7. Flashing lights do not drive SWD.** (A) Example EEG recording  
3 traces and associated spectrograms from a single *Syngap* <sup>$+/Δ-GAP$</sup>  rat before, during and  
4 exposure to flashing light stimuli. (B, C) Average SWD event count per genotype for the 10  
5 sec before (pre), during, and after (post) light exposure (WT left; *Syngap* <sup>$+/Δ-GAP$</sup>  right). Blue bar  
6 shading duration when flashing light was on. ( $n_{+/+} = 6$ ,  $n_{+/Δ-GAP} = 6$ ). *mean*  $\pm$  SE is noted. For  
7 *Syngap* <sup>$+/Δ-GAP$</sup> : one-way ANOVA  $F_{(2,15)} = 1.00$ ,  $p = 0.391$ .  
8

1 **Supplementary Table 1**

Figure	Task/ Measure	Population size	Values	Statistical test	Results / Comparisons
1D	Western blot quantification Full length	n <sub>+/+</sub> =4, n <sub>+/<math>\Delta</math>-GAP</sub> =4	+/ $\Delta$ : 1 $\pm$ 0.076 +/ $\Delta$ -GAP: 0.41 $\pm$ 0.04	Two-tailed unpaired t-test	t <sub>(6)</sub> =6.846, p=0.0005
	Western blot quantification total	n <sub>+/+</sub> =4, n <sub>+/<math>\Delta</math>-GAP</sub> =4	+/ $\Delta$ : 1 $\pm$ 0.061 +/ $\Delta$ -GAP: 0.87 $\pm$ 0.061	Two-tailed unpaired t-test	t <sub>(6)</sub> =1.483, p=0.1885
1E	Western blot quantification Full length	n <sub>+/+</sub> =3, n <sub>+/<math>\Delta</math>-GAP</sub> =3	+/ $\Delta$ : 1 $\pm$ 0.057 +/ $\Delta$ -GAP: 0.59 $\pm$ 0.07	Two-tailed unpaired t-test	t <sub>(4)</sub> =4.441, p=0.0113
	Western blot quantification total	n <sub>+/+</sub> =3, n <sub>+/<math>\Delta</math>-GAP</sub> =3	+/ $\Delta$ : 1 $\pm$ 0.055 +/ $\Delta$ -GAP: 0.98 $\pm$ 0.08	Two-tailed unpaired t-test	t <sub>(4)</sub> =0.1971, p=0.8534
2A	Fear conditioning conditioning	n <sub>+/+</sub> CSonly=7, n <sub>+/+</sub> CS-US=12, n <sub>+/<math>\Delta</math>-GAP</sub> CSonly=7, n <sub>+/<math>\Delta</math>-GAP</sub> CS-US=11		Three-way ANOVA	CS F <sub>(5,198)</sub> = 33.94, p<0.0001; genotype F <sub>(1,198)</sub> = 0.3902, p=0.5329; protocol F <sub>(1,198)</sub> = 203.3, p<0.0001; CS x genotype F <sub>(5,198)</sub> = 0.4439, p=0.8174; CS x protocol F <sub>(5,198)</sub> = 14.27, p<0.0001; genotype x protocol F <sub>(1,198)</sub> = 0.0473, p=0.8280; CS x genotype x protocol F <sub>(5,198)</sub> = 0.7156, p=0.6124
2B	Fear conditioning Pre-CS	n <sub>+/+</sub> CSonly=7, n <sub>+/+</sub> CS-US=12, n <sub>+/<math>\Delta</math>-GAP</sub> CSonly=7, n <sub>+/<math>\Delta</math>-GAP</sub> CS-US=11		Two-way ANOVA	genotype F <sub>(1,33)</sub> = 3.667, p=0.0642; protocol F <sub>(1,33)</sub> = 7.702, p=0.009; genotype x protocol F <sub>(1,33)</sub> = 2.407, p=0.1303
	Fear conditioning recall	n <sub>+/+</sub> CSonly=7, n <sub>+/+</sub> CS-US=12, n <sub>+/<math>\Delta</math>-GAP</sub> CSonly=7, n <sub>+/<math>\Delta</math>-GAP</sub> CS-US=11		Three-way ANOVA	CS F <sub>(11,396)</sub> = 2.465, p=0.0054; genotype F <sub>(1,396)</sub> = 91.16, p<0.0001; protocol F <sub>(1,396)</sub> = 943.3, p<0.0001; CS x genotype F <sub>(11,396)</sub> = 1.440, p=0.1524; CS x protocol F <sub>(11,396)</sub> = 7.391, p<0.0001; genotype x protocol F <sub>(1,396)</sub> = 84.73, p<0.0001; CS x genotype x protocol F <sub>(11,396)</sub> = 1.839, p=0.0461  post-hoc (for CS-US): CS1: +/+ vs +/ $\Delta$ -GAP p>0.9999 CS2: +/+ vs +/ $\Delta$ -GAP p=0.4268 CS3: +/+ vs +/ $\Delta$ -GAP p=0.7259 CS4: +/+ vs +/ $\Delta$ -GAP p=0.3514 CS5: +/+ vs +/ $\Delta$ -GAP p=0.0193 CS6: +/+ vs +/ $\Delta$ -GAP p=0.1363

					CS7: +/+ vs +/Δ-GAP $p < 0.0001$ CS8: +/+ vs +/Δ-GAP $p < 0.0001$ CS9: +/+ vs +/Δ-GAP $p = 0.0003$ CS10: +/+ vs +/Δ-GAP $p = 0.0063$ CS11: +/+ vs +/Δ-GAP $p < 0.0001$ CS12: +/+ vs +/Δ-GAP $p < 0.0001$
2C	Extinction index	$n_{+/+CS\text{only}}=7$ , $n_{+/+CS-US}=12$ , $n_{+/Δ-GAPCS\text{only}}=7$ , $n_{+/Δ-GAPCS-US}=11$		Two-way ANOVA	genotype $F_{(1,33)} = 5.653$ , $p = 0.0234$ ; protocol $F_{(1,33)} = 40.93$ , $p < 0.0001$ ; genotype x protocol $F_{(1,33)} = 2.198$ , $p = 0.1477$
2D	Fear conditioning recall	$n_{+/+CS\text{only}}=7$ , $n_{+/+CS-US}=12$ , $n_{+/Δ-GAPCS\text{only}}=7$ , $n_{+/Δ-GAPCS-US}=11$		Three-way ANOVA	CS $F_{(22,759)} = 8.008$ , $p < 0.0001$ ; genotype $F_{(1,759)} = 220.2$ , $p < 0.0001$ ; protocol $F_{(1,759)} = 1034$ , $p < 0.0001$ ; CS x genotype $F_{(22,759)} = 0.9397$ , $p = 0.5416$ ; CS x protocol $F_{(22,759)} = 9.205$ , $p < 0.0001$ ; genotype x protocol $F_{(1,759)} = 90.34$ , $p < 0.0001$ ; CS x genotype x protocol $F_{(22,759)} = 0.5502$ , $p = 0.9538$
2E	Modulation index overall	$n_{+/+CS\text{only}}=7$ , $n_{+/+CS-US}=12$ , $n_{+/Δ-GAPCS\text{only}}=7$ , $n_{+/Δ-GAPCS-US}=11$		Two-way ANOVA overall	genotype $F_{(1,33)} = 1.991$ , $p = 0.168$ ; protocol $F_{(1,33)} = 29.260$ , $p < 0.0001$ ; genotype x protocol $F_{(1,33)} = 5.551$ , $p = 0.025$  post-hoc (CS-US): +/+ vs +/Δ-GAP: $p = 0.007$ post-hoc (CS only): +/+ vs +/Δ-GAP: $p = 0.583$
			CSonly: +/+: $0.037 \pm 0.076$ +/Δ-GAP: $0.093 \pm 0.063$ CS-US: +/+: $0.494 \pm 0.034$ +/Δ-GAP: $0.272 \pm 0.063$	One-sample t-test Theoretical $\mu = 0$	CSonly: +/+ $t_{(6)} = 0.489$ , $p = 0.642$ +/Δ-GAP $t_{(6)} = 1.468$ , $p = 0.193$ CS-US: +/+ $t_{(11)} = 14.545$ , $p < 0.001$ +/Δ-GAP $t_{(10)} = 4.340$ , $p = 0.001$
				Two-way ANOVA timepoint (CS-US)	genotype $F_{(1,21)} = 8.270$ , $p = 0.009$ ; timepoint $F_{(1,21)} = 1.415$ , $p = 0.248$ ; genotype x timepoint $F_{(1,21)} = 4.920$ , $p = 0.038$  post-hoc (early): +/+ vs +/Δ-GAP: $p = 0.001$ post-hoc (late): +/+ vs +/Δ-GAP: $p = 0.607$
2F	Open Field Over time	$n_{+/+}=8$ , $n_{+/Δ-GAP}=10$		Two-way ANOVA	genotype $F_{(1,16)} = 5.660$ , $p = 0.0301$ ; effect of time $F_{(9,144)} = 60.04$ , $p < 0.0001$ ;

					genotype x time $F_{(9,144)}=1.235$ , $p<0.2782$
2G	Open Field	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=10$		Two-way ANOVA	day $F_{(1,16)}=16.34$ , $p=0.0009$ ; genotype $F_{(1,16)}=3.579$ , $p=0.0768$ ; day x genotype $F_{(1,16)}=1.653$ , $p=0.2169$
2H	Open Field	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=10$		Two-way ANOVA	genotype $F_{(1,16)}=2.633$ , $p=0.1242$ ; day $F_{(1,16)}=0.1767$ , $p=0.6798$ ; day x genotype $F_{(4,88)}=0.2019$ , $p=0.6592$
2I	Elevated Plus Maze	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=10$	$+/+$ : $16.79\pm 1.33$ $+/\Delta-GAP$ : $16.99\pm 1.2$	Two-tailed unpaired t-test	$t_{(16)}=0.1149$ , $p=0.9099$
2J	Elevated Plus Maze	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=10$	$+/+$ : $15.50\pm 1.68$ $+/\Delta-GAP$ : $13.6\pm 2.06$	Two-tailed unpaired t-test	$t_{(16)}=0.6892$ , $p=0.5006$
2K	Elevated Plus Maze	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=10$	$+/+$ : $97.11\pm 14.8$ $+/\Delta-GAP$ : $71.43\pm 14$	Two-tailed unpaired t-test	$t_{(16)}=1.273$ , $p=0.2212$
2L	Baseline rotarod	$n_{+/+}=12$ , $n_{+/\Delta-GAP}=12$		Two-way ANOVA	day $F_{(4,88)}=12.43$ , $p<0.0001$ ; genotype $F_{(1,22)}=1.606$ , $p=0.2183$ ; day x genotype $F_{(4,88)}=0.1084$ , $p=0.9793$
2M	Accelerating rotarod	$n_{+/+}=12$ , $n_{+/\Delta-GAP}=12$		Two-way ANOVA	day $F_{(4,88)}=4.757$ , $p=0.0016$ ; genotype $F_{(1,22)}=2.528$ , $p=0.1261$ ; day x genotype $F_{(4,88)}=0.0724$ , $p=0.9903$
3B	Watermaze Training - hidden	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=9$		Two-way ANOVA	day $F_{(5,75)}=16.49$ , $p<0.0001$ ; genotype $F_{(1,15)}=2.845$ , $p=0.1123$ ; interaction day x genotype $F_{(5,75)}=1.849$ , $p=0.1136$
3C	Watermaze % time in target	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=9$		Two-way ANOVA	day $F_{(5,75)}=16.49$ , $p<0.0001$ ; genotype $F_{(1,15)}=2.845$ , $p=0.1123$ ; interaction day x genotype $F_{(5,75)}=1.849$ , $p=0.1136$
3D	Watermaze Training - reversal	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=9$		Two-way ANOVA	day $F_{(5,75)}=29.48$ , $p<0.0001$ ; genotype $F_{(1,15)}=1.159$ , $p=0.2987$ ; interaction day x genotype $F_{(5,75)}=0.56$ , $p=0.7303$
3E	Watermaze % time in target	$n_{+/+}=8$ , $n_{+/\Delta-GAP}=9$		Two-way ANOVAs  ( <i>post-hoc</i> Sidak's multiple comparison test)	For $+/+$ (target vs old location): day $F_{(1,14)}=0.093$ , $p=0.7648$ ; location $F_{(1,14)}=8.422$ , $p=0.0116$ ; interaction day x location $F_{(1,14)}=5.342$ , $p=0.0037$ post-hoc (target vs old): P3: $p=0.6414$ P4: $p=0.0018$  For $+/\Delta-GAP$ (target vs old location): day $F_{(1,16)}=1.356$ , $p=0.2612$ ; location $F_{(1,16)}=36.62$ , $p<0.0001$ ; interaction day x location $F_{(1,16)}=24.36$ , $p=0.0001$ post-hoc (target vs old):

					P3: $p=0.3475$ P4: $p<0.0001$
3F	Watermaze Speed training	$n_{+/-}=8$ , $n_{+/\Delta-GAP}=9$		Two-way ANOVA	genotype $F_{(1,15)}=4.945$ , $p=0.0419$ ; day $F_{(5,75)}=3.580$ , $p=0.0059$ ; interaction day x genotype $F_{(5,75)}=0.7567$ , $p=0.5838$
3G	Watermaze Speed reversal	$n_{+/-}=8$ , $n_{+/\Delta-GAP}=9$		Two-way ANOVA	genotype $F_{(1,15)}=6.041$ , $p=0.0266$ ; day $F_{(5,75)}=4.714$ , $p=0.0008$ ; interaction day x genotype $F_{(5,75)}=1.885$ , $p=0.1070$
4B	Mean exploration	$n_{+/-}=5$ , $n_{+/\Delta-GAP}=8$		Two-way ANOVA	genotype $F_{(1,11)}=4.752$ , $p=0.0519$ ; task $F_{(3.587,$ $39.46)}=1.185$ , $p=0.3309$ ; interaction task x genotype $F_{(7,77)}=0.6130$ , $p=0.7436$
4C	Object Recognition	$n_{+/-}=5$ , $n_{+/\Delta-GAP}=8$	$+/-: 0.26\pm 0.079$ $+/\Delta-GAP: 0.43\pm 0.04$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(4)}=3.336$ , $p=0.0289$ $+/\Delta-GAP: t_{(7)}=9.632$ , $p<0.0001$
				Two-tailed unpaired t- test	$t_{(11)}=2.050$ , $p=0.0650$
4C	Object Context Recognition	$n_{+/-}=5$ , $n_{+/\Delta-GAP}=6$	$+/-: 0.38\pm 0.05$ $+/\Delta-GAP: 0.41\pm 0.10$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(4)}=8.082$ , $p=0.0013$ $+/\Delta-GAP: t_{(5)}=4.078$ , $p=0.0096$
				Two-tailed unpaired t- test	$t_{(9)}=0.2684$ , $p=0.7944$
4C	Object Place Recognition	$n_{+/-}=5$ , $n_{+/\Delta-GAP}=7$	$+/-: 0.30\pm 0.09$ $+/\Delta-GAP: 0.32\pm 0.09$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(4)}=3.140$ , $p=0.0348$ $+/\Delta-GAP: t_{(6)}=3.460$ , $p=0.0135$
				Two-tailed unpaired t- test	$t_{(10)}=0.1981$ , $p=0.8470$
4C	Object Place Context Recognition	$n_{+/-}=5$ , $n_{+/\Delta-GAP}=6$	$+/-: 0.38\pm 0.08$ $+/\Delta-GAP: 0.26\pm 0.06$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(4)}=4.491$ , $p=0.0109$ $+/\Delta-GAP: t_{(5)}=4.248$ , $p=0.0081$
				Two-tailed unpaired t- test	$t_{(9)}=1.122$ , $p=0.2910$
5B	Social interaction Time in chamber	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=14$			genotype $F_{(1,24)}=4.647$ , $p=0.0414$ ; stimulus $F_{(1,24)}=30.55$ , $p<0.0001$ ; interaction task x genotype $F_{(1,24)}=3.560$ , $p=0.0713$
5C	Sociability index	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=14$	$+/-: 0.47\pm 0.1$ $+/\Delta-GAP: 0.28\pm 0.07$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(11)}=4.649$ , $p=0.0007$ $+/\Delta-GAP: t_{(13)}=4.00$ , $p=0.0015$
				Two-tailed unpaired t- test	$t_{(24)}=1.561$ , $p=0.1317$
5D	Social interaction Time sniffing	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=14$		Two-way ANOVA	genotype $F_{(1,24)}=24.55$ , $p<0.0001$ ; stimulus $F_{(1,24)}=85.01$ , $p<0.0001$ ; interaction task x genotype $F_{(1,24)}=15.92$ , $p=0.0005$

				(post-hocs Bonferroni's multiple comparison test)	social vs empty: WT: $p < 0.0001$ HET: $p = 0.0015$  WT vs HET: social: $p < 0.0001$ empty: $p > 0.9999$
5E	Sociability index	$n_{+/+}=12,$ $n_{+/\Delta-GAP}=14$	$+/+ : 0.73 \pm 0.06$ $+/\Delta-GAP : 0.58 \pm 0.08$	One-sample t-test Theoretical $\mu = 0$	$+/+ : t_{(11)}=11.48, p < 0.0001$ $+/\Delta-GAP : t_{(13)}=7.592, p < 0.0001$
				Two-tailed unpaired t-test	$t_{(24)}=1.492, p=0.3976$
5F	Social preference Time in chamber	$n_{+/+}=10,$ $n_{+/\Delta-GAP}=10$			genotype $F_{(1,18)}=0.8293, p=0.3745$ ; stimulus $F_{(1,18)}=47.86, p < 0.0001$ ; interaction task x genotype $F_{(1,18)}=0.3826, p=0.5440$
5G	Social preference index	$n_{+/+}=10,$ $n_{+/\Delta-GAP}=10$	$+/+ : 0.47 \pm 0.14$ $+/\Delta-GAP : 0.33 \pm 0.15$	One-sample t-test Theoretical $\mu = 0$	$+/+ : t_{(9)}=5.807, p=0.0003$ $+/\Delta-GAP : t_{(9)}=2.996, p=0.0151$
				Two-tailed unpaired t-test	$t_{(18)}=0.9939, p=0.3334$
5H	Social preference Time sniffing	$n_{+/+}=10,$ $n_{+/\Delta-GAP}=10$			genotype $F_{(1,18)}=4.578, p=0.0463$ ; stimulus $F_{(1,18)}=6.678, p=0.0187$ ; interaction task x genotype $F_{(1,18)}=0.4159, p=0.5271$
5I	Social preference index	$n_{+/+}=10,$ $n_{+/\Delta-GAP}=10$	$+/+ : 0.21 \pm 0.09$ $+/\Delta-GAP : 0.13 \pm 0.14$	One-sample t-test Theoretical $\mu = 0$	$+/+ : t_{(9)}=2.277, p=0.0488$ $+/\Delta-GAP : t_{(9)}=0.9581, p=0.3630$
				Two-tailed unpaired t-test	$t_{(18)}=0.4513, p=0.6572$
6C	SWD proportion per genotype quantification	$n_{+/+}=12,$ $n_{+/\Delta-GAP}=12,$ Other=6	$+/+ : 2$ from 12 $+/\Delta-GAP : 9$ from 12 Other: 0 from 6	Chi-Sq Comparison of proportions	$\chi^2_{(2)} = 6.042, p=0.014$
6D	Spectral profile of SWD	$n_{+/+}=2,$ $n_{+/\Delta-GAP}=9$	$+/+ : 7.6 \pm 0.2$ $+/\Delta-GAP : 7.76 \pm 0.13$		
6E	Spectral band comparison of SWD	$n_{+/+}=2,$ $n_{+/\Delta-GAP}=9$	$+/+ :$ Delta: $4.67 \pm 0,$ Theta: $4.88 \pm 0.03$ Sigma: $4.61 \pm 0.06$ GammaL: $3.7 \pm 0.01,$ GammaH: $3.05 \pm 0.03$ $+/\Delta-GAP :$ Delta: $4.43 \pm 0.09,$ Theta: $4.75 \pm 0.08,$ Sigma: $4.59 \pm 0.09,$ Gamma L: $3.89 \pm 0.09,$ Gamma H: $3.26 \pm 0.08$	Two-tailed unpaired t-test and Mann-Whitney Rank Sum Test (for not-normal data)	Delta: $U=2, p=0.126$ Theta: $t_{(9)}=0.620, p=0.55$ Sigma: $t_{(9)}=0.0721, p=0.944$ Gamma Low $t_{(9)}=0.798, p=0.446$ Gamma High $U=3, p=0.195$

6F	Number of SWD	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=12$	$+/-: 8.3\pm 5.9$ $+/\Delta-GAP: 123.6\pm 32.2$	Mann-Whitney Rank Sum Test	$U = 23$ $p = 0.002$
6G	Cumulative frequency of SWD (number against duration)	$n_{+/-}=2$ , $n_{+/\Delta-GAP}=9$		Kolmogorov-Smirnov test	$D_{(130)} = 0.862, p < 0.001$
7B	SWD number by treatment	$n_{+/\Delta-GAP}=7$	Untreated: $55.2\pm 16.30$ ETX: $0.86\pm 0.5$ Saline: $89.4\pm 24.9$	One-way RM ANOVA (post-hoc two-tailed paired t-test - Holm-Sidak correction)	Effect of treatment day $F_{(2,12)}=9.25, p=0.004$ saline vs. ETX $t_{(6)}=4.25, p=0.003$ saline vs. untreated vs ETX $t_{(6)}=2.69, p=0.04$ untreated vs saline $t_{(6)}=1.56, p=0.146$
7C	SWD prevalence by day	$n_{+/\Delta-GAP}=7$	ETXvsPreETX: $-93.5 \pm -5.0$ PostETXvsPreETX: $-1.86 \pm -18.3$ SALvsPreSAL: $43.1 \pm -15.1$ PostSALvsPreSAL: $26.5 \pm -13.8$	One-way RM ANOVA (post-hoc two-tailed paired t-test - Holm-Sidak correction)	Effect of treatment day $F_{(3,18)}= 18.24, p < 0.001$
S2A	Marble burying time	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=12$	$+/-: 123.6\pm 34.07$ $+/\Delta-GAP: 47.63\pm 8.73$	Two-tailed unpaired t-test	$t_{(22)}=2.161, p=0.042$
S2B	Marble burying frequency	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=12$	$+/-: 34.17\pm 5.4$ $+/\Delta-GAP: 16.83\pm 3.76$	Two-tailed unpaired t-test	$t_{(22)}=2.634, p=0.0152$
S3A	Social interaction Time in chamber	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=14$			genotype $F_{(1,24)}=0.7652, p=0.39$ ; stimulus $F_{(1,24)}=62.64, p < 0.0001$ ; interaction task x genotype $F_{(1,24)}=5.04, p=0.0343$  social vs empty WT: $p < 0.0001$ HET: $p=0.0007$  WT vs HET social: $p=0.0444$ empty: $p=0.3221$
S3B	Sociability index	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=14$	$+/-: 0.644\pm 0.04$ $+/\Delta-GAP: 0.36\pm 0.12$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(11)}=16.46, p < 0.0001$ $+/\Delta-GAP: t_{(13)}=3.041, p=0.0095$
				Two-tailed unpaired t-test	$t_{(24)}=2.135, p=0.0432$
S3C	Social interaction Time sniffing	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=14$		Two-way ANOVA (post-hocs Bonferroni's multiple	genotype $F_{(1,24)}=25.03, p < 0.0001$ ; stimulus $F_{(1,24)}=178.3, p < 0.0001$ ; interaction task x genotype $F_{(1,24)}=21.89, p < 0.0001$  social vs empty WT: $p < 0.0001$

				comparison test)	HET: $p < 0.0001$ WT vs HET social: $p < 0.0001$ empty: $p > 0.9999$
S3D	Sociability index	$n_{+/-}=12,$ $n_{+/\Delta-GAP}=14$	$+/-: 0.86 \pm 0.04$ $+/\Delta-GAP: 0.81 \pm 0.05$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(11)}=24.26, p < 0.0001$ $+/\Delta-GAP: t_{(13)}=15.41, p < 0.0001$
				Two-tailed unpaired t-test	$t_{(24)}=0.8167, p=0.4221$
S3E	Social preference Time in chamber	$n_{+/-}=10,$ $n_{+/\Delta-GAP}=10$			genotype $F_{(1,18)}=0.6199, p=0.4413$ ; stimulus $F_{(1,18)}=75.62, p < 0.0001$ ; interaction task x genotype $F_{(1,18)}=0.554, p=0.4663$
S3F	Social preference index	$n_{+/-}=10,$ $n_{+/\Delta-GAP}=10$	$+/-: 0.56 \pm 0.08$ $+/\Delta-GAP: 0.47 \pm 0.09$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(9)}=6.615, p < 0.0001$ $+/\Delta-GAP: t_{(9)}=5.342, p=0.0005$
				Two-tailed unpaired t-test	$t_{(18)}=0.7958, p=0.4365$
S3G	Social preference Time sniffing	$n_{+/-}=10,$ $n_{+/\Delta-GAP}=10$			genotype $F_{(1,18)}=3.098, p=0.0954$ ; stimulus $F_{(1,18)}=11.11, p=0.0037$ ; interaction task x genotype $F_{(1,18)}=1.812, p=0.1950$
S3I	Social preference index	$n_{+/-}=10,$ $n_{+/\Delta-GAP}=10$	$+/-: 0.36 \pm 0.11$ $+/\Delta-GAP: 0.16 \pm 0.1$	One-sample t-test Theoretical $\mu = 0$	$+/-: t_{(9)}=3.153, p=0.0117$ $+/\Delta-GAP: t_{(9)}=01.647, p=0.1340$
				Two-tailed unpaired t-test	$t_{(18)}=1.284, p=0.2154$
S4C	Time sniffing	banana	$n_{+/-}=11,$ $n_{+/\Delta-GAP}=10$	Two-tailed unpaired t-test	$t_{(19)}=5.568, p < 0.0001$
		almond	$n_{+/-}=11,$ $n_{+/\Delta-GAP}=9$	Two-tailed unpaired t-test	$t_{(18)}=5.213, p < 0.0001$
S4F	Time sniffing	Social1	$n_{+/-}=9,$ $n_{+/\Delta-GAP}=5$	Two-tailed unpaired t-test	$t_{(12)}=2.427, p=0.0319$
		Social2	$n_{+/-}=8,$ $n_{+/\Delta-GAP}=5$	Two-tailed unpaired t-test	$t_{(11)}=0.5930, p=0.5652$
S5E	Number of SWD visual vs automatic	Visual = 24, Automatic=24	Visual: $143.9 \pm 29.9$ Auto: $159.4 \pm 37.3$	Paired t-test	$t_{(10)}= 1.624, p=0.135$
S5F	Number of SWD per genotype (Visual Count)	$n_{+/-}=12,$ $n_{+/\Delta-GAP}=12$	$+/-: 8.3 \pm 5.9$ $+/\Delta-GAP: 123.6 \pm 32.2$	Mann-Whitney Rank Sum Test	$U = 23 p = 0.002$
S5G	Number of SWD per genotype	$n_{+/-}=12,$ $n_{+/\Delta-GAP}=12$	$+/-: 10.54 \pm 7.9$ $+/\Delta-GAP: 135.5 \pm 39.1$	Mann-Whitney Rank Sum Test	$U = 25 p = 0.003$

	(Automatic Count)				
S6C	Number of SWD per min Wake ratio	$n_{+/-}=12$ , $n_{+/\Delta-GAP}=12$	$+/-: 0.04 \pm 0.03$ $+/\Delta-GAP: 0.65 \pm 0.16$	Two-tailed unpaired t-test	$t_{(22)}=3.794, p<0.001$
S7C	SWD count	$n_{+/\Delta-GAPpre-light}=6$ , $n_{+/\Delta-GAPlight}=6$ , $n_{+/\Delta-GAPpost-light}=6$		One-way ANOVA	Effect of light $F_{(2,15)}= 1.00$ , $p=0.391$

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## References

- 1  
2 Ahmadian, M. R., Stege, P., Scheffzek, K., & Wittinghofer, A. (1997, Sep). Confirmation of the arginine-  
3 finger hypothesis for the GAP-stimulated GTP-hydrolysis reaction of Ras. *Nat Struct Biol*, 4(9),  
4 686-689. <https://doi.org/10.1038/nsb0997-686>
- 5 Araki, Y., Hong, I., Gamache, T. R., Ju, S., Collado-Torres, L., Shin, J. H., & Haganir, R. L. (2020, Jun  
6 24). SynGAP isoforms differentially regulate synaptic plasticity and dendritic development.  
7 *Elife*, 9. <https://doi.org/10.7554/eLife.56273>
- 8 Araki, Y., Zeng, M., Zhang, M., & Haganir, R. L. (2015, Jan 7). Rapid dispersion of SynGAP from  
9 synaptic spines triggers AMPA receptor insertion and spine enlargement during LTP. *Neuron*,  
10 85(1), 173-189. <https://doi.org/10.1016/j.neuron.2014.12.023>
- 11 Berryer, M. H., Chattopadhyaya, B., Xing, P., Riebe, I., Bosoi, C., Sanon, N., Antoine-Bertrand, J.,  
12 Levesque, M., Avoli, M., Hamdan, F. F., Carmant, L., Lamarche-Vane, N., Lacaille, J. C.,  
13 Michaud, J. L., & Di Cristo, G. (2016, Nov 9). Decrease of SYNGAP1 in GABAergic cells impairs  
14 inhibitory synapse connectivity, synaptic inhibition and cognitive function. *Nat Commun*, 7,  
15 13340. <https://doi.org/10.1038/ncomms13340>
- 16 Berryer, M. H., Hamdan, F. F., Klitten, L. L., Moller, R. S., Carmant, L., Schwartzentruber, J., Patry, L.,  
17 Dobrzyniecka, S., Rochefort, D., Neugnot-Ceroli, M., Lacaille, J. C., Niu, Z., Eng, C. M., Yang,  
18 Y., Palardy, S., Belhumeur, C., Rouleau, G. A., Tommerup, N., Immken, L., Beauchamp, M. H.,  
19 Patel, G. S., Majewski, J., Tarnopolsky, M. A., Scheffzek, K., Hjalgrim, H., Michaud, J. L., & Di  
20 Cristo, G. (2013, Feb). Mutations in SYNGAP1 cause intellectual disability, autism, and a  
21 specific form of epilepsy by inducing haploinsufficiency. *Hum Mutat*, 34(2), 385-394.  
22 <https://doi.org/10.1002/humu.22248>
- 23 Blumenfeld, H. (2005). Cellular and network mechanisms of spike-wave seizures. *Epilepsia*, 46 Suppl  
24 9, 21-33. <https://doi.org/10.1111/j.1528-1167.2005.00311.x>
- 25 Carvill, G. L., Heavin, S. B., Yendle, S. C., McMahon, J. M., O'Roak, B. J., Cook, J., Khan, A.,  
26 Dorschner, M. O., Weaver, M., Calvert, S., Malone, S., Wallace, G., Stanley, T., Bye, A. M.,  
27 Bleasel, A., Howell, K. B., Kivity, S., Mackay, M. T., Rodriguez-Casero, V., Webster, R.,  
28 Korczyn, A., Afawi, Z., Zelnick, N., Lerman-Sagie, T., Lev, D., Moller, R. S., Gill, D., Andrade,  
29 D. M., Freeman, J. L., Sadleir, L. G., Shendure, J., Berkovic, S. F., Scheffer, I. E., & Mefford,  
30 H. C. (2013, Jul). Targeted resequencing in epileptic encephalopathies identifies de novo  
31 mutations in CHD2 and SYNGAP1. *Nat Genet*, 45(7), 825-830. <https://doi.org/10.1038/ng.2646>
- 32 Chen, H. J., Rojas-Soto, M., Oguni, A., & Kennedy, M. B. (1998, May). A synaptic Ras-GTPase  
33 activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron*, 20(5), 895-904.  
34 [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&listuids=9620694)  
35 [uids=9620694](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&listuids=9620694)
- 36 Childers, D., Skinner, D., & Kemeraït, R. C. (1977). The cepstrum: A guide to processing. *Proceedings*  
37 *of the IEEE*, 65, 1428-1443.
- 38 Clement, J. P., Ozkan, E. D., Aceti, M., Miller, C. A., & Rumbaugh, G. (2013, Jun 19). SYNGAP1 links  
39 the maturation rate of excitatory synapses to the duration of critical-period synaptic plasticity. *J*  
40 *Neurosci*, 33(25), 10447-10452. <https://doi.org/10.1523/JNEUROSCI.0765-13.2013>
- 41 Coenen, A. M., & Van Luijtelaar, E. L. (2003, Nov). Genetic animal models for absence epilepsy: a  
42 review of the WAG/Rij strain of rats. *Behav Genet*, 33(6), 635-655.  
43 <https://doi.org/10.1023/a:1026179013847>
- 44 Crawley, J. N., Belknap, J. K., Collins, A., Crabbe, J. C., Frankel, W., Henderson, N., Hitzemann, R. J.,  
45 Maxson, S. C., Miner, L. L., Silva, A. J., Wehner, J. M., Wynshaw-Boris, A., & Paylor, R. (1997,  
46 Jul). Behavioral phenotypes of inbred mouse strains: implications and recommendations for  
47 molecular studies. *Psychopharmacology (Berl)*, 132(2), 107-124.  
48 [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&listuids=9266608)  
49 [uids=9266608](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&listuids=9266608)
- 50 Creson, T. K., Rojas, C., Hwaun, E., Vaissiere, T., Kilinc, M., Jimenez-Gomez, A., Holder, J. L., Jr.,  
51 Tang, J., Colgin, L. L., Miller, C. A., & Rumbaugh, G. (2019, Apr 26). Re-expression of SynGAP  
52 protein in adulthood improves translatable measures of brain function and behavior. *Elife*, 8.  
53 <https://doi.org/10.7554/eLife.46752>
- 54 Deciphering Developmental Disorders, S. (2015, Mar 12). Large-scale discovery of novel genetic  
55 causes of developmental disorders. *Nature*, 519(7542), 223-228.  
56 <https://doi.org/10.1038/nature14135>
- 57 Deciphering Developmental Disorders, S. (2017, Feb 23). Prevalence and architecture of de novo  
58 mutations in developmental disorders. *Nature*, 542(7642), 433-438.  
59 <https://doi.org/10.1038/nature21062>

- 1 Geurts, A. M., Cost, G. J., Freyvert, Y., Zeitler, B., Miller, J. C., Choi, V. M., Jenkins, S. S., Wood, A.,  
2 Cui, X., Meng, X., Vincent, A., Lam, S., Michalkiewicz, M., Schilling, R., Foeckler, J., Kalloway,  
3 S., Weiler, H., Menoret, S., Anegon, I., Davis, G. D., Zhang, L., Rebar, E. J., Gregory, P. D.,  
4 Urnov, F. D., Jacob, H. J., & Buelow, R. (2009, Jul 24). Knockout rats via embryo microinjection  
5 of zinc-finger nucleases. *Science*, 325(5939), 433. <https://doi.org/10.1126/science.1172447>
- 6 Gou, G., Roca-Fernandez, A., Kilinc, M., Serrano, E., Reig-Viader, R., Araki, Y., Haganir, R. L., de  
7 Quintana-Schmidt, C., Rumbaugh, G., & Bayes, A. (2020, Feb 18). SynGAP splice variants  
8 display heterogeneous spatio-temporal expression and subcellular distribution in the  
9 developing mammalian brain. *J Neurochem*. <https://doi.org/10.1111/jnc.14988>
- 10 Guo, X., Hamilton, P. J., Reish, N. J., Sweatt, J. D., Miller, C. A., & Rumbaugh, G. (2009, Jun). Reduced  
11 expression of the NMDA receptor-interacting protein SynGAP causes behavioral abnormalities  
12 that model symptoms of Schizophrenia. *Neuropsychopharmacology*, 34(7), 1659-1672.  
13 <https://doi.org/10.1038/npp.2008.223>
- 14 Hamdan, F. F., Daoud, H., Piton, A., Gauthier, J., Dobrzyńska, S., Krebs, M. O., Joober, R., Lacaille,  
15 J. C., Nadeau, A., Milunsky, J. M., Wang, Z., Carmant, L., Mottron, L., Beauchamp, M. H.,  
16 Rouleau, G. A., & Michaud, J. L. (2011, May 1). De novo SYNGAP1 mutations in nonsyndromic  
17 intellectual disability and autism. *Biol Psychiatry*, 69(9), 898-901.  
18 <https://doi.org/10.1016/j.biopsych.2010.11.015>
- 19 Hamdan, F. F., Gauthier, J., Spiegelman, D., Noreau, A., Yang, Y., Pellerin, S., Dobrzyńska, S., Cote,  
20 M., Perreau-Linck, E., Carmant, L., D'Anjou, G., Fombonne, E., Addington, A. M., Rapoport, J.  
21 L., Delisi, L. E., Krebs, M. O., Mouaffak, F., Joober, R., Mottron, L., Drapeau, P., Marineau, C.,  
22 Lafreniere, R. G., Lacaille, J. C., Rouleau, G. A., Michaud, J. L., & Synapse to Disease, G.  
23 (2009, Feb 5). Mutations in SYNGAP1 in autosomal nonsyndromic mental retardation. *N Engl*  
24 *J Med*, 360(6), 599-605. <https://doi.org/10.1056/NEJMoa0805392>
- 25 Kim, J. H., Lee, H. K., Takamiya, K., & Haganir, R. L. (2003, Feb 15). The role of synaptic GTPase-  
26 activating protein in neuronal development and synaptic plasticity. *J Neurosci*, 23(4), 1119-  
27 1124. <http://www.ncbi.nlm.nih.gov/pubmed/12598599>
- 28 Kim, J. H., Liao, D., Lau, L. F., & Haganir, R. L. (1998, Apr). SynGAP: a synaptic RasGAP that  
29 associates with the PSD-95/SAP90 protein family. *Neuron*, 20(4), 683-691.  
30 [https://doi.org/10.1016/s0896-6273\(00\)81008-9](https://doi.org/10.1016/s0896-6273(00)81008-9)
- 31 Klitten, L. L., Moller, R. S., Nikanorova, M., Silaharoglu, A., Hjalgrim, H., & Tommerup, N. (2011, Dec).  
32 A balanced translocation disrupts SYNGAP1 in a patient with intellectual disability, speech  
33 impairment, and epilepsy with myoclonic absences (EMA). *Epilepsia*, 52(12), e190-193.  
34 <https://doi.org/10.1111/j.1528-1167.2011.03304.x>
- 35 Klose, A., Ahmadian, M. R., Schuelke, M., Scheffzek, K., Hoffmeyer, S., Gewies, A., Schmitz, F.,  
36 Kaufmann, D., Peters, H., Wittinghofer, A., & Nurnberg, P. (1998, Aug). Selective disactivation  
37 of neurofibromin GAP activity in neurofibromatosis type 1. *Hum Mol Genet*, 7(8), 1261-1268.  
38 <https://doi.org/10.1093/hmg/7.8.1261>
- 39 Knuesel, I., Elliott, A., Chen, H. J., Mansuy, I. M., & Kennedy, M. B. (2005, Feb). A role for synGAP in  
40 regulating neuronal apoptosis. *Eur J Neurosci*, 21(3), 611-621. <https://doi.org/10.1111/j.1460-9568.2005.03908.x>
- 41
- 42 Ko, J. (2017). Neuroanatomical Substrates of Rodent Social Behavior: The Medial Prefrontal Cortex  
43 and Its Projection Patterns. *Front Neural Circuits*, 11, 41.  
44 <https://doi.org/10.3389/fncir.2017.00041>
- 45 Komiyama, N. H., Watabe, A. M., Carlisle, H. J., Porter, K., Charlesworth, P., Monti, J., Strathdee, D.  
46 J., O'Carroll, C. M., Martin, S. J., Morris, R. G., O'Dell, T. J., & Grant, S. G. (2002, Nov 15).  
47 SynGAP regulates ERK/MAPK signaling, synaptic plasticity, and learning in the complex with  
48 postsynaptic density 95 and NMDA receptor. *J Neurosci*, 22(22), 9721-9732.  
49 <http://www.ncbi.nlm.nih.gov/pubmed/12427827>
- 50 Krapivinsky, G., Medina, I., Krapivinsky, L., Gapon, S., & Clapham, D. E. (2004, Aug 19). SynGAP-  
51 MUPP1-CaMKII synaptic complexes regulate p38 MAP kinase activity and NMDA receptor-  
52 dependent synaptic AMPA receptor potentiation. *Neuron*, 43(4), 563-574.  
53 <https://doi.org/10.1016/j.neuron.2004.08.003>
- 54 Kwon, C. H., Luikart, B. W., Powell, C. M., Zhou, J., Matheny, S. A., Zhang, W., Li, Y., Baker, S. J., &  
55 Parada, L. F. (2006, May 4). Pten regulates neuronal arborization and social interaction in mice.  
56 *Neuron*, 50(3), 377-388. <https://doi.org/10.1016/j.neuron.2006.03.023>
- 57 Letunic, I., & Bork, P. (2018, Jan 4). 20 years of the SMART protein domain annotation resource.  
58 *Nucleic Acids Res*, 46(D1), D493-D496. <https://doi.org/10.1093/nar/gkx922>

- 1 Li, W., Okano, A., Tian, Q. B., Nakayama, K., Furihata, T., Nawa, H., & Suzuki, T. (2001, Jun 15).  
2 Characterization of a novel synGAP isoform, synGAP-beta. *J Biol Chem*, 276(24), 21417-  
3 21424. <https://doi.org/10.1074/jbc.M010744200>
- 4 Mastro, T. L., Preza, A., Basu, S., Chattarji, S., Till, S. M., Kind, P. C., & Kennedy, M. B. (2020, Jan  
5 15). A sex difference in the response of the rodent postsynaptic density to synGAP  
6 haploinsufficiency. *Elife*, 9. <https://doi.org/10.7554/eLife.52656>
- 7 McMahon, A. C., Barnett, M. W., O'Leary, T. S., Stoney, P. N., Collins, M. O., Papadia, S., Choudhary,  
8 J. S., Komiyama, N. H., Grant, S. G., Hardingham, G. E., Wyllie, D. J., & Kind, P. C. (2012).  
9 SynGAP isoforms exert opposing effects on synaptic strength. *Nat Commun*, 3, 900.  
10 <https://doi.org/10.1038/ncomms1900>
- 11 Michaelson, S. D., Ozkan, E. D., Aceti, M., Maity, S., Llamosas, N., Weldon, M., Mizrachi, E., Vaissiere,  
12 T., Gaffield, M. A., Christie, J. M., Holder, J. L., Jr., Miller, C. A., & Rumbaugh, G. (2018, Dec).  
13 SYNGAP1 heterozygosity disrupts sensory processing by reducing touch-related activity within  
14 somatosensory cortex circuits. *Nat Neurosci*, 21(12), 1-13. [https://doi.org/10.1038/s41593-018-](https://doi.org/10.1038/s41593-018-0268-0)  
15 [0268-0](https://doi.org/10.1038/s41593-018-0268-0)
- 16 Mignot, C., von Stulpnagel, C., Nava, C., Ville, D., Sanlaville, D., Lesca, G., Rastetter, A., Gachet, B.,  
17 Marie, Y., Korenke, G. C., Borggraefe, I., Hoffmann-Zacharska, D., Szczepanik, E., Rudzka-  
18 Dybala, M., Yis, U., Caglayan, H., Isapof, A., Marey, I., Panagiotakaki, E., Korff, C., Rossier,  
19 E., Riess, A., Beck-Woedl, S., Rauch, A., Zweier, C., Hoyer, J., Reis, A., Mironov, M., Bobylova,  
20 M., Mukhin, K., Hernandez-Hernandez, L., Maher, B., Sisodiya, S., Kuhn, M., Glaeser, D.,  
21 Wechuysen, S., Myers, C. T., Mefford, H. C., Hortnagel, K., Biskup, S., Euro, E.-R. E. S. M. A.  
22 E. w. g., Lemke, J. R., Heron, D., Kluger, G., & Depienne, C. (2016, Mar 17). Genetic and  
23 neurodevelopmental spectrum of SYNGAP1-associated intellectual disability and epilepsy. *J*  
24 *Med Genet*. <https://doi.org/10.1136/jmedgenet-2015-103451>
- 25 Morris, R. G., Garrud, P., Rawlins, J. N., & O'Keefe, J. (1982, Jun 24). Place navigation impaired in rats  
26 with hippocampal lesions. *Nature*, 297(5868), 681-683.  
27 [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7088155)  
28 [\\_uids=7088155](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=7088155)
- 29 Muhia, M., Feldon, J., Knuesel, I., & Yee, B. K. (2009, Oct). Appetitively motivated instrumental learning  
30 in SynGAP heterozygous knockout mice. *Behav Neurosci*, 123(5), 1114-1128.  
31 <https://doi.org/10.1037/a0017118>
- 32 Muhia, M., Yee, B. K., Feldon, J., Markopoulos, F., & Knuesel, I. (2010, Feb). Disruption of  
33 hippocampus-regulated behavioural and cognitive processes by heterozygous constitutive  
34 deletion of SynGAP. *Eur J Neurosci*, 31(3), 529-543. [https://doi.org/10.1111/j.1460-](https://doi.org/10.1111/j.1460-9568.2010.07079.x)  
35 [9568.2010.07079.x](https://doi.org/10.1111/j.1460-9568.2010.07079.x)
- 36 Nakajima, R., Takao, K., Hattori, S., Shoji, H., Komiyama, N. H., Grant, S. G. N., & Miyakawa, T. (2019,  
37 Sep). Comprehensive behavioral analysis of heterozygous Syngap1 knockout mice.  
38 *Neuropsychopharmacol Rep*, 39(3), 223-237. <https://doi.org/10.1002/npr2.12073>
- 39 Ozkan, E. D., Creson, T. K., Kramar, E. A., Rojas, C., Seese, R. R., Babyan, A. H., Shi, Y., Lucero, R.,  
40 Xu, X., Noebels, J. L., Miller, C. A., Lynch, G., & Rumbaugh, G. (2014, Jun 18). Reduced  
41 cognition in Syngap1 mutants is caused by isolated damage within developing forebrain  
42 excitatory neurons. *Neuron*, 82(6), 1317-1333. <https://doi.org/10.1016/j.neuron.2014.05.015>
- 43 Parikshak, N. N., Luo, R., Zhang, A., Won, H., Lowe, J. K., Chandran, V., Horvath, S., & Geschwind,  
44 D. H. (2013, Nov 21). Integrative functional genomic analyses implicate specific molecular  
45 pathways and circuits in autism. *Cell*, 155(5), 1008-1021.  
46 <https://doi.org/10.1016/j.cell.2013.10.031>
- 47 Parker, M. J., Fryer, A. E., Shears, D. J., Lachlan, K. L., McKee, S. A., Magee, A. C., Mohammed, S.,  
48 Vasudevan, P. C., Park, S. M., Benoit, V., Lederer, D., Maystadt, I., Study, D., & FitzPatrick, D.  
49 R. (2015, Oct). De novo, heterozygous, loss-of-function mutations in SYNGAP1 cause a  
50 syndromic form of intellectual disability. *Am J Med Genet A*, 167A(10), 2231-2237.  
51 <https://doi.org/10.1002/ajmg.a.37189>
- 52 Pearce, P. S., Friedman, D., Lafrancois, J. J., Iyengar, S. S., Fenton, A. A., Maclusky, N. J., &  
53 Scharfman, H. E. (2014, Mar). Spike-wave discharges in adult Sprague-Dawley rats and their  
54 implications for animal models of temporal lobe epilepsy. *Epilepsy Behav*, 32, 121-131.  
55 <https://doi.org/10.1016/j.yebeh.2014.01.004>
- 56 Pena, V., Hothorn, M., Eberth, A., Kaschau, N., Parret, A., Gremer, L., Bonneau, F., Ahmadian, M. R.,  
57 & Scheffzek, K. (2008, Apr). The C2 domain of SynGAP is essential for stimulation of the Rap  
58 GTPase reaction. *EMBO Rep*, 9(4), 350-355. <https://doi.org/10.1038/embor.2008.20>
- 59 Pinto, D., Pagnamenta, A. T., Klei, L., Anney, R., Merico, D., Regan, R., Conroy, J., Magalhaes, T. R.,  
60 Correia, C., Abrahams, B. S., Almeida, J., Bacchelli, E., Bader, G. D., Bailey, A. J., Baird, G.,

- 1 Battaglia, A., Berney, T., Bolshakova, N., Bolte, S., Bolton, P. F., Bourgeron, T., Brennan, S.,  
2 Brian, J., Bryson, S. E., Carson, A. R., Casallo, G., Casey, J., Chung, B. H., Cochrane, L.,  
3 Corsello, C., Crawford, E. L., Crossett, A., Cytrynbaum, C., Dawson, G., de Jonge, M., Delorme,  
4 R., Drmic, I., Duketis, E., Duque, F., Estes, A., Farrar, P., Fernandez, B. A., Folstein, S. E.,  
5 Fombonne, E., Freitag, C. M., Gilbert, J., Gillberg, C., Glessner, J. T., Goldberg, J., Green, A.,  
6 Green, J., Guter, S. J., Hakonarson, H., Heron, E. A., Hill, M., Holt, R., Howe, J. L., Hughes,  
7 G., Hus, V., Iglizzi, R., Kim, C., Klauck, S. M., Kolevzon, A., Korvatska, O., Kustanovich, V.,  
8 Lajonchere, C. M., Lamb, J. A., Laskawiec, M., Leboyer, M., Le Couteur, A., Leventhal, B. L.,  
9 Lionel, A. C., Liu, X. Q., Lord, C., Lotspeich, L., Lund, S. C., Maestrini, E., Mahoney, W.,  
10 Mantoulan, C., Marshall, C. R., McConachie, H., McDougale, C. J., McGrath, J., McMahon, W.  
11 M., Merikangas, A., Migita, O., Minshew, N. J., Mirza, G. K., Munson, J., Nelson, S. F., Noakes,  
12 C., Noor, A., Nygren, G., Oliveira, G., Papanikolaou, K., Parr, J. R., Parrini, B., Paton, T.,  
13 Pickles, A., Pilorge, M., Piven, J., Ponting, C. P., Posey, D. J., Poustka, A., Poustka, F., Prasad,  
14 A., Ragoussis, J., Renshaw, K., Rickaby, J., Roberts, W., Roeder, K., Roge, B., Rutter, M. L.,  
15 Bierut, L. J., Rice, J. P., Salt, J., Sansom, K., Sato, D., Segurado, R., Sequeira, A. F., Senman,  
16 L., Shah, N., Sheffield, V. C., Soorya, L., Sousa, I., Stein, O., Sykes, N., Stoppioni, V.,  
17 Strawbridge, C., Tancredi, R., Tansey, K., Thiruvahindrapuram, B., Thompson, A. P.,  
18 Thomson, S., Tryfon, A., Tsiantis, J., Van Engeland, H., Vincent, J. B., Volkmar, F., Wallace,  
19 S., Wang, K., Wang, Z., Wassink, T. H., Webber, C., Weksberg, R., Wing, K., Wittemeyer, K.,  
20 Wood, S., Wu, J., Yaspan, B. L., Zurawiecki, D., Zwaigenbaum, L., Buxbaum, J. D., Cantor, R.  
21 M., Cook, E. H., Coon, H., Cuccaro, M. L., Devlin, B., Ennis, S., Gallagher, L., Geschwind, D.  
22 H., Gill, M., Haines, J. L., Hallmayer, J., Miller, J., Monaco, A. P., Nurnberger, J. I., Jr., Paterson,  
23 A. D., Pericak-Vance, M. A., Schellenberg, G. D., Szatmari, P., Vicente, A. M., Vieland, V. J.,  
24 Wijsman, E. M., Scherer, S. W., Sutcliffe, J. S., & Betancur, C. (2010, Jul 15). Functional impact  
25 of global rare copy number variation in autism spectrum disorders. *Nature*, 466(7304), 368-  
26 372. <https://doi.org/10.1038/nature09146>
- 27 Racine, R. J. (1972, Mar). Modification of seizure activity by electrical stimulation. II. Motor seizure.  
28 *Electroencephalogr Clin Neurophysiol*, 32(3), 281-294. [https://doi.org/10.1016/0013-4694\(72\)90177-0](https://doi.org/10.1016/0013-4694(72)90177-0)
- 29
- 30 Rodgers, K. M., Dudek, F. E., & Barth, D. S. (2015, Jun 17). Progressive, Seizure-Like, Spike-Wave  
31 Discharges Are Common in Both Injured and Uninjured Sprague-Dawley Rats: Implications for  
32 the Fluid Percussion Injury Model of Post-Traumatic Epilepsy. *J Neurosci*, 35(24), 9194-9204.  
33 <https://doi.org/10.1523/JNEUROSCI.0919-15.2015>
- 34 Rumbaugh, G., Adams, J. P., Kim, J. H., & Haganir, R. L. (2006, Mar 21). SynGAP regulates synaptic  
35 strength and mitogen-activated protein kinases in cultured neurons. *Proc Natl Acad Sci U S A*,  
36 103(12), 4344-4351. <https://doi.org/10.1073/pnas.0600084103>
- 37 Sarowar, T., Grabrucker, S., Fohr, K., Mangus, K., Eckert, M., Bockmann, J., Boeckers, T. M., &  
38 Grabrucker, A. M. (2016, Mar 11). Enlarged dendritic spines and pronounced neophobia in  
39 mice lacking the PSD protein RICH2. *Mol Brain*, 9, 28. <https://doi.org/10.1186/s13041-016-0206-6>
- 40
- 41 Satterstrom, F. K., Kosmicki, J. A., Wang, J., Breen, M. S., De Rubeis, S., An, J. Y., Peng, M., Collins,  
42 R., Grove, J., Klei, L., Stevens, C., Reichert, J., Mulhern, M. S., Artomov, M., Gerges, S.,  
43 Sheppard, B., Xu, X., Bhaduri, A., Norman, U., Brand, H., Schwartz, G., Nguyen, R., Guerrero,  
44 E. E., Dias, C., Autism Sequencing, C., i, P.-B. C., Betancur, C., Cook, E. H., Gallagher, L., Gill,  
45 M., Sutcliffe, J. S., Thurm, A., Zwick, M. E., Borglum, A. D., State, M. W., Cicek, A. E.,  
46 Talkowski, M. E., Cutler, D. J., Devlin, B., Sanders, S. J., Roeder, K., Daly, M. J., & Buxbaum,  
47 J. D. (2020, Feb 6). Large-Scale Exome Sequencing Study Implicates Both Developmental and  
48 Functional Changes in the Neurobiology of Autism. *Cell*, 180(3), 568-584 e523.  
49 <https://doi.org/10.1016/j.cell.2019.12.036>
- 50 Shaw, F. Z. (2004, Jan). Is spontaneous high-voltage rhythmic spike discharge in Long Evans rats an  
51 absence-like seizure activity? *J Neurophysiol*, 91(1), 63-77.  
52 <https://doi.org/10.1152/jn.00487.2003>
- 53 Shaw, F. Z. (2007, Jan). 7-12 Hz high-voltage rhythmic spike discharges in rats evaluated by  
54 antiepileptic drugs and flicker stimulation. *J Neurophysiol*, 97(1), 238-247.  
55 <https://doi.org/10.1152/jn.00340.2006>
- 56 Shin, L. M., & Liberzon, I. (2010, Jan). The neurocircuitry of fear, stress, and anxiety disorders.  
57 *Neuropsychopharmacology*, 35(1), 169-191.  
58 [http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&listuids=19625997)  
59 [uids=19625997](http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&listuids=19625997)

- 1 Siegle, J. H., Lopez, A. C., Patel, Y. A., Abramov, K., Ohayon, S., & Voigts, J. (2017, Aug). Open Ephys:  
2 an open-source, plugin-based platform for multichannel electrophysiology. *J Neural Eng*, 14(4),  
3 045003. <https://doi.org/10.1088/1741-2552/aa5eea>
- 4 Sullivan, B. J., Ammanuel, S., Kipnis, P. A., Araki, Y., Huganir, R. L., & Kadam, S. D. (2020, May 1).  
5 Low-Dose Perampanel Rescues Cortical Gamma Dysregulation Associated With Parvalbumin  
6 Interneuron GluA2 Upregulation in Epileptic Syngap1(+/-) Mice. *Biol Psychiatry*, 87(9), 829-  
7 842. <https://doi.org/10.1016/j.biopsych.2019.12.025>
- 8 Taylor, J. A., Reuter, J. D., Kubiak, R. A., Mufford, T. T., Booth, C. J., Dudek, F. E., & Barth, D. S. (2019,  
9 Jun 12). Spontaneous Recurrent Absence Seizure-like Events in Wild-Caught Rats. *J Neurosci*,  
10 39(24), 4829-4841. <https://doi.org/10.1523/JNEUROSCI.1167-18.2019>
- 11 Terzioglu, B., Aypak, C., Onat, F. Y., Kucukbrahimoglu, E., Ozkaynakci, A. E., & Goren, M. Z. (2006,  
12 Mar). The effects of ethosuximide on amino acids in genetic absence epilepsy rat model. *J*  
13 *Pharmacol Sci*, 100(3), 227-233. <https://doi.org/10.1254/jphs.fp0050691>
- 14 Till, S. M., Asiminas, A., Jackson, A. D., Katsanevaki, D., Barnes, S. A., Osterweil, E. K., Bear, M. F.,  
15 Chattarji, S., Wood, E. R., Wyllie, D. J., & Kind, P. C. (2015, Nov 1). Conserved hippocampal  
16 cellular pathophysiology but distinct behavioural deficits in a new rat model of FXS. *Hum Mol*  
17 *Genet*, 24(21), 5977-5984. <https://doi.org/10.1093/hmg/ddv299>
- 18 Vazquez, L. E., Chen, H. J., Sokolova, I., Knuesel, I., & Kennedy, M. B. (2004, Oct 6). SynGAP  
19 regulates spine formation. *J Neurosci*, 24(40), 8862-8872.  
20 <https://doi.org/10.1523/JNEUROSCI.3213-04.2004>
- 21 Vergnes, M., Marescaux, C., Micheletti, G., Reis, J., Depaulis, A., Rumbach, L., & Warter, J. M. (1982,  
22 Nov 16). Spontaneous paroxysmal electroclinical patterns in rat: a model of generalized non-  
23 convulsive epilepsy. *Neurosci Lett*, 33(1), 97-101. [https://doi.org/10.1016/0304-](https://doi.org/10.1016/0304-3940(82)90136-7)  
24 [3940\(82\)90136-7](https://doi.org/10.1016/0304-3940(82)90136-7)
- 25 Vlaskamp, D. R. M., Shaw, B. J., Burgess, R., Mei, D., Montomoli, M., Xie, H., Myers, C. T., Bennett,  
26 M. F., XiangWei, W., Williams, D., Maas, S. M., Brooks, A. S., Mancini, G. M. S., van de Laar,  
27 I., van Hagen, J. M., Ware, T. L., Webster, R. I., Malone, S., Berkovic, S. F., Kalnins, R. M.,  
28 Sicca, F., Korenke, G. C., van Ravenswaaij-Arts, C. M. A., Hildebrand, M. S., Mefford, H. C.,  
29 Jiang, Y., Guerrini, R., & Scheffer, I. E. (2019, Jan 8). SYNGAP1 encephalopathy: A distinctive  
30 generalized developmental and epileptic encephalopathy. *Neurology*, 92(2), e96-e107.  
31 <https://doi.org/10.1212/WNL.0000000000006729>
- 32 von Stulpnagel, C., Funke, C., Haberl, C., Hortnagel, K., Jungling, J., Weber, Y. G., Staudt, M., & Kluger,  
33 G. (2015, Aug). SYNGAP1 Mutation in Focal and Generalized Epilepsy: A Literature Overview  
34 and A Case Report with Special Aspects of the EEG. *Neuropediatrics*, 46(4), 287-291.  
35 <https://doi.org/10.1055/s-0035-1554098>
- 36 Walkup, W. G., Mastro, T. L., Schenker, L. T., Vielmetter, J., Hu, R., Iancu, A., Reghunathan, M.,  
37 Bannon, B. D., & Kennedy, M. B. (2016, Sep 13). A model for regulation by SynGAP-alpha1 of  
38 binding of synaptic proteins to PDZ-domain 'Slots' in the postsynaptic density. *Elife*, 5.  
39 <https://doi.org/10.7554/eLife.16813>
- 40 Walkup, W. G. t., Washburn, L., Sweredoski, M. J., Carlisle, H. J., Graham, R. L., Hess, S., & Kennedy,  
41 M. B. (2015, Feb 20). Phosphorylation of synaptic GTPase-activating protein (synGAP) by  
42 Ca<sup>2+</sup>/calmodulin-dependent protein kinase II (CaMKII) and cyclin-dependent kinase 5 (CDK5)  
43 alters the ratio of its GAP activity toward Ras and Rap GTPases. *J Biol Chem*, 290(8), 4908-  
44 4927. <https://doi.org/10.1074/jbc.M114.614420>
- 45 Weldon, M., Kilinc, M., Lloyd Holder, J., Jr., & Rumbaugh, G. (2018, Feb 5). The first international  
46 conference on SYNGAP1-related brain disorders: a stakeholder meeting of families,  
47 researchers, clinicians, and regulators. *J Neurodev Disord*, 10(1), 6.  
48 <https://doi.org/10.1186/s11689-018-9225-1>
- 49 Wiest, M. C., & Nicolelis, M. A. (2003, Sep). Behavioral detection of tactile stimuli during 7-12 Hz cortical  
50 oscillations in awake rats. *Nat Neurosci*, 6(9), 913-914. <https://doi.org/10.1038/nn1107>
- 51 Yang, M., & Crawley, J. N. (2009, Jul). Simple behavioral assessment of mouse olfaction. *Curr Protoc*  
52 *Neurosci*, Chapter 8, Unit 8 24. <https://doi.org/10.1002/0471142301.ns0824s48>
- 53 Yang, M., Silverman, J. L., & Crawley, J. N. (2011, Jul). Automated three-chambered social approach  
54 task for mice. *Curr Protoc Neurosci*, Chapter 8, Unit 8 26.  
55 <https://doi.org/10.1002/0471142301.ns0826s56>
- 56 Zeng, M., Shang, Y., Araki, Y., Guo, T., Huganir, R. L., & Zhang, M. (2016, Aug 25). Phase Transition  
57 in Postsynaptic Densities Underlies Formation of Synaptic Complexes and Synaptic Plasticity.  
58 *Cell*, 166(5), 1163-1175 e1112. <https://doi.org/10.1016/j.cell.2016.07.008>
- 59 Zimmerman, F. T., & Burgemeister, B. B. (1958, Oct). A new drug for petit mal epilepsy. *Neurology*,  
60 8(10), 769-775. <https://doi.org/10.1212/wnl.8.10.769>