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Novel behavioural characteristics of the superoxide dismutase 1 G93A ($SOD1^{G93A}$) mouse model of amyotrophic lateral sclerosis include sex-dependent phenotypes

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Abstract

Amyotrophic lateral sclerosis (ALS) involves the rapid degeneration of upper and lower motor neurons leading to weakening and paralysis of voluntary movements. Mutations in *copper-zinc superoxide dismutase 1* (*SOD1*) are a known genetic cause of ALS, and the *SOD1* ^{G93A} mouse has been used extensively to investigate molecular mechanisms in ALS. In recent years, evidence suggests that ALS and frontotemporal dementia form a spectrum disorder ranging from motor to cognitive dysfunctions. Thus, we tested male and female *SOD1* ^{G93A} mice for the first time before the onset of debilitating motor impairments in behavioural domains relevant to both ALS and frontotemporal dementia. *SOD1* ^{G93A} males displayed reduced locomotion, exploration and increased anxiety-like behaviours compared with control males. Intermediate-term spatial memory was impaired in *SOD1* ^{G93A} females, whereas long-term spatial memory deficits as well as lower acoustic startle response, and prepulse inhibition were identified in *SOD1* ^{G93A} mice of both sexes compared with respective controls. Interestingly, *SOD1* ^{G93A} males exhibited an increased conditioned cue *freezing* response. *Nosing* behaviours were also elevated in both male and female *SOD1* ^{G93A} when assessed in social paradigms. In conclusion, *SOD1* ^{G93A} mice exhibit a variety of sex-specific behavioural deficits beyond motor impairments supporting the notion of an ALS-frontotemporal spectrum disorder. Thus, *SOD1* ^{G93A} mice may represent a useful model to test the efficacy of therapeutic interventions on clinical symptoms in addition to declining motor abilities.

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Novel behavioural characteristics of the *SOD1^{G93A}* mouse model of amyotrophic lateral sclerosis

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Abstract

Amyotrophic lateral sclerosis (ALS) involves the rapid degeneration of upper and lower motor neurons leading to weakening and paralysis of voluntary movements. Mutations *in copper-zinc superoxide dismutase 1* (*SOD1*) are a known genetic cause of ALS and the *SOD1*^{G93A} mouse has been used extensively to investigate molecular mechanisms in ALS. In recent years evidence suggests that ALS and frontotemporal dementia (FTD) form a spectrum disorder ranging from motor to cognitive dysfunctions. Thus, we tested male and female *SOD1*^{G93A} mice for the first time prior to the onset of debilitating motor impairments in behavioural domains relevant to both ALS and FTD.

SOD1^{G93A} males displayed reduce locomotion, exploration and increased anxiety-like behaviours compared to control males. Intermediate-term spatial memory was impaired in *SOD1*^{G93A} females, while long-term spatial memory deficits as well as lower acoustic startle response and prepulse inhibition were identified in *SOD1*^{G93A} mice of both sexes compared to respective controls. Interestingly, *SOD1*^{G93A} males exhibited an increased conditioned cue *freezing* response. *Nosing* behaviours were also elevated in both male and female *SOD1*^{G93A} when assessed in social paradigms.

In conclusion, *SOD1*^{G93A} mice exhibit a variety of sex-specific behavioural deficits beyond motor impairments supporting the notion of an ALS-frontotemporal spectrum disorder. Thus, *SOD1*^{G93A} mice may represent a useful model to test the efficacy of therapeutic interventions on clinical symptoms in addition to declining motor abilities.

Keywords: Amyotrophic lateral sclerosis, cognition, dementia, *SOD1*^{G93A}, behaviour, memory, prepulse inhibition

Highlights

SOD1^{G93A} mice had sex-dependent behavioural deficits beyond motor impairment

SOD1^{G93A} males exhibited elevated anxiety and fear-associated *freezing*

SOD1^{G93A} mice showed defective long-term memory and sensorimotor gating but more social *nosing*

1. Introduction

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease, is a rapidly progressing neurodegenerative disease which leads to muscular atrophy via the degeneration of both upper and lower motor neurons. Mechanistically, ALS has been linked to oxidative stress (Barber & Shaw, 2010; Ferrante et al., 1997), protein misfolding (Piao et al., 2003; Wang, Johnson, Agar, & Agar, 2008), protein homeostasis imbalance, dysfunction of the serotonergic (M. R. Turner et al., 2005) and dopaminergic system (Przedborski et al., 1996; Vogels, Veltman, Oyen, & Horstink, 2000); changes which extend beyond the motor cortex. Indeed, degeneration and dysfunction of the frontal cortex has been established in ALS cases (Sharon Abrahams et al., 2005; Usman et al., 2011), as well as abnormalities in the basal ganglia (Machts et al., 2015) and mid-cingulate cortex (Sudharshan et al., 2011). These changes are consistent with the cognitive deficits that have been observed in ALS patients, including deficits in executive function and verbal fluency (Abrahams, Leigh, & Goldstein, 2005; Abrahams et al., 2000). The presentation of cognitive and other behavioural impairment in ALS further supports studies showing genetic overlap between FTD and ALS (DeJesus-Hernandez et al., 2011), and the idea that these disorders make up the ALS-frontotemporal spectrum disorder, which includes motor and cognitive symptoms (Strong et al., 2017).

The majority of ALS cases are sporadic (90-95%), the remaining 5-10% of ALS cases are inherited or familial (fALS). Accounting for approximately 20% of fALS, mutations in *copper-zinc superoxide dismutase 1* (*SOD1*) are one of the primary genetic causes of fALS with over 100 different mutations in this protein currently identified (Parton et al., 2002; Bradley J Turner & Talbot, 2008). SOD1 is a ubiquitous cytoplasmic enzyme that catalyzes the breakdown of reactive oxygen species (ROS) preventing harmful oxidative stress to neurons (Rosen et al., 1993). It is also noteworthy that $SOD1^{G93A}$ associated ALS cases appear to share similar disease

pathology as sporadic ALS (Synofzik, Fernández-Santiago, Maetzler, Schöls, & Andersen, 2010).

The most commonly used mouse model for investigating *SOD1* mutations has been the *SOD1*^{G93A} transgenic mouse model (Gurney et al., 1994). *SOD1*^{G93A} transgenic mice overexpress human mutant *SOD1* (glycine to alanine at residue 93) and develop a relatively early disease onset at approximately 90-110 days (Gurney et al., 1994), after which there is rapid motor function decline with transgenic mice typically surviving until around 4-5 months of age (Zang & Cheema, 2002). *SOD1*^{G93A} transgenic mice also develop ALS-relevant pathology such as progressive gliosis, loss of motor neurons and exhibit a rapid decline in body mass (Gurney et al., 1994). Copy number variation of the mutant *SOD1* transgene can alter the severity of the phenotype (Acevedo-Arozena et al., 2011) and cage enrichment is also known to attenuate the motor phenotype of this mouse model (Stam et al., 2008).

Importantly, the impact of the *SOD1*^{G93A} mutation on cognitive and other behavioural domains beyond motor deficits has been largely overlooked. In addition, the effect of sex on the behavioural phenotype of *SOD1*^{G93A} transgenic mice has mostly been ignored despite the fact that gender effects have been found in human ALS patients (McCombe & Henderson, 2010) and that male *SOD1*^{G93A} mice have a more rapid progression of motor dysfunction compared to females (Choi et al., 2008). Thus, in the present study, we tested male and female *SOD1*^{G93A} transgenic mice in behavioural domains not considered previously and started testing before the onset of debilitating motor impairments.

2. Materials and methods

2.1 Animals

Experimental animals were male and female heterozygous *Superoxide dismutase 1 G93A* mutant (*SOD1*^{G93A}) and wild type-like (WT) control littermates bred at the Australian BioResources (ABR Moss Vale, Australia). Genotyping was performed post weaning (postnatal day 21) by polymerase chain reaction amplification. Breeding colonies at ABR were housed in individually ventilated (IVC) cages (Type Mouse Version 1; Airlaw, Smithfield, Australia; air change: 90-120 times per hour averaged; passive exhaust ventilation system). The test mice were transported to the animal facility at the Western Sydney University (WSU) Campbelltown campus, where the mice were housed in groups of 2-3 in IVC cages (GM500 Green, Techniplast Australia Pty Ltd, Rydalmere, Australia) under a 12:12 hour light:dark cycle (white light illumination from 0900 and red light illumination from 2100) using corncob bedding (PuraCob Premium: Able Scientific, Perth, Australia), tissue and 'crinkle nest' for nesting (with no enriching structures), and provided water and standard lab chow *ad-libitum*. Cages were changed fortnightly. For the social preference test, sex-matched, adult A/J mice from the Animal Resources Centre (ARC: Cunning Vale, Australia) were sent to the WSU animal facility and used as social conspecifics.

All research and animal care procedures were approved by the Western Sydney University Animal Care and Ethics Committee (#A11748) and were in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.4 Behavioural tests

Mice were tested in a number of behavioural paradigms with relevance to ALS and FTD prior to the expected onset of motor impairments to avoid motor deficits becoming a major

confounder of test outcomes. Test biography and test age of the two test cohorts are outlined in Table 1.

2.4.1 Accelerod (Cohort 1)

To assess motor functions including balance as published previously (Karl, Pabst, & von Horsten, 2003), mice were first trained on the accelerod apparatus (ENV-574M, MED Associates Inc., St Albans, VT, USA) at a fixed speed of 12 rpm for two minutes, this training was repeated a total of three times to ensure that the mice were able to stay on the rod without falling off at this speed. Training was only performed prior to the first test. On the test day, mice were placed on the accelerod after which an accelerating program was started (4-40 rpm over 300 sec). The time the mouse spent successfully running on top of the rotating rod was recorded. This was done twice with an ITI of 1 hour, the average latency to loop/fall was analysed.

2.4.2 Pole test (Cohort 1)

To assess motor functions including grip strength mice were placed (facing upwards) on a vertical pole (diameter: 1 cm; length: 51 cm) as described previously (Karl et al., 2003). The time to turn around, and the time to reach the bottom of the pole was recorded. A 120s cut-off time was given to mice which did not turn or reach the bottom.

2.4.3 Open Field (OF) (Cohort 1)

To assess locomotive, explorative, and anxiety-related behaviours, mice were placed into an infrared photobeam controlled OF test chamber (MED Associates Inc., St Albans, VT, USA) for 30 minutes as described previously (Shang, Talmage, & Karl, 2017). The test area (43.2 cm x 43.2 cm) was divided into a central and peripheral zone (MED software coordinates for central zone: 3/3, 3/13, 13/3, 13/13); total distance travelled, and vertical activity were automatically measured. Total centre zone time and the ratio of central distance travelled vs total distance travelled were used to identify anxiety-related behaviours.

2.4.4 Y-Maze (Cohort 1)

The Y-maze was used to assess intermediate-term spatial memory [intermediate-term memory differentiated from short-term memory as described (Stough, Shobe, & Carew, 2006; Taglialatela, Hogan, Zhang, & Dineley, 2009)]. The apparatus was Y-shaped (three arms 7.5 cm wide, 22 cm long, 19 cm high – at 120 degrees to each other) with external visual cues placed on the wall above the apparatus similar to that previously published (Olaya, Heusner, Matsumoto, Shannon Weickert, & Karl, 2018). In the first trial mice were placed in the centre of the Y-maze with one arm closed off, and allowed to explore for 10 min. After 30 min, mice were returned to the apparatus with all three arms open and allowed to explore for a further 10 min. Entries and distance travelled in the 'novel' and 'familial' arms were recorded by Any-Maze™ tracking software.

2.4.5 Social Preference Test (SPT) (Cohort 1)

The SPT was used to measure social approach behaviour (i.e. sociability) and social recognition memory (Moy et al., 2004) as previously published with minor alterations (Cheng, Low, Logge, Garner, & Karl, 2014). The apparatus consists of three connected chambers (16.5 cm x 19 cm per chamber): a central chamber with clear Plexiglas dividing walls with square passages (height: 4 cm and width: 4 cm). One circular enclosure (height: 16 cm, width: 8 cm; bars spaced 1 cm apart) was placed into each outer chamber to allow contact between mice but prevent fighting. Fresh bedding was added to all chambers prior to each test trial. Test animals were isolated for 1 hour prior to testing in a clean cage with fresh nesting material. Test mice were then allowed to habituate to the apparatus for 5 minutes before being removed from the test apparatus prior to the sociability trial. For the test of sociability, an unfamiliar social conspecific (i.e. a sex-matched, adult A/J mouse), was placed in one of the two opponent outer chambers in a quasi-randomised manner before the test mouse was return to the apparatus and allowed to explore the whole apparatus freely for 10 minutes. Finally, test animals were

observed in a 10 minutes social recognition test trial in which the test mouse was again removed from the apparatus while a second unfamiliar 'novel' opponent A/J mouse was placed in the previously empty chamber. The test mouse was placed into the apparatus and allowed to explore either the familiar mouse (from the previous trial) or the novel, unfamiliar mouse for another 10 minutes. Any-Maze^{TM} tracking software was used to determine the time the test mice spent in the different chambers during the trial.

2.4.6 Fear Conditioning (FC) (Cohort 1)

FC results from the association of a previously neutral stimulus (e.g. a tone) with an aversive stimulus (e.g. a foot shock) (Owen, Logue, Rasmussen, & Wehner, 1997). The test was carried out as previously published (Olaya et al., 2018). The FC task occurred over 3 days. On the first day (conditioning trial), mice were placed in a test chamber (NIR-022MD, ENV-005FPU-M, MED Associates Inc., St Albans, VT, USA) with a vanilla scent (Queen™ imitation vanilla essence) cue for 7 minutes. After an initial 2 min period, an 80dB conditioned stimulus (CS) was presented for 30 seconds co-terminating with a 0.4 mA 2 second foot shock (unconditioned stimulus). The same tone/foot shock pairing occurred again 2 min later. On the second day of testing (context trial), mice were returned to the apparatus for 7 minutes with the vanilla scent cue and no tone. On the third day of testing (cue trial), mice were placed in an altered environment (i.e. a triangular plastic insert added to the chamber to change its overall shape and scent removed) for 9 minutes. At time = 2 mins the CS was continuously presented for 5 minutes with the test concluding 2mins after termination of the CS presentation. Total time spent *freezing* in all tests was measured using the video-freeze^{TM} software (software settings: threshold = 15).

2.4.6 Prepulse inhibition (PPI) (Cohort 1)

PPI was used to test for sensorimotor gating (the attenuation of the startle response by a non-startling stimulus) (Paylor & Crawley, 1997). The PPI protocol was carried out as previously

described (Cheng et al., 2014). The test protocol used a startle pulse of 120dB, three different prepulse intensities (74, 82 and 86dB) and inter stimulus intervals of 32, 64, 128 and 256 ms.

2.4.7 Novel object recognition test (NOR) (Cohort 2)

Object recognition memory in the NOR is demonstrated by the animal's ability to distinguish between familiar and unfamiliar objects [rodents have an innate preference towards novelty (Dere, Huston, & De Souza Silva, 2007)]. The NOR was conducted over 2 days in a similar protocol previously published (Cheng et al., 2014). On day 1, mice were habituated to the empty test arena (35 x 35 x 30 cm grey plastic). On day 2 (test day - two trials), mice were placed into the arena with two identical objects for 10 min (LEGO Duplo animals or blocks) for test trial 1. After a 20 min inter-trial interval, one of the now 'familiar' objects from test trial 1 was replaced with a 'novel' object (i.e. a LEGO Duplo animal different to test trial 1), and the mouse was allowed to explore the familial and novel object for 10 min. The objects and their locations were counterbalanced across genotypes. Video footage of each trial was recorded using AnyMaze^{TM} tracking software, and the time spent *nosing* and *rearing* on the objects were quantified by an experimenter blind to the genotypes of the animals. The percentage of time spent *nosing* + *rearing* towards the novel object indicated object recognition memory (% novel object recognition) and was calculated using (novel object *nosing* + *rearing* time) / (novel + familiar object *nosing* + *rearing* time) × 100.

2.4.8 Social interaction (SI) (Cohort 2)

The social interaction test was employed to measure spontaneous social behaviour between a pair of unfamiliar mice as previously published (Olaya et al., 2018). One day prior to testing A/JArc mice were habituated to the test area for 10 min. Test animals were placed inside a grey plastic arena (35 x 35 x 30 cm) together with an unfamiliar, sex-matched adult A/JArc standard opponent mouse and allowed to explore the environment and each other freely for 10 min. Frequency and duration of active socio-positive behaviours including *nosing, ano-genital*

sniffing, allo-grooming, following and *climbing over/under* were scored manually. *Nosing* was scored when the test mouse had its snout directed towards the A/JArc mouse and was 1 cm or less away from the standard opponent's body. *Total active social time* was calculated as total time spent engaging in any of the socio-positive behaviours listed above.

2.4.9 Cheeseboard (Cohort 2)

The cheeseboard (a dry land equivalent of the Morris water maze) was used to test spatial learning and memory (Llano Lopez, Hauser, Feldon, Gargiulo, & Yee, 2010). Details of the test protocol are described in Cheng et. al (2014). Briefly, mice were deprived of food beginning 1 day prior to habituation and kept at 85-90% starting bodyweight during testing. Mice were habituated to a flat 110 cm diameter board for two days (3 x 2 min trials, 15 min ITI). During training, mice were placed on a 110 cm diameter board containing 32 wells (8 x 4 well rows radiating from the centre of the board), one well containing the food reward of diluted sweetened condensed milk. The time for the mouse to find the reward was recorded. If the mice did not find the reward before the 120 s cut-off they were placed near the well (and given a time of 120 s). This was repeated 3 time per day with an ITI of 15 min. Training was conducted over 7 days in males and 9 days in females to allow sufficient training of control mice. On the 8th day in males and 10th day in females a probe trial was conducted where no wells contained the food reward. Mice were allowed to explore the board once for 2 min. The time spent and distance travelled in 1 of 8 zones containing the target well was recorded by Any-Maze^{TM} tracking software.

2.5 Statistical analysis

SOD1^{G93A} mice have a sex-dependent onset of motor degeneration (Choi et al., 2008) and we detected main sex effects as well as 'sex' by 'genotype' interactions in major behavioural parameters (exploration, anxiety-like behaviours and fear-associated *freezing*; outlined in

Supplementary Table 1). Thus, all data were split by sex and two-way repeated measures (RM) ANOVAs were utilized to analyse the main between subject effect of 'genotype' and the within subject effects of 'time' (OF, CB, FC), 'prepulse', 'startle pulse', 'startle block' (PPI) and 'cue' (FC) in each sex. A 'genotype' main effect was further investigated in CB and PPI by splitting data by either training day or prepulse and using a one-way ANOVA to compare WT and *SOD1*^{G93A}. One sample t-tests were also used for Y-maze, SPT, NOR and CB probe to determine whether the percentage of time or distance involved in a specific behavior was above chance levels. Differences were regarded as statistically significant if $p < 0.05$. Data are shown as means ± standard error of means (SEM). F-values and degrees of freedom are presented for ANOVAs and significant genotype effects *versus* WT are shown in figures and tables as '*' (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.0001$) Significant 'time' by 'genotype' interactions are shown in figures as '$^{+}$' ($^{+}p < 0.05$, $^{++}p < 0.01$). All analyses were performed in IBM SPSS Statistics v24.

3. Results

Two-way RM ANOVA found a significant effect of 'sex' on bodyweight [$F(1,34) = 115.383$; $p < 0.0001$], with males having a higher bodyweight compared to females (Supplementary Figure 1A-B). Split by sex, a 'time' by 'genotype' interaction in both male and female bodyweight was found [male: $F(1,85) = 34.63$; $p < 0.0001$; female: $F(1,85) = 29.08$; $p < 0.0001$], with *SOD1*^{G93A} mice failing to gain weight from PND96 onwards (Supplementary Figure 1A-B). One-way ANOVA identified a significant difference in bodyweight between WT and *SOD1*^{G93A} from PND125 onwards in males and PND96 onwards in females (Supplementary Figure 1A-B).

3.1 Motor functions

A 'sex' by 'genotype' interaction effect was detected in the 'latency to turn' on the pole test at PND74, [$F(1,34) = 4.87$; $p = 0.034$] where *SOD1*^{G93A} males needed longer to turn compared to WT males while *SOD1*^{G93A} females showed a reduced latency to turn compared to WT females (Table 2). However, split by sex, no main effect of 'genotype' was found in the pole test when analysing latencies to turn and to reach the bottom in either sex at either age (all p's > 0.05) (Table 2).

In the accelerod task no significant effect of genotype on motor performance of male mice was found at either test age (all p's > 0.05). However, one-way ANOVA found a significant effect of 'genotype' in female mice [PND74: $F(1, 18) = 8.543$; $p = 0.009$ – PND94: $F(1, 18) = 11.725$; $p = 0.003$], as *SOD1*^{G93A} transgenic females showed poorer motor co-ordination compared to WT females at both test ages (Table 2).

3.2 Locomotion and exploration

In the open field, two-way ANOVA revealed a significant 'sex' by 'genotype' effect on the total distance travelled [$F(1,34) = 6.916$; $p = 0.013$] (Table 3). *SOD1*^{G93A} males travelled less than WT males while *SOD1*^{G93A} females travelled a similar distance compared to WT females. Split by sex, one-way ANOVA revealed a significant effect of 'genotype' on total distance travelled [$F(1,17) = 17.328$; $p = 0.001$] and OF exploration [$F(1,17) = 12.31$; $p = 0.003$] with male *SOD1*^{G93A} transgenic mice travelling less and showing less *rearing* compared to their WT littermates (Table 3). However, habituation to the OF arena was similar, as no 'time' by 'genotype' interaction was found in male mice ($p > 0.05$) (Figure 1A).

In females a 'time' by 'genotype' interaction described a moderately slower habituation to the OF in *SOD1*^{G93A} mice when compared to WT [$F(5,85 = 2.57$; $p = 0.032$] (Figure 1B) which appeared to be most evident in the first 10 min of the test. Total distance travelled and *rearing* frequency of females were not different between genotypes (all p's > 0.05; Table 3).

3.3 Anxiety

Two-way ANOVA found a significant effect of 'sex' on OF centre time [$F(1,34) = 12.754$; $p = 0.001$] and centre zone distance ratio [$F(1,34) = 4.32$; $p = 0.045$], with males spending more time and travelling relatively further in the centre zone compared to females. Split by sex, one-way ANOVA found a main effect of 'genotype' on OF centre time [$F(1,17) = 10.17$; $p = 0.005$] and centre distance ratio [$F(1,17) = 5.38$; $p = 0.033$] with *SOD1*^{G93A} transgenic males exhibiting more anxiety-like behaviours than controls (Table 3). No genotype effects were evident in females (all p's > 0.05, Table 3).

3.4 Spatial memory

Three-way RM ANOVA found a significant effect of 'sex' on the latency to find the reward in the first trial of CB training [$F(1,48) = 8.822$; $p = 0.005$] with females taking significantly

longer to find the reward compared to males (Figure 2C-D). Thus, all cognitive data were split by sex.

3.4.1 Y-maze: There were no main effects of 'genotype' on the total distance travelled in the novel arm, or total entries into the novel arm in either male or female mice (all *p*'s > 0.05; Table 4). One sample t-tests revealed that males regardless of genotype had a preference for the novel arm (all *p's* for percentage novel arm entries and distance < 0.05; Table 4). However, *SOD1*^{G93A} transgenic females failed to develop such a preference [WT - entries: t(10) = 4.53, *p* = 0.001; WT - distance: t(10) = 4.47, *p* = 0.001; *SOD1*^{G93A} - distance: t(7) = 2.95, *p* = 0.106; *SOD1*^{G93A} - entries: t(7) = 2.15, *p* = 0.068] (Table 4).

3.4.2 Cheeseboard (CB): Two-way RM ANOVA revealed a main effect of 'genotype' in both male [F(1,21) = 4.464; *p* = 0.047] and female [F(1,27) = 5.197; *p* = 0.031] mice, with *SOD1*^{G93A} transgenic mice exhibiting an overall higher latency to find the food reward compared to WT when averaged across the three daily training trials (Figure 2A-B). Importantly, a 'time' by 'genotype' interaction in female mice [F(8,216) = 3.493; *p* = 0.001] indicated that *SOD1*^{G93A} transgenic females acquired the task slower than their WT littermates, in particular at the later stages of training (training day 7, 8 and 9) (Figure 2B). This task acquisition impairment was not observed in male mice ('time' by 'genotype': *p* > 0.05). Interestingly, examining the first trial of each training day as a measure of long-term memory (i.e. 24-h test delay), 'time' by 'genotype' interactions in both male [F(6,126) = 3.564; *p* = 0.003] and female [F(8,216) = 4.453; *p* < 0.0001] mice were evident highlighting a learning deficit in both sexes of *SOD1*^{G93A} mice (Figure 2C-D). Again, this was evident in the later stages of training (training day 7, 8, 9 in females, and 5, 6 and 7 in males) (Figure 2C-D). This deficit was specific to long-term memory as analysing the average latency to find the reward during trails two and three (15 min ITI) did not reveal any significant differences in either sex (all *p's* > 0.05; data not shown). In

the probe trial, none of the mice regardless of sex or genotype showed a preference for the target zone (one sample t-test: all p's > 0.05; Table 4).

Average distance travelled and speed were also evaluated: the average distance travelled during training was not significantly different across experimental groups (all p's > 0.05; data not shown). However, *SOD1*^{G93A} males did have a lower average speed across training days (average of three trials) when compared to WT [F(1,21) = 4.578; p = 0.044] (Supplementary Table 2). Average speed when considering trial 1 only was not significantly different between *SOD1*^{G93A} and WT in either males or females (all p's > 0.05) (Supplementary Table 2).

3.5 Social domains

3.5.1 Sociability and social recognition memory: SOD1^{G93A} transgenic males failed to show a preference for the chamber containing a mouse [t(9) = 0.939, p = 0.372] whereas sociability was intact in WT mice [t(8) = 4.303, p = 0.003] (Figure 3A). All females regardless of genotype showed intact sociability, i.e. having a preference for the chamber containing a mouse [WT: t(9) = 4.014, p = 0.003; *SOD1*^{G93A}: t(8) = 3.276, p = 0.014] (Figure 3B). All mice exhibited intact social recognition memory as they all had a preference for *nosing* a novel mouse (all p*'s* < 0.05) (Figure 3C-D).

3.5.2 Social interaction: SOD1^{G93A} transgenic males showed WT-like levels of total active social interaction time whereas transgenic females engaged for longer in social behaviours compared to respective WT females [F(1,27) = 5.594; p = 0.025] (Table 4). Analysing social behaviours individually, a 'genotype' effect was observed in both male [F(1,21) = 4.645; p = 0.043] and female mice [F(1,27) = 5.122; p = 0.032] with *SOD1*^{G93A} transgenic mice spending more time *nosing* the standard opponent mouse compared to control mice (Table 4).

3.6 Prepulse inhibition (PPI)

3.6.1 Startle response and habituation: Two-way RM ANOVA showed that all mice responded to increasing startle pulse intensities ['startle pulse': males: $F(2,34) = 36.193$; $p < 0.0001$ – females: $F(2,32) = 54.393$; $p < 0.0001$]. However, an interaction of 'startle intensity' and 'genotype' in both sexes [males: $F(2,34) = 13.172$; $p < 0.0001$ – females: $F(2,32) = 5.124$; $p = 0.012$] suggested that this correlation was significantly weaker in $SOD1^{G93A}$ transgenic mice (Figure 4A-B). Furthermore, a significantly weaker acoustic startle response to 120dB was observed in $SOD1^{G93A}$ mice [males: $F(1,18) = 16.09$; $p = 0.001$, females: $F(1,17) = 6.292$; $p = 0.023$] (Figure 4A-B). Startle habituation to repeated presentation of 120dB startle blocks was evident in all mice regardless of sex and genotype (data not shown).

3.6.2 Prepulse inhibition: Mice of all experimental groups responded to increasing prepulse intensities [RM ANOVA for 'prepulse': males: $F(2,34) = 138.568$; $p < 0.0001$ - females: $F(2,32) = 61.860$; $p < 0.0001$] (Figure 4C-D). However, an interaction of 'prepulse intensity' and 'genotype' indicated that this response was impaired in $SOD1^{G93A}$ transgenic males [$F(2,34) = 4.619$; $p = 0.017$], a phenomenon not seen in females ('prepulse intensity' by 'genotype': $p > 0.05$). Male and female $SOD1^{G93A}$ mice had overall lower PPI compared to WT ['genotype' effect: male - $F(1,17) = 8.431$; $p = 0.01$, female - $F(1,16) = 18.791$; $p = 0.001$] (Figure 4C-D). Split by prepulse intensity, $SOD1^{G93A}$ males had significantly lower percent PPI at 82dB [$F(1,18) = 8.47$; $p = 0.01$] and 86dB [$F(1,18) = 13.34$; $p = 0.002$] when compared to WT (Figure 4C). Female $SOD1^{G93A}$ had lower percent PPI at 74dB [$F(1,17) = 6.69$; $p = 0.02$], 82dB [$F(1,17) = 17.98$; $p = 0.001$] and 86dB [$F(1,17) = 34.96$; $p < 0.0001$] (Figure 4D).

3.7 Fear conditioning

Three-way RM ANOVA found an effect of 'sex' on contextual *freezing* time [$F(1,34) = 6.861$; $p = 0.013$], where females spent significantly longer *freezing* compared to males (Figure 5A-B). Thus, data were split by sex.

3.7.1 Conditioning: Two-way RM ANOVA detected a main effect of 'time' on *freezing* [males: $F(6,102) = 9.208$; $p < 0.0001$, females: : $F(6,102) = 13.565$; $p < 0.0001$] with all mice increasing *freezing* behaviour as a response to the foot shocks (Figure 5A-B). Importantly, a 'time' by 'genotype' interaction was found in males [$F(6,102) = 3.05$; $p = 0.009$], with *SOD1*^{G93A} transgenic males *freezing* increasing more over time compared to WT males (Figure 5A).

3.7.2 Context: Split by sex, two-way RM ANOVA comparing the total freezing in the first 2 min of the conditioning test (i.e. baseline before tone / shock exposure) to the first 2 min of the context test found a significant effect of 'test' with all mice increasing *freezing* in response to the context [male: $F(1,17) = 20.49$; $p < 0.0001$, female: $F(1,17) = 22.39$; $p < 0.0001$] (Table 3). Importantly, two-way RM ANOVA found an interaction of 'time' and 'genotype' in contextual *freezing* across 1-min blocks in males with *SOD1*^{G93A} transgenic mice showing an increase in *freezing* across time compared to WT males [$F(6,102) = 2.40$; $p = 0.033$], in particular in the later stages of contextual conditioning testing (i.e. 6th and 7th minute; Figure 5C). This effect of *SOD1*^{G93A} was absent in female mice ($p > 0.05$) (Figure 5D).

3.7.3 Cue: Two-way RM ANOVA for average *freezing* prior and during cue presentation found a significant effect of 'cue' on *freezing* in both males [$F(1,17) = 51.585$; $p < 0.0001$] and females [$F(1,16) = 79.726$; $p < 0.0001$], indicating all mice responded to the cue (Figure 5E-F). However, a 'cue' by 'genotype' interaction in males indicated that *SOD1*^{G93A} transgenic males showed an increased *freezing* response to the cue when compared to WT males [$F(1,17) = 15.173$; $p = 0.01$] (Figure 5E). In line with this, *SOD1*^{G93A} transgenic males also exhibited higher overall *freezing* levels during cue presentation [$F(1,17) = 14.48$; $p = 0.001$], a phenomenon absent in female mice ($p > 0.05$; Table 5).

3.8 Object recognition memory

Novel object: A significant preference for novel object exploration was found in WT and *SOD1*^{G93A} transgenic mice of both sexes (all p's < 0.05; Supplementary Figure 1A-B).

4. Discussion

We have investigated the behavioural impact of the *SOD1*^{G93A} mutation in both male and female mice. *SOD1*^{G93A} mice exhibit motor degeneration beginning at approximately 120 days of age, and therefore the majority of behavioural phenotyping was carried out in younger mice, before motor dysfunction could significantly impact mobility during testing. The *SOD1*^{G93A} mutation increased *nosing* social interaction, attenuated the acoustic startle response, impaired prepulse inhibition to acoustic startle and impaired long-term spatial memory in the cheeseboard task in both male and female mice. In some cases, the cognitive changes were sex-dependent with only female *SOD1*^{G93A} mice showing a spatial memory deficit in the Y-maze. Compared to WT littermates, male *SOD1*^{G93A} mice had decreased locomotion and exploration, and increased anxiety in the open field. *SOD1*^{G93A} males also showed elevated *freezing* in response to fear conditioning compared to WT; these changes were not observed in female *SOD1*^{G93A} mice.

Locomotion and exploration (*rearing*) in the open field was lower in male *SOD1*^{G93A} mice compared to WT. A previous study did not find a hypo-locomotive phenotype in younger (PND56) *SOD1*^{G93A} males (Quarta, Bravi, Scambi, Mariotti, & Minciacchi, 2015), suggesting this phenotype is progressive. Importantly, the impact of motor impairments on this result can be largely excluded as *SOD1*^{G93A} males did not show a deficit in accelerod or pole test performance when compared to WT males at the age of OF testing. These findings may be linked to early dysfunction in the nucleus accumbens, a region known to be involved with locomotion and motivation (Pijnenburg, Honig, & Van Rossum, 1975; Pulvirenti, Berrier, Kreifeldt, & Koob, 1994). Interestingly, reduced dopamine levels have been found in the nucleus accumbens of end stage *SOD1*^{G93A} mice (at 140 days of age but not at 28 days) and may explain the reduced locomotor and exploratory behaviors in younger *SOD1*^{G93A} males. The OF phenotype of *SOD1*^{G93A} females was not affected, potentially due to protective nature

of female sex hormones on neurological processes in female *SOD1*^{G93A} (Choi et al., 2008), e.g. the neuroprotective properties of 17-β estradiol (Culmsee et al., 1999; Singer, Figueroa-Masot, Batchelor, & Dorsa, 1999).

Anxiety-like behaviors were elevated in male *SOD1*^{G93A} mice in the OF in line with a previous study on presymptomatic *SOD1*^{G93A} male mice (Quarta et al., 2015). In that study an observed loss of hippocampal inhibitory GABAergic interneurons was identified as a potential mechanism (Quarta et al., 2015), and deactivation of these neurons has indeed been shown to elevate anxiety in rats (Temel, Blokland, & Lim, 2012).. Fear-associated *freezing* was also elevated in *SOD1*^{G93A} males compared to WT in fear conditioning, context and cue trials. *SOD1*^{G93A} female mice did not exhibit an anxiety-like OF although it is known that estrogen increases anxiety and fear-related behaviours (Morgan & Pfaff, 2001), which is consistent with the lower OF centre zone time and elevated *freezing* time detected in our females compared to males. Importantly, anxiety is observed in ALS patients as well (Kurt, Nijboer, Matuz, & Kübler, 2007; Vignola et al., 2008).

Male and female *SOD1*^{G93A} mice showed a long-term spatial memory deficit, whereas the intermediate-term task acquisition performance (i.e. using a 15 min ITI in the CB) was intact in all mice. Interestingly, *SOD1*^{G93A} females also exhibited a spatial memory deficit in the Y-maze which uses a similar ITI. A previous study using the Barnes maze also identified an initially slower spatial memory learning in *SOD1*^{G93A} male mice, however, *SOD1*^{G93A} learnt as quickly as WT in proceeding training trials (Quarta et al., 2015). The motivation factor of the Barnes maze (i.e. fear) is significantly different compared to the CB (i.e. hunger), which may explain the differences observed. Memory deficits in *SOD1*^{G93A} mice may arise from hippocampal dysfunction, as the region has previously been identified as having elevated oxidative stress levels and altered calcium signaling in these mice (Cha et al., 2000; Chung et

al., 2005). Hippocampal degeneration is also evident in ALS patients (Takeda, Uchihara, Arai, Mizutani, & Iwata, 2009), as are deficits in memory (Mantovan et al., 2003).

Social interaction *nosing* time was robustly elevated in both *SOD1*^{G93A} male and female mice, an observation described for the first time in *SOD1*^{G93A} mice. In younger *SOD1*^{G93A} mice (PND82), sociability was not elevated above WT levels in the social preference test, suggesting increased social interaction develops later in the disease progression. Increased striatal cAMP has been shown to increase social interaction in *cyclic nucleotide phosphodiesterase 10A* deficient mice without altering anxiety-like behaviours (Sano, Nagai, Miyakawa, Shigemoto, & Yokoi, 2008), however, this is likely to be related to elevated dopamine signaling. This is inconsistent with finding in *SOD1*^{G93A} mice (Kostic et al., 1997) and human ALS (Vogels et al., 2000) which describe a depression of the dopaminergic system. Additionally, apathy is commonly observed in ALS (Caga et al., 2018; Radakovic et al., 2016), inconsistent with the increased social interaction phenotype in our *SOD1*^{G93A} mice. Further studies are required to elucidate the unexpected social interaction phenotype of *SOD1*^{G93A} mice.

In agreement with our findings, lower acoustic startle has previously been observed in *SOD1*^{G93A} mice (Acevedo-Arozena et al., 2011). This may be attributed to muscle weakness that precedes severe motor degeneration. Our study also shows a robust PPI deficit in both male and female mice that was previously not observed in a low copy number variant of the *SOD1*^{G93A} mouse (Acevedo-Arozena et al., 2011). To our knowledge this is the first description of a PPI deficit in the *SOD1*^{G93A} mouse model, ALS mouse model or human ALS. Serotonin receptor modulation impacts on PPI in mice, with $5HT_{1A}$ activation increasing PPI, and $5HT_{1B}$ activation decreasing PPI (Dulawa, Gross, Stark, Hen, & Geyer, 2000). Changes in the serotonergic system are also implicated in ALS with reduced $5HT_{1A}$ binding in ALS patients (M. R. Turner et al., 2005) and delayed motor phenotype in *SOD1*^{G93A} mice treated with serotonin precursors (B. J. Turner, Lopes, & Cheema, 2003). These results give further support

to potential serotonergic dysfunction in *SOD1*^{G93A} mice and ALS, and highlights a neurological deficit that requires further investigation in human patients.

In summary, behavioural deficits are evident in *SOD1*^{G93A} mice prior to the onset of a severe motor phenotype, some of which resemble phenotypes in other mouse models of neurodegeneration with dementia. Furthermore, sex-dependent findings suggest that sex hormones may play a neuroprotective role to ameliorate the presentation of these phenotypes in female *SOD1*^{G93A}. To conclude, *SOD1*^{G93A} mice show several behavioural deficits related to symptoms observed in human ALS patients, therefore examining altered behavioural phenotypes (prior to onset of significant motor dysfunction) may provide a useful, non-invasive biomarker of therapeutic efficacy in future studies.

5. References

Abrahams, S., Goldstein, L. H., Suckling, J., Ng, V., Simmons, A., Chitnis, X., . . . Leigh, P. N. (2005). Frontotemporal white matter changesin amyotrophic lateral sclerosis. *Journal of Neurology, 252*(3), 321-331. doi: 10.1007/s00415-005-0646-x

Abrahams, S., Leigh, P. N., & Goldstein, L. H. (2005). Cognitive change in ALS: a prospective study. *Neurology, 64*(7), 1222-1226. doi: 10.1212/01.wnl.0000156519.41681.27

Abrahams, S., Leigh, P. N., Harvey, A., Vythelingum, G. N., Grise, D., & Goldstein, L. H. (2000). Verbal fluency and executive dysfunction in amyotrophic lateral sclerosis (ALS). *Neuropsychologia, 38*(6), 734-747.

Acevedo-Arozena, A., Kalmar, B., Essa, S., Ricketts, T., Joyce, P., Kent, R., . . . Fisher, E. M. (2011). A comprehensive assessment of the SOD1G93A low-copy transgenic mouse, which models human amyotrophic lateral sclerosis. *Dis Model Mech, 4*(5), 686-700. doi: 10.1242/dmm.007237

Barber, S. C., & Shaw, P. J. (2010). Oxidative stress in ALS: Key role in motor neuron injury and therapeutic target. *Free Radical Biology and Medicine, 48*(5), 629-641. doi: https://doi.org/10.1016/j.freeradbiomed.2009.11.018

Caga, J., Hsieh, S., Highton-Williamson, E., Zoing, M. C., Ramsey, E., Devenney, E., . . . Kiernan, M. C. (2018). Apathy and its impact on patient outcome in amyotrophic lateral sclerosis. *Journal of Neurology, 265*(1), 187-193. doi: 10.1007/s00415-017-8688-4

Cha, C. I., Chung, Y. H., Shin, C.-m., Shin, D. H., Kim, Y. S., Gurney, M. E., & Lee, K. W. (2000). Immunocytochemical study on the distribution of nitrotyrosine in the brain of the transgenic mice expressing a human Cu/Zn SOD mutation. *Brain Research, 853*(1), 156-161. doi: https://doi.org/10.1016/S0006-8993(99)02302-1

Cheng, D., Low, J. K., Logge, W., Garner, B., & Karl, T. (2014). Novel behavioural characteristics of female APPSwe/PS1ΔE9 double transgenic mice. *Behavioural brain research, 260*, 111-118. doi: https://doi.org/10.1016/j.bbr.2013.11.046

Choi, C. I., Lee, Y. D., Gwag, B. J., Cho, S. I., Kim, S. S., & Suh-Kim, H. (2008). Effects of estrogen on lifespan and motor functions in female hSOD1 G93A transgenic mice. *J Neurol Sci, 268*(1-2), 40-47. doi: 10.1016/j.jns.2007.10.024

Chung, Y. H., Joo, K. M., Nam, R. H., Cho, M. H., Kim, D. J., Lee, W. B., & Cha, C. I. (2005). Decreased expression of calretinin in the cerebral cortex and hippocampus of SOD1G93A transgenic mice. *Brain Research, 1035*(1), 105-109. doi: https://doi.org/10.1016/j.brainres.2004.12.022

Culmsee, C., Vedder, H., Ravati, A., Junker, V., Otto, D., Ahlemeyer, B., . . . Krieglstein, J. (1999). Neuroprotection by estrogens in a mouse model of focal cerebral ischemia and in cultured neurons: evidence for a receptor-independent antioxidative mechanism. *J Cereb Blood Flow Metab, 19*(11), 1263-1269. doi: 10.1097/00004647-199911000-00011

DeJesus-Hernandez, M., Mackenzie, Ian R., Boeve, Bradley F., Boxer, Adam L., Baker, M., Rutherford, Nicola J., . . . Rademakers, R. (2011). Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. *Neuron, 72*(2), 245-256. doi: https://doi.org/10.1016/j.neuron.2011.09.011

Dere, E., Huston, J. P., & De Souza Silva, M. A. (2007). The pharmacology, neuroanatomy and neurogenetics of one-trial object recognition in rodents. *Neurosci Biobehav Rev, 31*(5), 673-704. doi: 10.1016/j.neubiorev.2007.01.005

Dulawa, S. C., Gross, C., Stark, K. L., Hen, R., & Geyer, M. A. (2000). Knockout Mice Reveal Opposite Roles for Serotonin 1A and 1B Receptors in Prepulse Inhibition. *Neuropsychopharmacology, 22*(6), 650-659. doi: http://dx.doi.org/10.1016/S0893-133X(99)00164-5

Dunn, R. W., Corbett, R., & Fielding, S. (1989). Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. *European Journal of Pharmacology, 169*(1), 1-10. doi: https://doi.org/10.1016/0014-2999(89)90811-X

Ferrante, R. J., Browne, S. E., Shinobu, L. A., Bowling, A. C., Baik, M. J., MacGarvey, U., . . . Beal, M. F. (1997). Evidence of Increased Oxidative Damage in Both Sporadic and Familial Amyotrophic

Lateral Sclerosis. *Journal of neurochemistry, 69*(5), 2064-2074. doi: 10.1046/j.1471-4159.1997.69052064.x

Gurney, M. E., Pu, H., Chiu, A. Y., Mauro, C. D. C., Polchow, C. Y., Alexander, D. D., . . . Siddique, T. (1994). Motor Neuron Degeneration in Mice That Express a Human Cu,Zn Superoxide Dismutase Mutation. *Science, 264*(5166), 1772-1775.

Karl, T., Pabst, R., & von Horsten, S. (2003). Behavioral phenotyping of mice in pharmacological and toxicological research. *Exp Toxicol Pathol, 55*(1), 69-83. doi: 10.1078/0940-2993-00301

Kostic, V., Gurney, M. E., Deng, H.-X., Siddique, T., Epstein, C. J., & Przedborski, S. (1997). Midbrain dopaminergic neuronal degeneration in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Annals of neurology, 41*(4), 497-504. doi: doi:10.1002/ana.410410413

Kurt, A., Nijboer, F., Matuz, T., & Kübler, A. (2007). Depression and Anxiety in Individuals with Amyotrophic Lateral Sclerosis. *CNS Drugs, 21*(4), 279-291. doi: 10.2165/00023210-200721040-00003

Llano Lopez, L., Hauser, J., Feldon, J., Gargiulo, P. A., & Yee, B. K. (2010). Evaluating spatial memory function in mice: A within-subjects comparison between the water maze test and its adaptation to dry land. *Behavioural brain research, 209*(1), 85-92. doi: https://doi.org/10.1016/j.bbr.2010.01.020

Machts, J., Loewe, K., Kaufmann, J., Jakubiczka, S., Abdulla, S., Petri, S., . . . Bede, P. (2015). Basal ganglia pathology in ALS is associated with neuropsychological deficits. *Neurology, 85*(15), 1301-1309. doi: 10.1212/wnl.0000000000002017

Mantovan, M. C., Baggio, L., Barba, G. D., Smith, P., Pegoraro, E., Soraru', G., . . . Angelini, C. (2003). Memory deficits and retrieval processes in ALS1. *European Journal of Neurology, 10*(3), 221-227. doi: doi:10.1046/j.1468-1331.2003.00607.x

McCombe, P. A., & Henderson, R. D. (2010). Effects of gender in amyotrophic lateral sclerosis. *Gend Med, 7*(6), 557-570. doi: 10.1016/j.genm.2010.11.010

Morgan, M. A., & Pfaff, D. W. (2001). Effects of Estrogen on Activity and Fear-Related Behaviors in Mice. *Hormones and Behavior, 40*(4), 472-482. doi: https://doi.org/10.1006/hbeh.2001.1716

Moy, S. S., Nadler, J. J., Perez, A., Barbaro, R. P., Johns, J. M., Magnuson, T. R., . . . Crawley, J. N. (2004). Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. *Genes, Brain and Behavior, 3*(5), 287-302. doi: 10.1111/j.1601-1848.2004.00076.x

Olaya, J. C., Heusner, C. L., Matsumoto, M., Shannon Weickert, C., & Karl, T. (2018). Schizophrenia-relevant behaviours of female mice overexpressing neuregulin 1 type III. *Behavioural brain research, 353*, 227-235. doi: https://doi.org/10.1016/j.bbr.2018.03.026

Owen, E. H., Logue, S. F., Rasmussen, D. L., & Wehner, J. M. (1997). Assessment of learning by the Morris water task and fear conditioning in inbred mouse strains and F1 hybrids: implications of genetic background for single gene mutations and quantitative trait loci analyses. *Neuroscience, 80*(4), 1087-1099. doi: https://doi.org/10.1016/S0306-4522(97)00165-6

Parton, M. J., Broom, W., Andersen, P. M., Al-Chalabi, A., Nigel Leigh, P., Powell, J. F., . . . Consortium, D. A. S. A. (2002). D90A-SOD1 mediated amyotrophic lateral sclerosis: A single founder for all cases with evidence for a Cis-acting disease modifier in the recessive haplotype. *Human mutation, 20*(6), 473-473.

Paylor, R., & Crawley, J. N. (1997). Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology, 132*(2), 169-180. doi: 10.1007/s002130050333

Piao, Y.-S., Wakabayashi, K., Kakita, A., Yamada, M., Hayashi, S., Morita, T., . . . Takahashi, H. (2003). Neuropathology with Clinical Correlations of Sporadic Amyotrophic Lateral Sclerosis: 102 Autopsy Cases Examined Between 1962 and 2000. *Brain Pathology, 13*(1), 10-22. doi: 10.1111/j.1750-3639.2003.tb00002.x

Pijnenburg, A. J. J., Honig, W. M. M., & Van Rossum, J. M. (1975). Inhibition of d-amphetamine-induced locomotor activity by injection of haloperidol into the nucleus accumbens of the rat. *Psychopharmacologia, 41*(2), 87-95. doi: 10.1007/BF00421062

Przedborski, S., Dhawan, V., Donaldson, D. M., Murphy, P. L., McKenna-Yasek, D., Mandel, F. S., . . . Eidelberg, D. (1996). Nigrostriatal dopaminergic function in familial amyotrophic lateral sclerosis patients with and without copper/zinc superoxide dismutase mutations. *Neurology, 47*(6), 1546-1551.

Pulvirenti, L., Berrier, R., Kreifeldt, M., & Koob, G. K. (1994). Modulation of locomotor activity by NMDA receptors in the nucleus accumbens core and shell regions of the rat. *Brain Res, 664*(1), 231-236. doi: https://doi.org/10.1016/0006-8993(94)91977-1

Quarta, E., Bravi, R., Scambi, I., Mariotti, R., & Minciacchi, D. (2015). Increased anxiety-like behavior and selective learning impairments are concomitant to loss of hippocampal interneurons in the presymptomatic SOD1(G93A) ALS mouse model. *J Comp Neurol, 523*(11), 1622-1638. doi: 10.1002/cne.23759

Radakovic, R., Stephenson, L., Colville, S., Swingler, R., Chandran, S., & Abrahams, S. (2016). Multidimensional apathy in ALS: validation of the Dimensional Apathy Scale. *Journal of Neurology, Neurosurgery & Psychiatry, 87*(6), 663. doi: 10.1136/jnnp-2015-310772

Rosen, D. R., Siddiquef, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentatif, A., . . . Cayabyabi, A. (1993). Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic. *Nature, 362*, 4.

Sano, H., Nagai, Y., Miyakawa, T., Shigemoto, R., & Yokoi, M. (2008). Increased social interaction in mice deficient of the striatal medium spiny neuron-specific phosphodiesterase 10A2. *J Neurochem, 105*(2), 546-556. doi: 10.1111/j.1471-4159.2007.05152.x

Shang, K., Talmage, D. A., & Karl, T. (2017). Parent-of-origin effects on schizophrenia-relevant behaviours of type III neuregulin 1 mutant mice. *Behavioural brain research, 332*, 250-258. doi: https://doi.org/10.1016/j.bbr.2017.05.057

Singer, C. A., Figueroa-Masot, X. A., Batchelor, R. H., & Dorsa, D. M. (1999). The Mitogen-Activated Protein Kinase Pathway Mediates Estrogen Neuroprotection after Glutamate Toxicity in Primary Cortical Neurons. *The Journal of Neuroscience, 19*(7), 2455-2463. doi: 10.1523/jneurosci.19-07-02455.1999

Stam, N. C., Nithianantharajah, J., Howard, M. L., Atkin, J. D., Cheema, S. S., & Hannan, A. J. (2008). Sex-specific behavioural effects of environmental enrichment in a transgenic mouse model of amyotrophic lateral sclerosis. *European Journal of Neuroscience, 28*(4), 717-723.

Stough, S., Shobe, J. L., & Carew, T. J. (2006). Intermediate-term processes in memory formation. *Current Opinion in Neurobiology, 16*(6), 672-678. doi: https://doi.org/10.1016/j.conb.2006.10.009

Strong, M. J., Abrahams, S., Goldstein, L. H., Woolley, S., McLaughlin, P., Snowden, J., . . . Turner, M. R. (2017). Amyotrophic lateral sclerosis - frontotemporal spectrum disorder (ALS-FTSD): Revised diagnostic criteria. *Amyotroph Lateral Scler Frontotemporal Degener, 18*(3-4), 153-174. doi: 10.1080/21678421.2016.1267768

Sudharshan, N., Hanstock, C., Hui, B., Pyra, T., Johnston, W., & Kalra, S. (2011). Degeneration of the Mid-Cingulate Cortex in Amyotrophic Lateral Sclerosis Detected In Vivo with MR Spectroscopy. *American Journal of Neuroradiology, 32*(2), 403-407. doi: 10.3174/ajnr.A2289

Synofzik, M., Fernández-Santiago, R., Maetzler, W., Schöls, L., & Andersen, P. M. (2010). The human G93A SOD1 phenotype closely resembles sporadic amyotrophic lateral sclerosis. *Journal of Neurology, Neurosurgery & Psychiatry, 81*(7), 764-767. doi: 10.1136/jnnp.2009.181719

Taglialatela, G., Hogan, D., Zhang, W.-R., & Dineley, K. T. (2009). Intermediate- and long-term recognition memory deficits in Tg2576 mice are reversed with acute calcineurin inhibition. *Behavioural brain research, 200*(1), 95-99. doi: https://doi.org/10.1016/j.bbr.2008.12.034

Takeda, T., Uchihara, T., Arai, N., Mizutani, T., & Iwata, M. (2009). Progression of hippocampal degeneration in amyotrophic lateral sclerosis with or without memory impairment: distinction from Alzheimer disease. *Acta Neuropathol, 117*(1), 35-44. doi: 10.1007/s00401-008-0447-2

Temel, Y., Blokland, A., & Lim, L. W. (2012). Deactivation of the parvalbumin-positive interneurons in the hippocampus after fear-like behaviour following electrical stimulation of the dorsolateral periaqueductal gray of rats. *Behavioural brain research, 233*(2), 322-325. doi: https://doi.org/10.1016/j.bbr.2012.05.029

Turner, B. J., Lopes, E. C., & Cheema, S. S. (2003). The serotonin precursor 5-hydroxytryptophan delays neuromuscular disease in murine familial amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord, 4*(3), 171-176.

Turner, B. J., & Talbot, K. (2008). Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Progress in neurobiology, 85*(1), 94-134.

Turner, M. R., Rabiner, E. A., Hammers, A., Al-Chalabi, A., Grasby, P. M., Shaw, C. E., . . . Leigh, P. N. (2005). [11C]-WAY100635 PET demonstrates marked 5-HT1A receptor changes in sporadic ALS. *Brain, 128*(Pt 4), 896-905. doi: 10.1093/brain/awh428

Usman, U., Choi, C., Camicioli, R., Seres, P., Lynch, M., Sekhon, R., . . . Kalra, S. (2011). Mesial Prefrontal Cortex Degeneration in Amyotrophic Lateral Sclerosis: A High-Field Proton MR Spectroscopy Study. *American Journal of Neuroradiology, 32*(9), 1677-1680. doi: 10.3174/ajnr.A2590

Vignola, A., Guzzo, A., Calvo, A., Moglia, C., Pessia, A., Cavallo, E., . . . Chiò, A. (2008). Anxiety undermines quality of life in ALS patients and caregivers. *European Journal of Neurology, 15*(11), 1231-1236. doi: 10.1111/j.1468-1331.2008.02303.x

Vogels, O. J. M., Veltman, J., Oyen, W. J. G., & Horstink, M. W. I. M. (2000). Decreased striatal dopamine D2 receptor binding in amyotrophic lateral sclerosis (ALS) and multiple system atrophy (MSA): D2 receptor down-regulation versus striatal cell degeneration. *J Neurol Sci, 180*(1), 62-65. doi: https://doi.org/10.1016/S0022-510X(00)00429-9

Wang, Q., Johnson, J. L., Agar, N. Y., & Agar, J. N. (2008). Protein aggregation and protein instability govern familial amyotrophic lateral sclerosis patient survival. *PLoS Biol, 6*(7), e170. doi: 10.1371/journal.pbio.0060170

Zang, D., & Cheema, S. (2002). Degeneration of corticospinal and bulbospinal systems in the superoxide dismutase 1 G93A G1H transgenic mouse model of familial amyotrophic lateral sclerosis. *Neuroscience letters, 332*(2), 99-102.

Acknowledgements

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6. Figure legends

Figure 1A-B: Locomotor behaviour in a 30-min open field test. **A-B)** Ambulatory distance [cm] across 5-min blocks, for male **(A)** and female **(B)** *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-10 per group). Data are shown as mean ± SEM.

Figure 2A-D: Long-term and intermediate-term spatial learning and memory in the cheeseboard task. **A-B)** The latency to find a food reward averaged across three trials per day and **C-D)** the first trial per day in male **(A,C)** and female **(B,D)** *SOD1*^{G93A} and wild type-like (WT) mice (n = 11-17 per group). A significant effect of 'sex' was found in the latency to find the reward average across three trials per day ($p = 0.005$). Data are shown as mean ± SEM. Two-way ANOVA 'time' by 'genotype' interaction $^{++}p < 0.01$, $^{+++}p < 0.0001$, one-way ANOVA 'genotype' effect $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$.

Figure 3A-D: Sociability and social recognition memory test. **A-B)** Percent time spent in a chamber containing a standard opponent (sex matched AJ) vs an empty chamber, and **C-D)** percent time spent *nosing* a novel standard opponent vs time spent *nosing* a familiar mouse in male **(A,C)** and female **(B,D)** *SOD1*^{G93A} and wild type-like (WT) mice (n= 9-10 per group). Data are shown as mean ± SEM. One sample t-test vs 50% chance $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$.

Figure 4A-D: Acoustic startle and prepulse inhibition. **A-B)** Acoustic startle response to 120 dB [arbritary units] and **C-D)** prepulse inhibition [%] at 74, 82 and 86 dB prepulse intensities averaged across inter stimulus intervals are shown for male **(A,C)** and female **(B,D)** *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-10 per group). Data are shown as mean ± SEM. Two-way

ANOVA ‘startle pulse/prepulse’ by ‘genotype’ interaction $^{+}p < 0.05$, $^{+++}p < 0.0001$. One-way ANOVA $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.0001$.

Figure 5A-F: Fear associated learning during fear conditioning. **A-B)** Conditioning, **C-D)** context test and **E-F)** cue test in male **(A,C,E)** and female **(B,D,F)** *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-10 per group). An effect of ‘sex’ was found in contextual *freezing* time ($p = 0.013$). Data are shown as mean ± SEM. Two-way ANOVA ‘time’ by ‘genotype’ interaction $^{+}p < 0.05$ Two-way ANOVA ‘cue’ by ‘genotype’ interaction $^{++}p < 0.01$. One-way ANOVA $^{*}p < 0.05$.

	Behavioural test	Age (postnatal days ± 4)
Cohort 1	Motor function (Accelerod, pole test)	74
	Open field	76
	Y maze	80
	Social preference	82
	PPI	86
	Motor function (Accelerod, pole test)	94
	Fear conditioning	96
	Bodyweight	96-138
Cohort 2	Novel object recognition	116
	Social interaction	120
	Cheeseboard	131

Table 1: The order of behavioural testing and age of *SOD1*^{G93A} and wild type-like (WT) mice. Cohort 1: n = 9-10 per group; Cohort 2: n = 11-17 per group.

	WT Male	*SOD1* Male	WT female	*SOD1* female
PND74 Accelerod latency [s]	164.9 ± 9.36	161.1 ± 7.44	182.5 ± 9.64	146.7 ± 5.27**
PND94 Accelerod latency [s]	172.2 ± 13.8	146.5 ± 10.4	181.6 ± 10.7	134.6 ± 6.43**
PND74 Pole test – latency to turn [s]	16.2 ± 4.9	29.2 ± 6.5	26.1 ± 7.1	12.8 ± 3.3
PND94 Pole test – latency to turn [s]	11.1 ± 3.2	17.2 ± 5.0	10.2 ± 3.1	7.8 ± 2.3
PND74 Pole test – latency to bottom [s]	24.5 ± 4.9	36.2 ± 5.7	31.0 ± 6.2	21.6 ± 3.7
PND94 Pole test – latency to bottom [s]	16.9 ± 3.4	23.7 ± 4.9	16.2 ± 3.4	15.0 ± 3.5

Table 2 Motor co-ordination in male and female *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-10 per group). A 'sex' by 'genotype' interaction effect was found in 'time to turn' on the pole test at postnatal day (PND) 74 ($p = 0.034$). Data are shown as mean ± SEM. One-way ANOVA **$p < 0.01$ vs WT of the corresponding sex.

	WT Male	*SOD1* Male	WT female	*SOD1* female
Open field *Rearing* frequency [n]	371.2 ± 22.8	307.4 ± 15.6**	279.5 ± 27.0	240.3 ± 31.8
Open field Total distance [cm]	10626 ± 820	6392 ± 622**	8162 ± 610	7448 ± 572
Open field Centre time [s]	306.1 ± 21.5	204.4 ± 23.3**	174.4 ± 19.9	177.9 ± 23.4
Open field Centre distance ratio [%]	32.6 ± 2.1	26.2 ± 1.8*	25.6 ± 2.2	24.4 ± 2.3

Table 3: Open field exploration, locomotion and anxiety measures in male and female *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-10 per group). A 'sex' by 'genotype' effect was found in open field distance ($p = 0.013$). A significant effect of 'sex' was found in centre time ($p = 0.001$) and centre zone distance ratio ($p = 0.045$). Data are shown as mean ± SEM. One-way ANOVA *$p < 0.05$ **$p < 0.01$ vs WT of the corresponding sex.

	WT Male	*SOD1* Male	WT female	*SOD1* female
Y-maze (percentage distance in novel arm [%])	$44.1 \pm 2.4^{++}$	$40.7 \pm 2.1^{++}$	$42.9 \pm 2.1^{++}$	40.1 ± 3.6
Y-maze (percentage entries into novel arm [%])	$42.1 \pm 1.6^{++}$	$40.0 \pm 2.2^{+}$	$43.0 \pm 2.1^{++}$	40.1 ± 3.1
Cheeseboard probe (percentage time in target zone [%])	16.1 ± 3.7	21.6 ± 5.2	16.0 ± 3.0	21.1 ± 6.7
Social interaction Total active social time [s]	70.6 ± 5.9	78.0 ± 5.2	59.8 ± 3.6	78.0 ± 7.6*
Social interaction *Nosing* time [s]	34.6 ± 5.6	53.2 ± 6.6*	35.4 ± 4.0	52.4 ± 6.9*

Table 4: Spatial memory and sociability measures in male and female *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-17 per group). Data are shown as mean ± SEM. One-way ANOVA *$p < 0.05$ vs WT of the corresponding sex. One sample t-test vs 33.3% chance $^{+}p < 0.05$, $^{++}p < 0.01$, $^{+++}p < 0.0001$.

	Male	Male	*SOD1* Male	WT female	*SOD1* female
				± 0	0.29 ± 0.19
				5 ± 3.0	14.4 ± 3.0
Fear conditioning Total context *freezing* [s]	18.4 ± 7.9		38.9 ± 8.0	54.9 ± 10.7	58.5 ± 13.9
Fear conditioning Cue presentation *freezing* [s]	25.3 ± 9.3		76.3 ± 14.5**	106.8 ± 9.1	83.1 ± 17.8

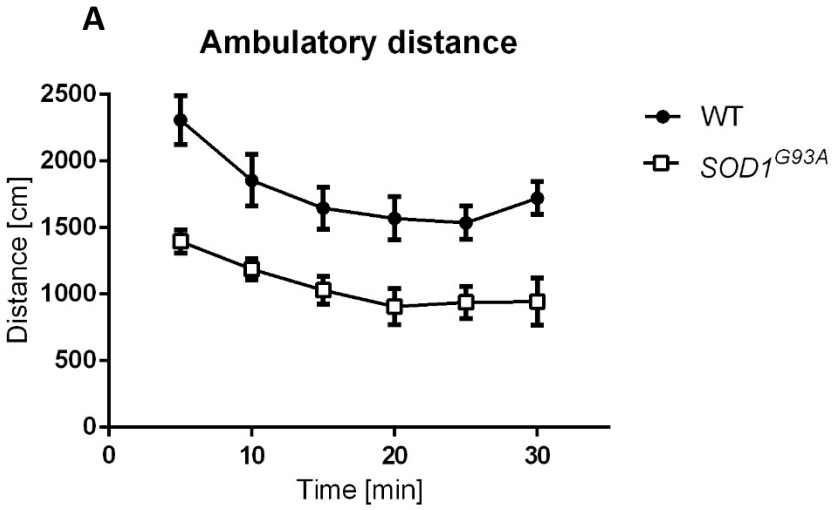


Table 5: *Freezing* time in the first 2 min of fear conditioning and context trial. Total *freezing* in contextual fear and conditioned cued fear (during cue presentation) in *SOD1*^{G93A} and wild type-like (WT) mice (n = 9-10 per group). Data are shown as mean ± SEM. RM ANOVA main effect of 'genotype' $^{**}p < 0.01$ vs WT of corresponding sex.

Figure 1

Female

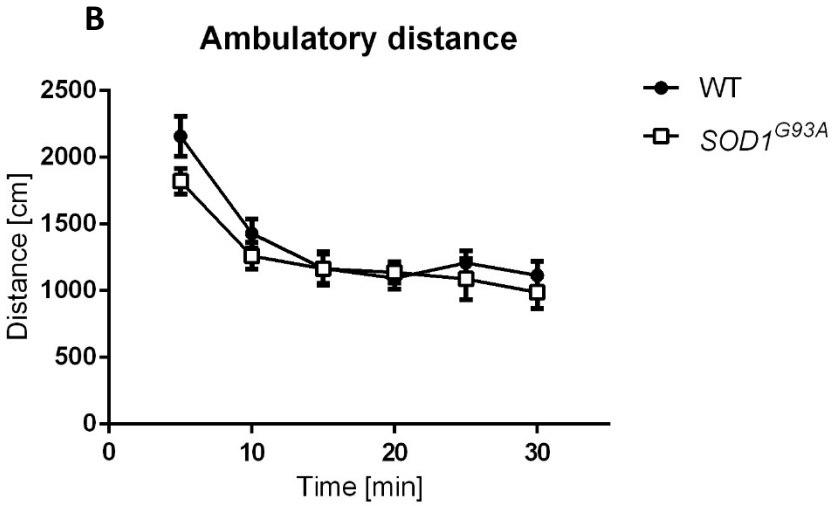
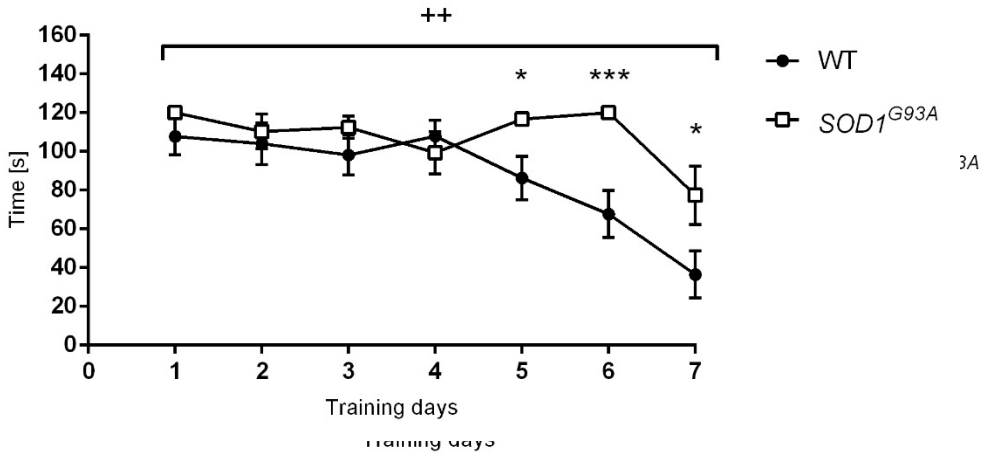

B
Ambulatory distance
2500
2000
1500
1000
500
0
Distance [cm]
0
10
20
30
Time [min]
WT
SOD1G93A

Figure 2

Male

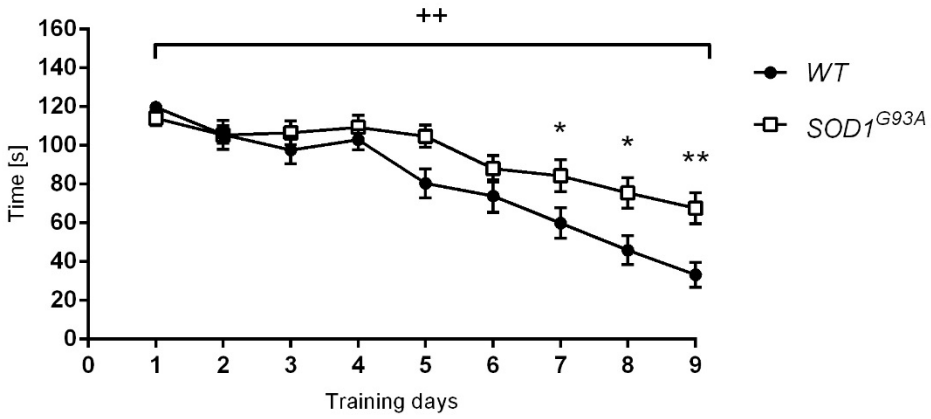
C **Latency to find the food reward: Trial 1**



Female

B

Latency to find the food reward: Average of 3 trials per day

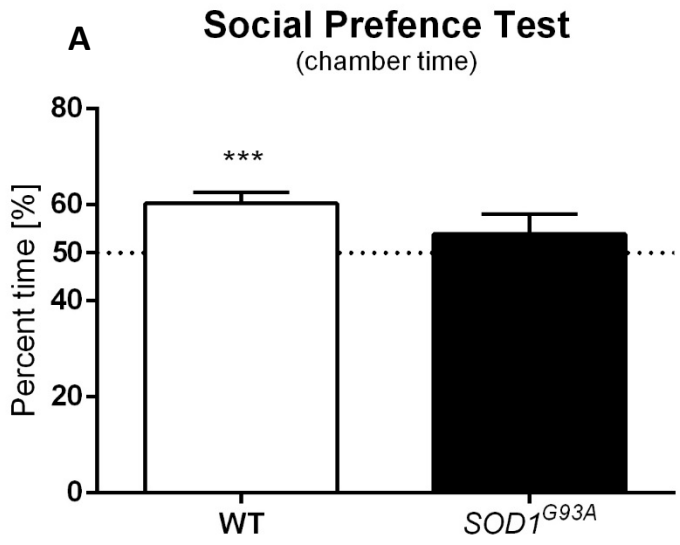


Female

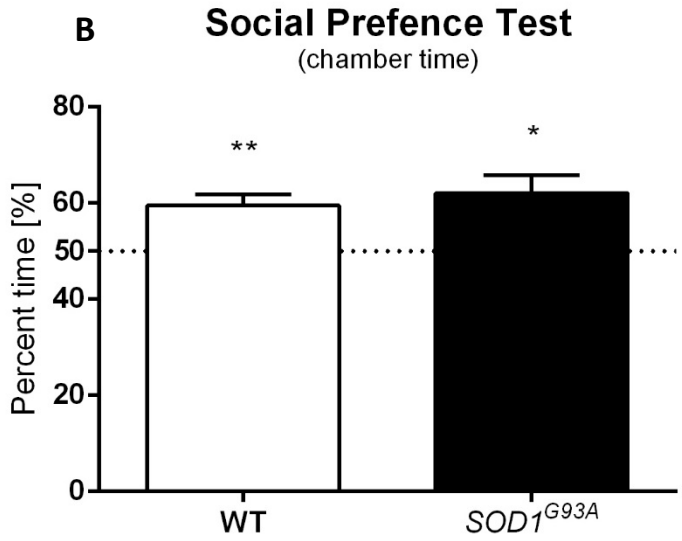

D
Latency to find the food reward: Trial 1
+++
*
*
**
WT
SOD1G93A
Time [s]
Training days
0
20
40
60
80
100
120
140
160
0
1
2
3
4
5
6
7
8
9

Figure 3

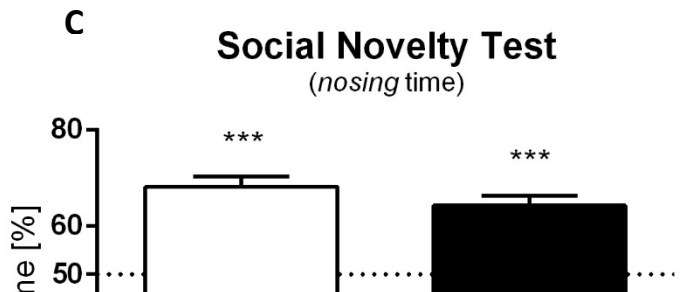
Male



Female



Male



Female

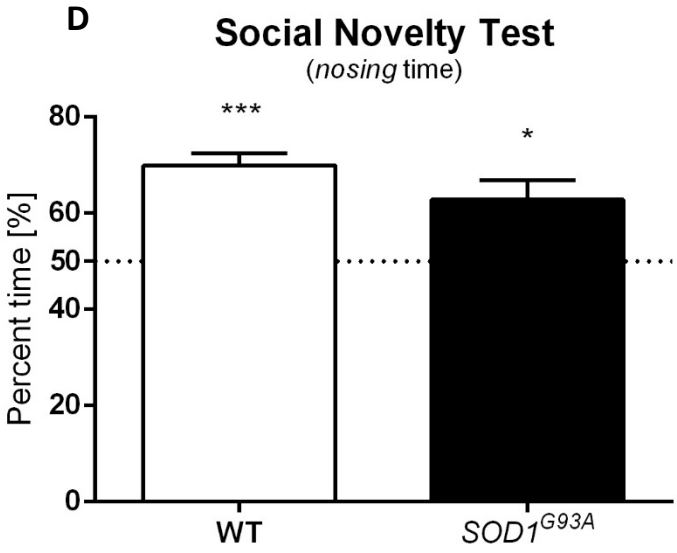
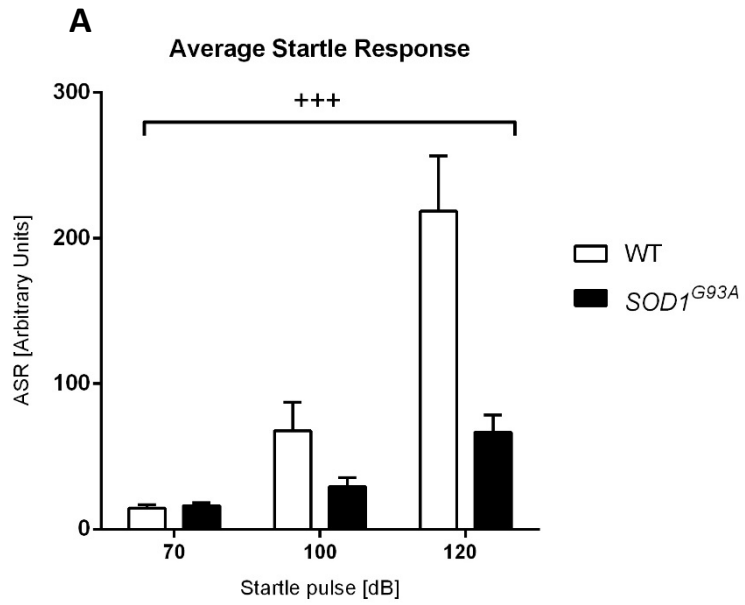
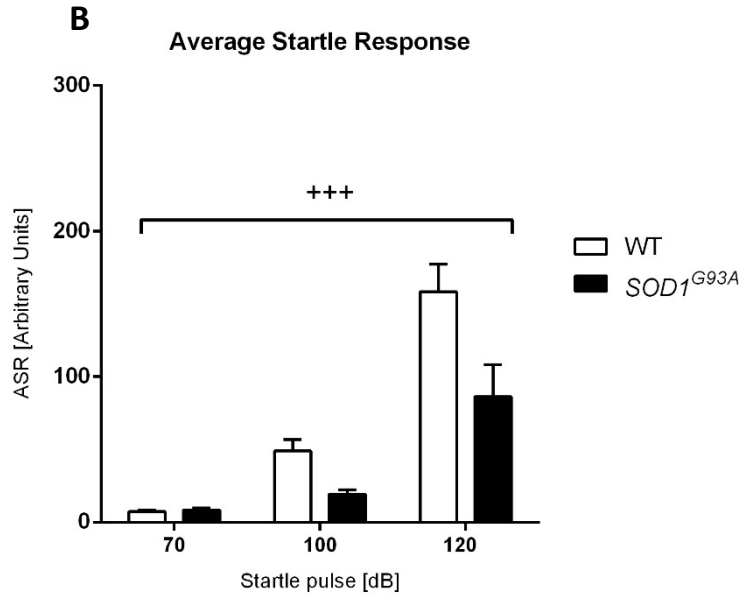

D
Social Novelty Test
(nosing time)
***
*
80
60
50
40
20
0
Percent time [%]
WT
SOD1G93A

Figure 4

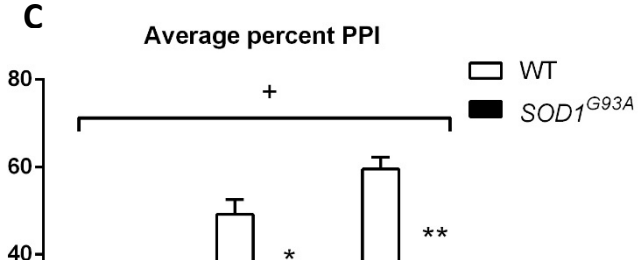
Male



Female



Male



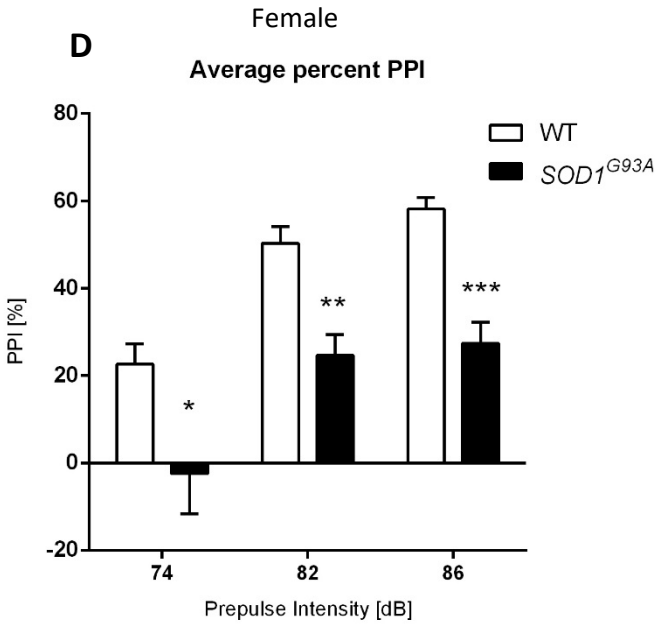
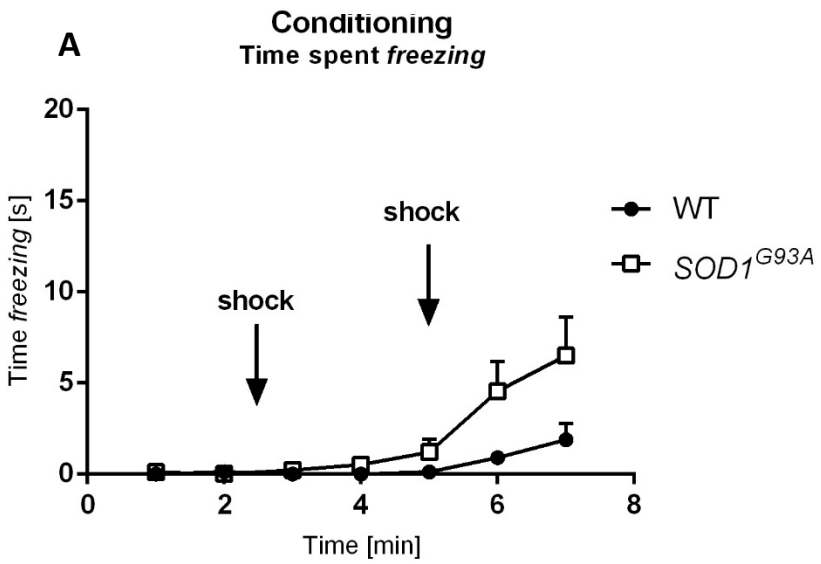
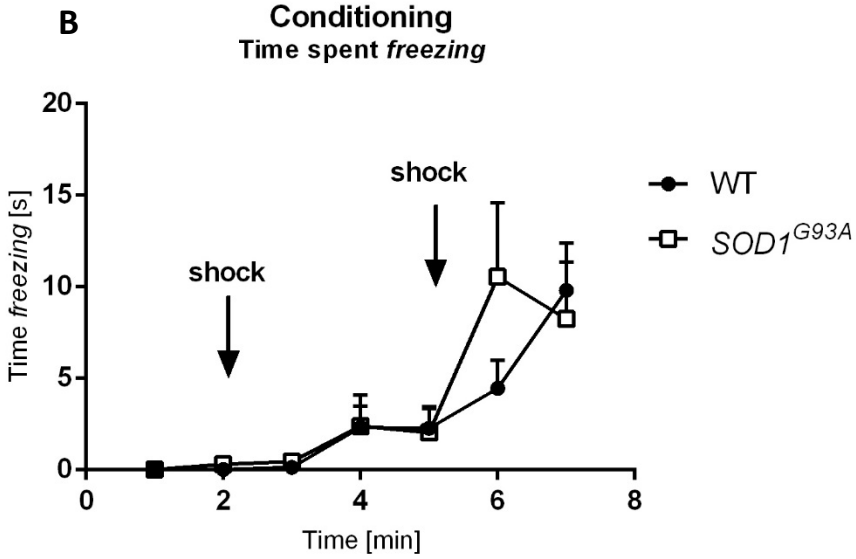

Female
D
Average percent PPI
WT
SOD1G93A
80
60
40
20
0
-20
PPI [%]
*
**
***
74
82
86
Prepulse Intensity [dB]

Figure 5

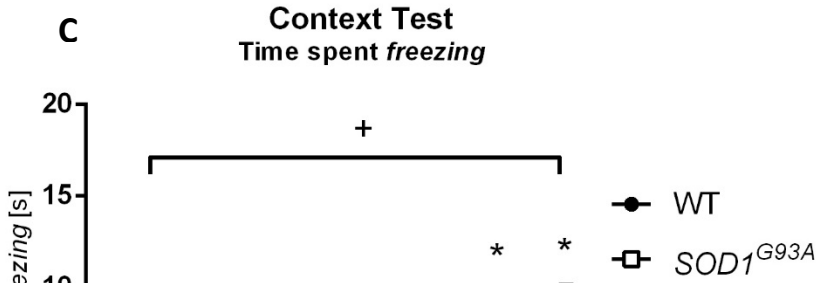
Male



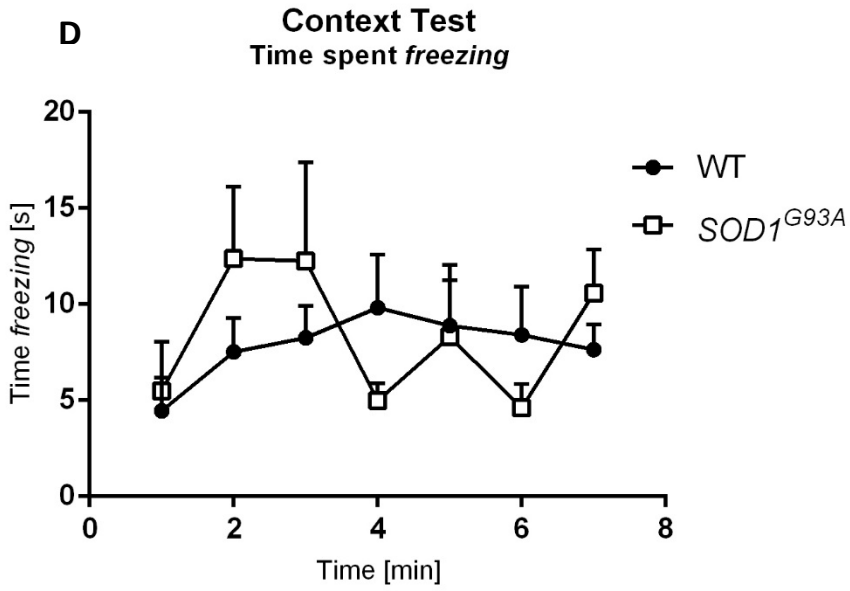
Female



Male

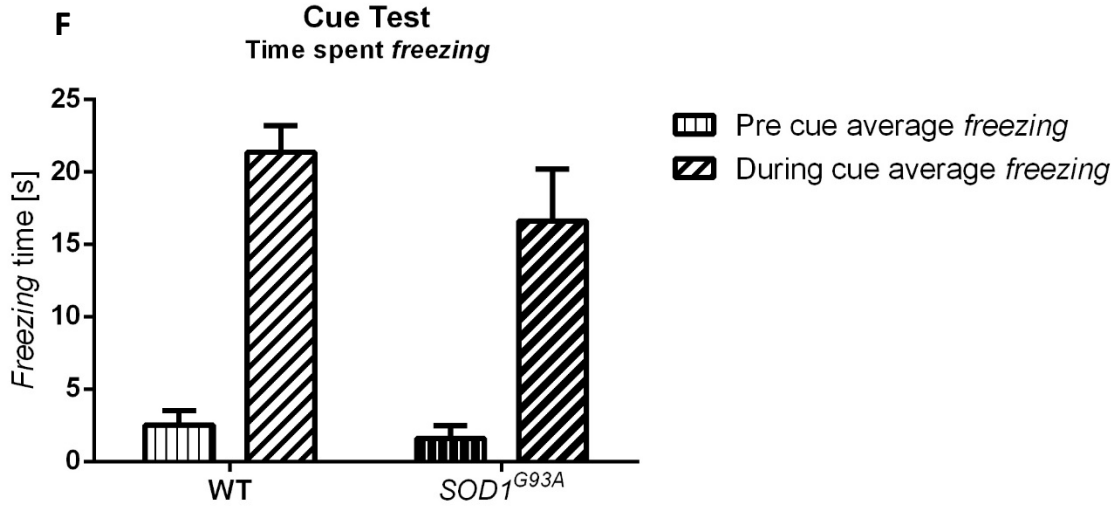


Female


D
Context Test
Time spent freezing
Time freezing [s]
20
15
10
5
0
0
2
4
6
8
Time [min]
WT
SOD1G93A

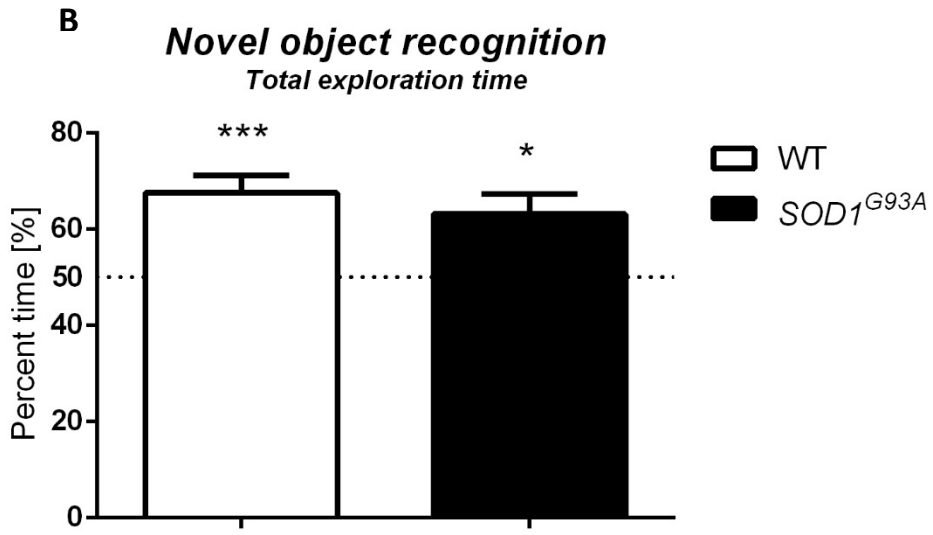
Male

Female



Supplementary Figure 1

Female


B
Novel object recognition
Total exploration time
Percent time [%]
80
60
50
40
20
0
***
*
WT
SOD1G93A

Supplementary Table 1: *P* values from two-way ANOVA analysing main effects of 'sex' and 'sex' by 'genotype' in *SOD1*^{G93A} mice compared to WT. n.s = not significant.

Behavioural test	Sex effect	Sex*genotype effect
Accelerod PND 74	n.s	n.s
Accelerod PND 94	n.s	n.s
Pole test total time PND 74	n.s	n.s
Pole test total time PND 94	n.s	n.s
Pole test time to turn PND 74	n.s	0.034
Pole test time to turn PND 94	n.s	n.s
Open field locomotion	n.s	0.013
Open field *rearing*	0.002	n.s
Open field centre time	0.001	0.023
Open field centre distance ratio	0.045	0.223
Y maze percent novel arm distance	n.s	n.s
Y maze percent novel arm entries	n.s	n.s
Sociability	n.s	n.s
Social recognition memory	n.s	n.s
Total active social interaction	n.s	n.s
Social interaction *nosing* time	n.s	n.s
Acoustic startle response	n.s	n.s
Percent PPI	n.s	n.s
Freezing time conditioning	0.018	n.s
Freezing time context	0.013	n.s
Freezing time cue	<0.0001	0.004
Novel object recognition percent *nosing* + *rearing* time	n.s	n.s

Cheeseboard time to find reward – average three trials per day	0.001	n.s
Cheeseboard time to find reward – first trial	0.005	n.s
Cheeseboard probe –percent time in target zone	n.s	n.s

	WT Male	*SOD1* Male	WT female	*SOD1* female
Cheeseboard average speed (first trial) [cm/s]	3.35 ± 0.57	2.23 ± 0.55	2.95 ± 0.60	1.66 ± 0.55
Cheeseboard average speed (3 trials) [cm/s]	4.06 ± 0.46	2.98 ± 0.48*	3.84 ± 0.40	2.84 ± 0.47

Supplementary Table 2: Average distance travelled, and average speed during training trials of the cheeseboard task in male and female $SOD1^{G93A}$ and WT mice. Data are shown as mean ± SEM. One-way ANOVA *$p < 0.05$ vs WT of corresponding sex.

Supplementary Figure 1A-B: Bodyweight in male **(A)** and female **(B)** *SOD1*^{G93A} and wild type-like (WT) mice measured from postnatal day (PND) 96-138 (n = 9-10 per group). There was an overall sex effect between males and females ($p < 0.0001$). Data are shown as mean ± SEM. Two-way RM ANOVA 'time' by 'genotype' interaction $^{+++}p < 0.0001$. One-way ANOVA *$p < 0.05$ **$p < 0.01$, ***$p < 0.0001$ vs WT of the corresponding sex.