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# Impaired working memory, cognitive flexibility and reward processing in mice genetically lacking Gpr88: evidence for a key role for Gpr88 in multiple cortico-striatal-thalamic circuits

David M Thomson[1], Rebecca L Openshaw[1], Emma J Mitchell[1], Marianna Kouskou[1], Mark J Millan[2], Clotilde Mannoury la Cour[2], Brian J Morris [3] and Judith A Pratt[1].

1. Strathclyde Institute of Pharmacy and Biomedical Science, University of Strathclyde, Glasgow, G4 0RE UK; 2. Centre for Therapeutic Innovation-CNS, Institute de Recherche Servier, Croissy-sur-Seine, France; 3. Institute of Neuroscience and Psychology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G12 8QQ, UK

Corresponding author:
Judith A Pratt

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***Abstract***

The GPR88 orphan G protein-coupled receptor is expressed throughout the striatum, being preferentially localized in medium spiny neurons. It is also present in lower densities in frontal cortex and thalamus. Rare mutations in humans suggest a role in cognition and motor function, while common variants are associated with psychosis. Here we evaluate the influence of genetic deletion of GPR88 upon performance in translational tasks interrogating motivation, reward evaluation and cognitive function. In an automated radial arm maze 'N-back' working memory task, *Gpr88* KO mice showed impaired correct responding, suggesting a role for GPR88 receptors in working memory circuitry. Associative learning performance was similar to wildtype controls in a touchscreen task but performance was impaired at the reversal learning stage, suggesting cognitive inflexibility. *Gpr88* KO mice showed higher breakpoints, reduced latencies and lengthened session time in a progressive ratio task consistent with enhanced motivation. Simultaneously, locomotor hyperactivity was apparent in this task, supporting previous findings of actions of GPR88 in a cortico-striatal-thalamic motor loop. Evidence for a role of GPR88 in reward processing was demonstrated in a touchscreen-based equivalent of the Iowa gambling task. Although both *Gpr88* KO and wildtype mice showed a preference for an optimum contingency choice, *Gpr88* KO mice selected more risky choices at the expense of more advantageous lower risk options. Together these novel data suggest that striatal GPR88 receptors influence activity in a range of procedures integrated by prefrontal, orbitofrontal and anterior cingulate cortico-striatal- thalamic loops leading to altered cognitive, motivational and reward evaluation processes.

***Introduction***

The *GPR88* gene was discovered some 20 years ago and encodes an orphan G protein-coupled receptor, GPR88, which is enriched throughout the dorsal and ventral striatum (including the nucleus accumbens), and is also present in lower levels in the cortex, thalamus and amygdala [1-3]. Within the striatum, GPR88 is expressed in GABAergic medium spiny neurons and is therefore positioned to modulate cortico-striatal thalamic loops. These circuits play an important role in the control of movement, whereby GABAergic medium spiny neurones of the striatum (caudate putamen) receive glutamatergic inputs from motor and somatosensory cortices. This information is then transmitted and processed in the globus pallidus and substantia nigra, and relayed to specific area of the thalamus (ventrolateral). The thalamus in turn 'filters' the signals, which are passed back to the cortex and lead to appropriate motor function [4,5].

While initial work in this field focussed mainly on motor function, current thinking ascribes a role for basal ganglia-thalamocortical pathways not only in mediating goal-directed behaviours through movement, but also in the motivational, emotional and cognitive processes that ultimately drive actions to achieve a particular outcome [6]. These different functions are associated with topographical inputs to the striatum from separate parts of the frontal cortex, which are organised in a series of parallel large-scale anatomical loops [4,5,7,8]. These loops include a dorsolateral prefrontal cortex (PFC) - striatal (dorsolateral caudate)- globus pallidus- mediodorsal thalamus- loop implicated in working memory and executive function, an anterior cingulate-striatal (ventral striatum) ventral pallidum-mediodorsal thalamus loop implicated in a diverse range of processes involved in cognitive and emotional control, and a lateral orbitofrontal cortex (OFC) -striatal (ventromedial caudate) -globus pallidus/substantia nigra-anterior ventral thalamus/mediodorsal thalamus loop implicated in reversal learning and adapting behaviour to the most rewarding outcome. A medial OFC loop is involved in encoding reward value, and these OFC loops also play a role in emotional processing [9,4,6,10-12,8,13-15].

From their localisation in striatal output cells, GPR88 receptors are strategically sited to modulate the function of several cortico-striato-thalamic loops, suggesting possible relevance as a target for the treatment of neuropsychiatric disorders. Indeed the *GPR88* gene has been genetically associated with psychosis [16], although it is not currently clear whether its expression and/or activity is altered in idiopathic patients [17]. Currently-available treatments for schizophrenia are centred on the blockade of postsynaptic populations of dopamine (mainly D2) receptors in mesolimbic structures, for the amelioration of positive symptoms. However, interference with dopaminergic signalling in other regions like the basal ganglia and frontal cortex may lead to unwanted motor and cognitive-side effects [18]. Further, despite the integration of serotonergic, dopamine D3 and other properties, control of negative symptoms and the cognitive impairment of schizophrenia is poor [19-23]. The enriched location of GPR88 in the striatum offers a potentially attractive alternative target for modulating cortico-striatal-thalamic circuitry dysfunctional in schizophrenia and accordingly acheiving a broad-based control of symptoms.

Evaluation of behaviours in mice deficient in the *Gpr88* gene offers a means to determine a role for GPR88 in symptoms relevant to schizophrenia. Previous studies of GPR88 have mainly focused on phenotypic changes related to the positive symptoms of schizophrenia, such as enhanced locomotor sensitivity to amphetamine and elevated apomorphine-induced climbing [2,24-26]. In contrast, there is limited knowledge of the role of *GPR88* in the cognitive deficits and the negative symptoms of schizophrenia. A contribution to the cognitive impairments would be consistent with the discovery that a rare mutation in the *GPR88* gene in humans causes intellectual disability, in addition to motor dysfunction [27].

An enduring challenge in preclinical research has been to adopt translationally relevant paradigms that tap into analagous cognitive domains, underpinned by similar neural systems, in humans [28]. Rodent models for these aspects of schizophrenia-like behaviours have been developed in recent years to move beyond those that show limited translation, to ones that mirror more closely the neural systems recruited in the domain investigated [28-31]. Given the importance of the aforementioned functionally segregated 'cortico-striatal-thalamic loops' in mediating distinct elements of behaviours relevant to cognitive and negative symptoms, and the pivotal role of the striatum in these loops, we have examined the importance of GPR88 in a range of translational behavioural tasks in which these loops are recruited. In particular, we focus on assessment of *Gpr88* KO mice in tasks for working memory, motivation, reward evaluation, reversal learning, and decision making.

## *Materials and Methods*

### Experimental subjects

~~*Gpr88* KO mice[2] were obtained from the Jackson laboratory (strain #022510) and maintained congenic on C57/Bl6J background, with wild-type (WT) C57/Bl6J mice as controls.~~

*Gpr88* KO mice[2] were obtained from the Jackson laboratory (strain #022510) and maintained congenic on C57BL/6J background, with wild-type (WT) C57BL/6J mice, from the same recipient inbred substrain, as controls.

Animals were group (working memory, progressive ratio, gambling tasks) or single (visual discrimination task) housed, and maintained on a 12 hour light dark cycle (lights on 7-8am, except for the gambling task, lights on 10pm). Prior to behavioural testing animals followed a similar habituation protocol; animals were handled for 5 minutes each day and placed on a food restriction diet to reduce their body weight to 85-90% of their free feeding weight over a period of one week prior to the commencement of the task. Typically mice were 8-10 weeks of age at the beginning of the studies. The following numbers were employed: working memory task (10 male *Gpr88* KO mice and 10 WT male controls), progressive ratio task (9 female and 7 male *Gpr88* KO, 9 female and 7 male WT mice), visual discrimination and reversal learning study (8 male *Gpr88* KO and 8 male WT C57BL/6J control mice), gambling task (4 female and 5 male *Gpr88* KO, 5 female and 4 male WT mice). Testing occurred in the light phase for all tasks except for the gambling task when mice were tested in the dark phase.

### *Working memory*

The "N-back" task is a test of working memory that can be translated from rodents to humans - patients with schizophrenia show robust impairments in N-back task performance (particularly at more demanding levels of cognitive demand), in association with hypoactivation of PFC-striatal networks [32-34]. The rodent adaption of the task employs a radial arm maze to test performance at increasing levels of working memory load [35]. The mouse N-back task is designed to measure working memory in the mouse by presenting 3 pairs of arms in a pseudorandom order, where the mouse must select the previously non-selected arm in a non-match to sample fashion. This requires that the animal hold in memory previous interactions with each pair of arms in order to correctly select the previously non selected arm in successive presentation of each pair. Proactive interference from other arm pair presentations requires that the animal correctly recall these interactions in a temporal order that is specific for each arm pair presentation. Deficits in working memory would be revealed as reductions in overall percentage corrects for each level of the task [35,36].

The N-back test was performed using two fully automated 8 arm radial mazes (Med Associates Inc., St Albans, Vermont), for the present task only 6 arms were utilised, as previously described in [35]. Each arm was 36.8 cm long 30.5 cm, with an opaque floor and transparent Perspex walls connected by an octagonal hub 116.8 x 116.8 cm, height 30.5 cm. The entrance of each runway was regulated by an automated guillotine door and IR detectors were used to

identify ingress and egress from the maze arms. At the end of each arm an automated pellet dispenser was located which delivered a single reward pellet (Test Diet, formula 5TUL 14mg). For habituation, all animals were given 3 days of free access to the maze where all arms were accessible and 2 pellets placed in the food hoppers at the end of each arm. Following the "free access" period, the animals were given 2 days of the full task where the testing period was restricted to 30 minutes. Following this 2 day "introductory period" all animals were then tested for a further 16 days, incorporating the full test schedule of 24 trials, with equal numbers of N-back trials. The 6 arms were allocated to 1 of 3 pairs of arms (pair A, B and C) which were presented in a random order. Following an initial presentation of a given pair (free choice; rewarded) a reward was delivered only when the alternative arm was selected from that selected in the previous presentation of the arm pair. The N-back set was then constructed from the individual trial set by the number of intervening alternative arm pair presentations.

***Progressive Ratio task***

Progressive ratio tasks, in which animals work to obtain a food reward, are operant assays that measure motivation. Animals are required to press a panel or make a 'nose poke' an increased number of times over successive trials to obtain a food reward. A 'break point' is defined as the first criterion the animal is unable to complete and provides an index of 'avolition'.

Initially animals were given a 15 minute session with all lights off and the food hopper baited with 140 µl of YAZOO$^{tm}$ strawberry milk shake. Following this habituation phase, all animals were placed onto a fixed ratio of 1:1 task, in the multi-hole operant boxes with the central hole (5) open and all other nose- poke holes blocked. Mice were trained for 12 days until they were consistently completing 30 trials in less than 30 minutes. Any animals failing to reach this target were removed from the study (n = 2).

The response schedule, after Young et al. [37], was designed such that at each stage of the ratio, the animals were given three repeats of the stage before progressing onto the next stage. The stages were: 1, 2, 4, 7, 11, 16, 22, 29, 37, 46, 56 and 67 nose pokes. For each stage the animal was required to nose-poke the central hole and then enter the reward delivery hopper, this constituted a single shuttle. The initial trial was started with a hopper entry to begin the session. At this stage the animals were rewarded for completing the ratio criteria with 70µl of the liquid reward. All mice were tested for 60 minutes or until no response in either the nose-poke hole or the food hopper was recorded over a 3 minute period. The breakpoint was recorded as the final ratio attained over the 60 minute period. The mice were tested in the progressive ratio task for 12 days, following the initial 12 days of testing; a further three days of testing were conducted as extinction trials, run in the same manner as the previous test days with the exception of reward delivery which was omitted on these days.

***Paired Associate Learning and Reversal Learning Task***

The visual discrimination and reversal learning task in the touchscreen apparatus enables the dissociation of several cognitive processes. The mouse is presented with two distinct visual stimuli and learns that a nosepoke towards one stimulus is rewarded while a response to the other is not. Once the animals have learnt the stimulus-reward paired association, the reversal phase of the task takes place. In this phase, the visual stimulus that was rewarded previously is no longer rewarded and the one that was not previously rewarded is now rewarded. Hence performance on this task involves the ability to learn stimulus-reward associations and during the reversal phase to suppress previously learned responses while learning the new stimulus reward contingencies. Motivational factors, readiness to engage in new responses as well as the attention to stimulus-reward relationships can also impact upon behavioural performance.

Following initial habituation training mice were given access to the liquid reward (strawberry milkshake YAZOO tm) in the home cage for 3 days prior to the beginning of testing in the touchscreen apparatus (Campden instruments Ltd). All mice were presented with a Touchscreen consisting of 2 response panels (7.5cm X 7.5cm) where the stimulus was displayed.

S+ S-

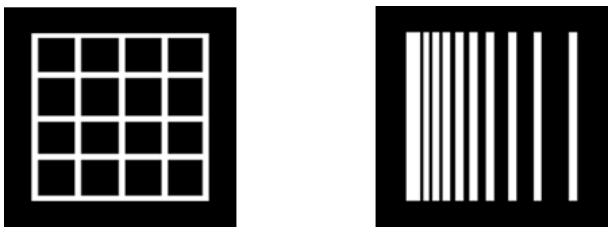

*Figure 1 shows the stimulus used for the current pairwise discrimination task rewarded stimulus = S+, punished stimulus = S-*

Initially, random images were displayed in one of the two panels with the other side left blank, until following a delay period where the image was removed and a reward delivered. If the animal touched a displayed stimulus during the presentation period the reward was delivered immediately. Touches to the blank panel were not punished. In the next stage (5 days), mice had to touch the screen before a reward was delivered, incorrect responses were not punished, while for the following 3 days, with the same procedure, an incorrect response was now punished with a 5 second house light on.
Thereafter, the mice were required to touch the panel that corresponds to the S+ reward stimulus (see Figure 1). Touches to the correct panel following stimulus presentation were rewarded with 30µl of strawberry milkshake (YAZOO$^{tm}$) delivered to the food hopper, simultaneously a reward tone was sounded that was equal in duration to the reward delivery period. Touches to the incorrect stimulus were punished with a 5 second lights on and the trial reset. All following incorrect trials were regarded as correction trials and not scored against the final percentage of total correct trials. A correct response triggered a reward delivery sequence and the next trial in the overall sequence of 30 trials was then initiated. All animals were required to complete the 30 trial set in a 60 minute period. Following acquisition of correct responding (>75% correct over 3 days) the stimulus/reward contingency of the target stimulus were switched such that the previously rewarded S+ stimulus was now punished and vice versa for the S- stimulus.

***Gambling task***
In order to assess risk/reward evaluation, a translational touchscreen-based equivalent of the Iowa gambling task (IGT) was employed. Mice were encouraged to work for a liquid reward (YAZOO$^{TM}$ strawberry milkshake) at 7µl per reward. The main touchscreen was covered by a 5x1 mask, which limited access to the touchscreen to 4 discrete apertures, with only apertures 1, 2, 4, 5 rewarded. Rewards delivered were dependent on predetermined contingencies (see Figure 2).

*Must touch training sessions*
After habituation, the stimulus (a white square) was displayed randomly in one of the 4 apertures one at a time, in a pseudo randomly chosen position, such that the stimulus was not displayed in the same position more than 3 times in a row. After a delay (30sec) the image was removed and a reward delivered (accompanied by illumination of the tray light). Entry to collect the reward turned off the tray light. Immediately following tray entry another image was displayed. If the mouse touched the screen image location whilst that image was displayed, the image was removed and 3 x food rewards were delivered immediately. Collection of this reward again started the next image. Touch training was performed with the house light off. Criterion for advancement onto the next stage was 30 trials in 30 min. All animals were trained for 2 days. Next, the white square was presented in each of the 4 stimuli grid positions (grid positions 1, 2, 4 & 5). The mouse must touch one of the stimuli to elicit a food response, while no food reward was delivered without a response. Criterion for advancement was to complete 40 responses in a 30-min period; all mice were trained for a total of 5 days of training.

*Touch basic training sessions*
The touch basic portion of training consisted of a similar method to the must touch sessions with the exception that with improvement animals are moved onto increasing levels of difficulty levels (sessions 9-12) until there are consistently completing sessions with >80% accuracy and <20% omissions over two consecutive days, criteria for advancement onto the forced choices training sessions was completion of criteria at the level of session 12. Animals completed a total of 53 sessions at this stage (Table 1).

| 'set-up' | Session 09 | Session 10 | Session 11 | Session 12 |
|---|---|---|---|---|
| Session Length | 30 min | 30 min | 30 min | 30 min |
| Stimulus Duration | 32 s | 16 s | 10 s | 10 s |
| Limited Hold | 37 s | 21 s | 13 s | 10 s |

*Table 1 shows the task parameters for progression from session 09-12 of the 'touch basic' task.*

*Forced choice stage of task*
The "forced choice" stage familiarises the mice to the reward/punishment contingencies for each response aperture. This schedule was run in darkness, with time out periods being signalled by illumination of the house light. The session began by delivering a liquid reward and turning on the tray light. When the mouse exited the food magazine the first

trial began. Following this tray release, an inter-trial interval (5sec) began, at the end of which a stimulus (white square) was presented in one of the 4 stimuli grid positions (grid positions 1, 2, 4 & 5) on the touchscreen. The sequence of presentations of the stimuli was a pseudorandom schedule such that there were 5 presentations at each spatial location within each block of 20 trials. (there was a further restriction to prevent the stimuli being presented in the same grid position more than 3 times in a row). The subject had to respond within a time period (10sec). An incorrect response, i.e. touching a location other than where the stimulus was presented, resulted in no action. Failure to respond within the limited hold period was classed as an omission. The tray light was then illuminated to signal the start of the next trial. The next trial began when the tray was entered and then exited. A correct response, touching at the location in which the stimulus is presented within the limited hold period, resulted in either a reward or punishment according to contingencies associated with a particular response aperture used in the final task (see Figure 2), all animals were given 3 days of training to learn the different response attributes.

*Free choice stage of task*

For the final, free choice, stage, mice initiate a trial by nose poking an illuminated reward tray. On leaving the reward tray the tray light was extinguished and an inter trial interval (ITI) begins (5s). If the screen was touched during the ITI, this was classed as a premature response and punished with a time out (5s), during which time the house light is reversed. At the end of the ITI white squares appeared in grid windows 1, 2, 4 & 5 of the 5 x 1 mask for 10 seconds (stimulus duration). The mouse was required to respond to one of the white squares. Failure to respond within the limited hold period was classed as an omission, at which point the white squares were removed and the tray light illuminated to signal the start of the next trial. The chance of reward, and the amount of reward delivered, varied according to which location was touched (Figure 2). All animals were given 10 days of testing to show preferred contingencies.

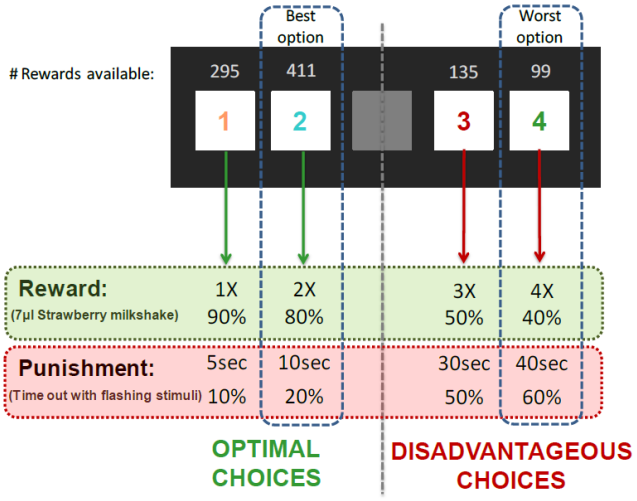


*Figure 2 Reward contingencies for each of 4 choices available during the gambling task.*

**Statistical analysis**

In all cases, data were analysed by repeated measures ANOVA with post-hoc Tukey tests, or ANOVA with post-hoc Fisher test for overall performance in the Gambling task (non-repeated measures) (Minitab or SPSS), following Box-Cox normalisation where data deviated substantially from a normal distribution.

## Results

### Working memory

In both WT and *Gpr88* KO mice, there was a 'N-back'-dependent decrease in performance with increasing task difficulty, such that wild-type mice achieved over 80% correct responding at a N-back of zero, ~60% correct responding at the more difficult N-back of 2 and closer to (although still significantly better than) chance levels of 50% performance at the most difficult 'N back levels' of 3 and 4. *Gpr88* KO mice did not achieve a similar level of responding to WT mice and showed impairments at all levels of task difficulty compared to WT mice (Figure 3).

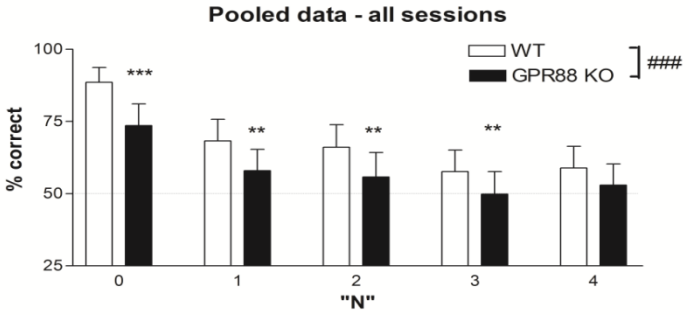


***Figure 3:*** *Gpr88 KO mice show working memory deficits in the N-back task. Results (% correct responses) at each N-back (N) level are shown as the mean ± SEM (n=10/group) over the 16 sessions of testing. ### p<0.0001 genotype main effect (ANOVA); *** pp<0.0001; **p<0.01 vs corresponding WT group, same "N"*

Investigation of performance at each of the 'N-back' levels showed at N = 0, the lowest level of task difficulty (essentially equivalent to a T-maze based non-match to sample test), *Gpr88* KO mice were significantly poorer at the task compared to wild types; at N = 0 there was a significant effect of genotype ($F_{1,18}$ = 20.37 p = 0.0001) (Figure 3). This pattern of deficiency for the *Gpr88* KO mice continued for the next three levels of the N-back task; N = 1, significant effect of genotype ($F_{1,18}$ = 7.19 p = 0.01); N = 2 significant effect of genotype ($F_{1,18}$ = 8.44 p = 0.009); N = 3 significant effect of genotype ($F_{1,18}$ = 12.79 p = 0.002). As expected, the lowest level of overall performance was observed at the most difficult N-back (N = 4), where the effect of genotype approached significance ($F_{1,18}$ = 3.290 p = 0.08) (Figure 3).

### Progressive Ratio task of motivation

### Breakpoint

*Gpr88* KO mice showed markedly higher breakpoints in comparison to WTs (main effect of genotype ($F_{1,28}$ = 24.29 p = 0.0001)) (Fig 4A). No significant effect of gender was detected. A similar pattern of results was obtained from a separate analysis of the 3 extinction trial days (Figure 4A), with a significant effect of day ($F_{2,56}$ = 15.30 p = 0.0001) and a main effect of genotype ($F_{1,28}$ = 22.06 p = 0.0001). This suggests that both groups were aware of the removal of the reward, as evidenced by the reduction in trials completed across days. Increased levels of trials completed by the GPR88 KO mice were still evident during the 3 day extinction period.

### Locomotor activity

Both male and female *Gpr88* KO mice showed elevated levels of locomotor activity in comparison to the WT mice (Figure 4B) (significant effect of day ($F_{11,286}$ = 4.31 p = 0.0001), and of genotype ($F_{1,26}$ = 28.07 p = 0.0001)). A similar pattern of results was seen in the 3 day extinction trials with overall trial rates being reduced for all groups (main effect of day $F_{2,48}$ = 23.42 p = 0.023) although the *Gpr88* KO mice showed increased locomotor activity across these days compared to WTs (main effect of genotype $F_{1,24}$ = 23.42 p = 0.0001)

### Session time

*Gpr88* KO mice sessions lasted significantly longer than those for WT mice (Figure 4C), indicating that *Gpr88* KO mice were willing to work for longer on the task before hitting a breakpoint. There was a main effect of genotype ($F_{1,26}$ = 23.57 p = 0.0001), but again no gender effects were seen for this measure. For the final 3 days of extinction trials no effect of day was seen, but the pre-existing difference between GPR88 KO and WT mice remained during the extinction trial period (main effect of genotype $F_{1,24}$ = 14.15 p = 0.001).

**Shuttle latency**

For shuttle latency, a main effect of day was seen during the first 12 days of progressive ratio testing ($F_{11,286}$ = 8.21 p = 0.0001) and a main effect of genotype ($F_{1,26}$ = 14.75 p = 0.001) with *Gpr88* KO mice producing reduced latencies to complete a single shuttle compared to the WT mice (Figure 4D). This was true for both genders. For the 3 days of extinction trials a similar pattern was seen, with a significant effect of day showing increased shuttle latencies for all groups across days ($F_{2,44}$ = 6.01 p = 0.005) and a main effect of genotype, with *Gpr88* KO mice showing reduced latencies compared to WTs.

In summary *Gpr88* KO mice showed an ability to work longer and harder, compared to control mice, in the progressive ratio task.

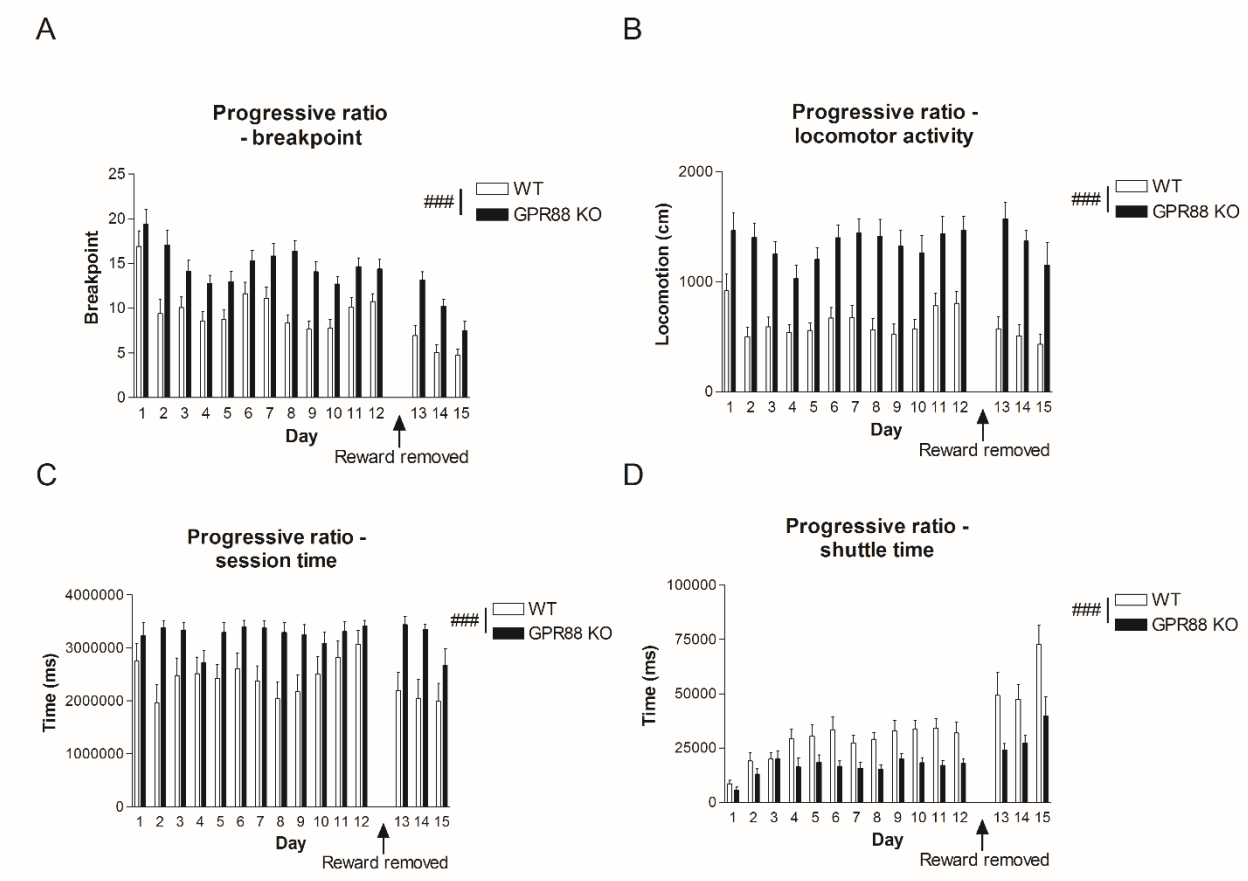


***Figure 4:*** *Gpr88 KO mice show improved motivation-like behaviour in a Progressive Ratio task compared to WT control mice. Breakpoint ratio (A), locomotion (B), session time (C) and shuttle latency (D) during the progressive ratio task, including the final 3 days of extinction trials in WT and Gpr88 KO mice. Results shown are mean ± SEM. ### p<0.001, ANOVA main effect of genotype.*

**Paired Associate learning and Reversal learning**

*Gpr88* KO mice were able to learn the Paired Associate learning task very effectively.Scores of perecentage correct responses, show that the *Gpr88* KO mice were in fact superior in performance compared to WT controls during the first block of 3 day sessions *($F_{1,14}$ = 4.54 p = 0.05)*. Following this the acquisitions rates converge, with both groups showing comparable levels of percentage correct responding over the final three 3-day blocks (Figure 5A) of discrimination learning.

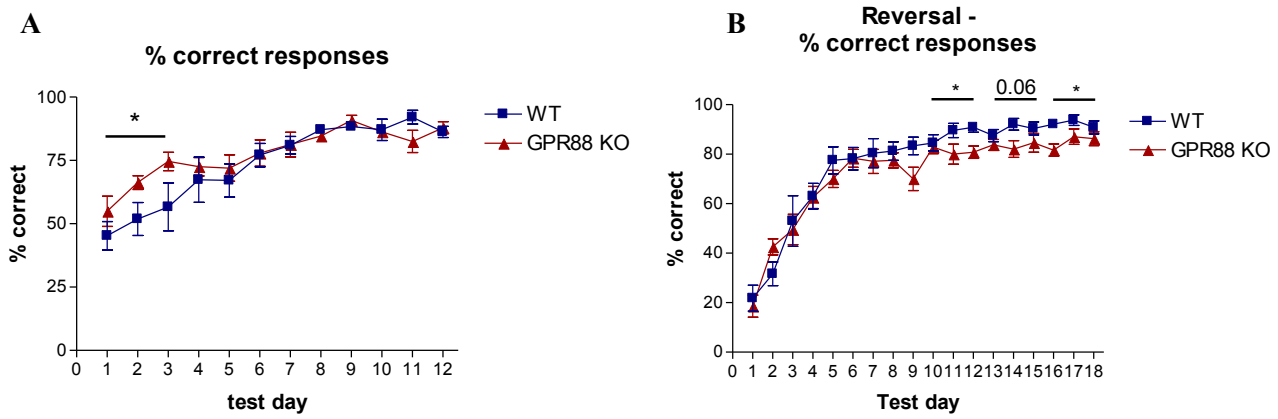


*Figure 5. Performance of WT control and Gpr88 KO mice in a paired associate learning and reversal learning task. A) Percentage correct rates over 12 days of initial discrimination learning. * ANOVA (days 1-3) effect of genotype B) Percentage correct rates in reversal phase of task, across all six 3-day test blocks. * p<0.05, ANOVA genotype effect. Where results approached significance, the relevant p value is shown. Results shown as mean ±SEM.*

As seen in Figure 5B, both groups showed the predicted reduction in percentage correct responses due to the reversal manipulation, and showed similar rates of re-acquisition during the initial 3 blocks of 3-day testing. However, as both groups approached peak performance rates, *Gpr88* KO mice however failed to reattain their pre-reversal levels of accuracy, and were significantly poorer at the task in comparison to wild types during block 4 *( F1,14 = 4.98 p = 0.04)*. This difference approached significance for block 5 (*F1,14 = 3.87 p = 0.06)* and was again present for the final block of 3 days *(block 6: $F_{1,14}$ = 5.03 p = 0.04)*, where *Gpr88* KO mice were still significantly worse at the task in comparison to wild type controls.

**Gambling Task**

**Free choice stage of task.**

This is a key stage of the task for evaluating risk/reward. A clear preference for advantageous contingencies P1 and P2 was seen at the commencement of the 10 days of free choice testing (all animals had been pre-exposed to a forced choice of these contingencies, and so were aware of their risk/reward balance). P1 choices remained stable across the 10 days (Figure 6A), with both groups slowly increasing their preference for P2 choices (Figure 6B) at the expense of P3 and P4 (Figure 6C,D) choices, which decline slightly across the 10 days. Over the full 10 day period, there were clear differences between the frequency of selection of the different options (ANOVA F(3,710 = 5.58, p=0.003; P2 vs P3 or P4, p<0.001, P2 vs P1, p=0.06, post-hoc Fisher test). *Gpr88* KO mice showed a slightly reduced preference for the 2nd best option (P1) (*F(1,179)=7.28, p=0.008)*, and an elevated preference for the riskiest, and overall least advantageous option (P4) ( *F(1,179) = 14.61, p<0.001*) (Figure 6A,D).

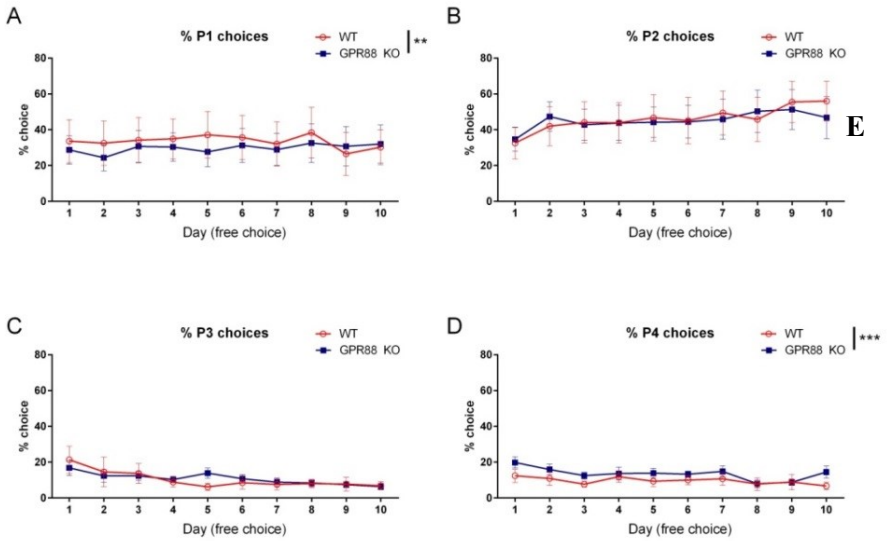


***Figure 6:*** *Gpr88 KO mice show altered risk/reward preference in the mouse Iowa Gambling Task, exhibiting a reduced preference for the 2nd best option (P1) and an elevated preference for the riskiest, and overall least advantageous option (P4).* ***A-D:*** *contingency choices across the 4 contingency options, for P1(A), P2 (B), P3 (C) and P4 (D), for each day over the 10 days of free choice testing. ** p<0.01, *** p<0.001, ANOVA main effect of genotype. Data shown as mean ±SEM; n=9/group.*

**Advantageous choices**

Using the conventional compound measure for performance (% advantageous choices - P1+P2) (Figure 7A), *Gpr88* KO mice again showed a clear impairment, with lower selection of advantageous choices (*F(1,179)=18.75, p<0.001)*.

**Premature responses**

*Gpr88* mice also commit significantly more premature errors compared to wild type mice (*F(1,107)=53.80, p<0.001)***.** This may be related to impaired inhibitory control processes but may also be linked to the hyperactivity previously noted with this genotype.

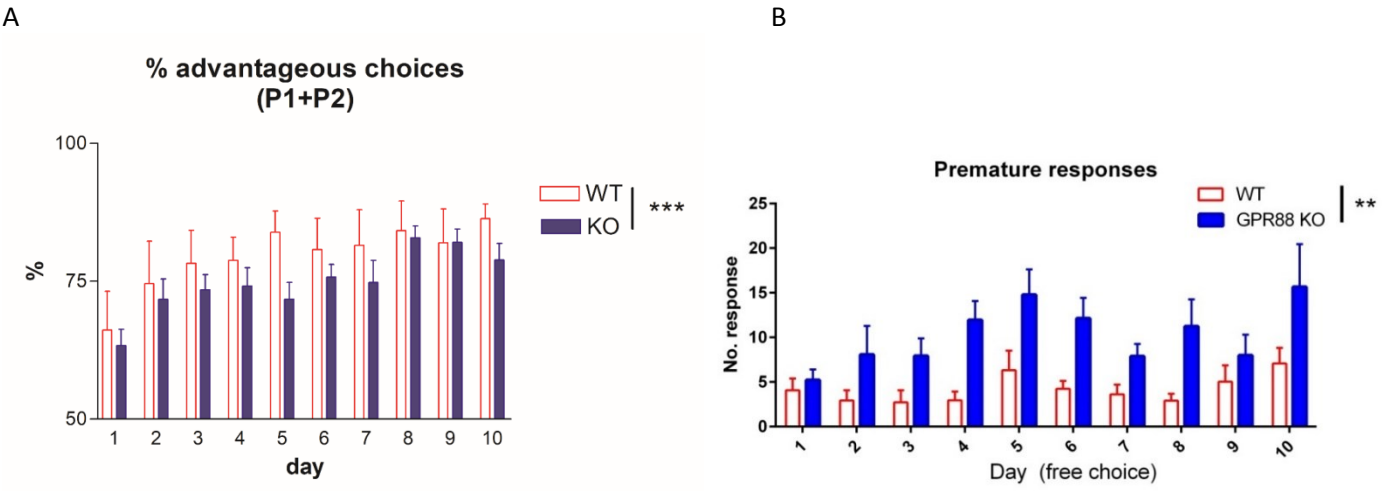


***Figure 7:*** *Performance of WT control and Gpr88 KO mice* in the mouse IOWA Gambling Task*. Advantageous choices (A) and premature response (B) over the 10 days of free choice testing, for WT and GPR88 KO mice. Data shown as mean ±SEM; n=9/group***.** **** ANOVA main effect of genotype p<0.001, ** ANOVA main effect of genotype*

**Discussion**

We demonstrate that *Gpr88* KO mice showed abnormalities across a range of cognitive domains, providing novel evidence for the involvement of GPR88 receptors in working memory, motivation, reversal learning and in the processing of reward, using a range of translational tasks. These findings are in line with neoranatomical expression patterns in rodents and humans along with human genetic data. Taken together these data demonstrate the importance of GPR88 in striatal efferent pathways and their influence on behaviours integrated by several cortico-striatal thalamic networks. Consistent with previous findings[2,24-26], we note enhanced locomotor activity in mice deficient in GPR88, implying a role in modulaton of the 'motor' cortico-striatal-thalamic loop. Importantly, we show for the first time disruption in behaviours related to cortico-striatal-thalamic loops of the orbitofrontal- anterior cingulate- and prefrontal-cortices.

**Working memory**

Working memory deficits are a robust feature of schizophrenia [38,39], but there are challenges in modelling similar processes in rodent models. Working memory paradigms are often conceptualised in different ways in humans and rodents [40]. In rodents, a common feature of working memory tasks is to assess performance deficits, as the delay over which a memory is held increases However, delay-dependent performance deficits are not convincingly observed in schizophrenia [41]. Hence it is important to select a working memory task where the animal and human characteristics overlap. One such task is the 'N-back' task which has the feature of 'interference control'. This can be defined as 'the processes involved in protecting the contents of working memory from interference from either competing representations or external stimuli' (see [40]). We therefore adopted a rodent adaption of the task that employs a radial arm maze to test performance at increasing levels of working memory load [35].

Our findings show clear reductions in working memory performance in WT and GPR88 KO mice as the working memory load is increased. While all animals were capable of performing above chance, the percentage of correct responses for the *Gpr88* KO mice was significantly lower compared to the wild type controls. This suggests that lack of functional GPR88 receptors compromises working memory function, and that *Gpr88* KO mice are potentially useful for modelling the cognitive deficits associated with psychiatric conditions . In humans, patients show robust impairments in "N back" tasks in association with hypoactivation of PFC-striatal networks [32,33]. Similarly in preclinical studies a cortico-striatal circuit incoportaing the mediodorsal thalamus is reported to subserve working memory [11]. Hence the present data suggest that GPR88 receptors may play an important role in working memory circuitry. Importantly this may be related to an impact of GPR88 in the PFC-striatal-mediodorsal thalamus cognitive loop.

**Progressive Ratio task**

Avolition is arguably the negative symptom component that is most relevant to schizophrenia. Patients are less likely to anticipate or predict that a future event will be pleasurable, and hence are less motivated to seek out pleasurable experiences [19,42].

The neurobiological mechanisms of motivational impairments in patients are believed to reflect aberrant cortical-striatal network function [43,44]. In the progressive ratio task, *Gpr88* KO mice showed higher breakpoints suggesting that they were more motivativated to obtain rewards. They also worked longer compared to the WTs. Additionally, mice had reduced shuttle time, which may imply

increased motivation. Potentially this may relate to an action of GPR88 on the medial OFC-striatal-mediodorsal thalamus loop, which is important in encoding the relative value of a stimulus [9].

We originally anticipated that *Gpr88* KO mice would show reduced motivation (analogous to the avolition that is characteristic of patients with schizophrenia[19], and hence would show lower levels of performance in this test (lower breakpoints). On the contrary, the *Gpr88* KO mice demonstated elevated breakpoints suggesting higher levels of motivation. By comparison, *Gpr88* KO mice showed increased motivation to work for an alcohol reward in an operant task but not for natural rewards and performed similarly to WTs in a progressive ratio task based upon lever presses rather than nosepokes [45,46].
However, it is possible that other factors resulting from the neurobiological dysfunction caused by lack of GPR88 preclude simple interpretation of these findings. One possibility is hyperlocomotor activity,which may have lead them to 'accidentally' nosepoke in the progressive ratio paradigm, whereas a paradigm requiring lever presses, as employed by Rainwater et al [46] may be less prone to unwanted pressing. Hyperlocomotor activity has been reported previously [2] and is likely to arise from an action of GPR88 in a corticostriatal 'motor' loop. A role of dopamine in the hyperactivity of *Gpr88* KO mice is not completely clear. Reports suggest unchanged [2,47] or decreased [24,25] DA levels in the striatum of *Gpr88* KO mice.

Another possibility is that the mice show a hyper-dopaminergic phenotype in the absence of elevated dopamine activity. This could be due to an action downstream of dopamine, for example reflecting dysregulation of striatal output pathways which are regulated powerfully by dopamine modulation of glutamatergic signalling [48]. There is indeed evidence to suggest that effects of dopamine in corticostriatal loops are mediated via GPR88 [49]. The data suggest that the endogenous agonist for the GPR88 receptor is involved in dampening dopamine activity in cortico-striato-thalamic circuits. The increased breakpoint observed in the GPR88 KO mice may be another manifestation of a hyperdopamine-like phenotype of the strain. Indeed, increased dopaminergic tone in mice, induced by dopamine transporter antagonism or amphetamine administration, also leads to increased breakpoint in this test [50-52].

During the 3 days of extinction trials in the progressive ratio task both genotypes showed a decline in breakpoint suggesting that context-dependent relearning is intact in *Gpr88* KO mice. Arguably subregions of the mPFC are important in the extinction of natural rewards[53], suggesting that this circuitry is functionally intact in *Gpr88* KO mice. Interestingly, DAT KO mice show enhanced responding in the extinction phase of a progressive ratio task compared to WT mice, indicating the importance of DA [54]. In summary, *Gpr88* KO mice show show higher breakpoints, reduced latencies and lengthened session time in a progressive ratio task consistent with enhanced motivation, although there remains the possibility that hyperactivity was a confounding factor in these behavioural outputs.

**Paired Associate Learning and Reversal learning**

The touchscreen-based pairwise discrimination/reversal learning task was used to probe possible alterations in cognitive function in *Gpr88* KO mice. In corresponding human cognitive assessments, patients with schizophrenia reportedly show impaired ability at the reversal stage, but not at the visual discrimination stage [55], although other studies detect slight impairments in simple visual discrimination as well as reversal [56,57].

*Gpr88* KO mice were able to acquire the task to similar performance levels as WT mice, albeit we observed a generally faster performance of the task relative to controls at early stages of task acquisition. In contrast, *Gpr88* KO mice showed reduced levels of performance in in the reversal stage. This is interesting considering the impaired performance of patients with schizophrenia in the reversal

phase of analogous tasks [55-57]. In this study *Gpr88* KO mice showed similar performance to WT mice in the early post reversal trials indicating that perseverative responding was intact. However, in the later post reversal trials the *Gpr88* KO mice consistently underperformed compared to WT, indicating deficits in the learning of new associations. Potentially this could suggest that the *Gpr88* KO mice are unable to maintain a new response strategy during reversal learning, consistent with deficits in dorsomedial striatal inactivation [58]. In line with these findings, *Gpr88* KO mice were slower to shift from a turn-based strategy to a cue-based strategy in a water based U-maze task [2]. However, direct comparisons of behavioural outputs from the maze and touchscreen tasks should be treated with caution as the cognitive processes to perform them may differ. The deficit observed in the present touchscreen study could be related to dysfunctional dopamine regulation of striatofugal pathways, since reversal learning in patients with schizophrenia is associated with altered striatal activation [59], and monkeys with striatum-specific depletion of dopamine also show impaired reversal learning in an analogous touchscreen task [60]. It is possible that increased dopamine activity contributes to the impaired reversal learning performance in *Gpr88* KO mice, since amphetamine impairs reversal learning in rats [61]. Taken together these data implicate an impact of GPR88 on a cortico-thalamic loop involving the lateral orbital cortex, which is well documented to play a role in reversal learning and adapting behaviour to choose a previously unrewarded stimulus [9].

The touchscreen task used here is relatively novel, and has not been widely used in mice. Hence there is only limited information available concerning reversal learning performance in other schizophrenia-related mouse models. Certainly the NMDA class of glutamate receptor plays a role, since mice lacking either the GluN2B or GluN2A NMDA receptor subunit show impairments in both acquisition and reversal stages [62,63]. AMPA receptors may also be important, since genetic deletion of AMPA receptor subunits facilitates reversal learning [64 65]. Interestingly, there is some evidence for altered (enhanced) AMPA receptor function in GPR88 mice [2], so this may contribute to the reversal learning impairment that we observe. Equally, it would be worthwhile in the future to assess whether there is a concurrent alteration in GluN2A/GluN2B receptor function in these mice.

In summary, *Gpr88* KO mice show increased movement and responding, with reduced processing time taken before a behavioural response is released, along with a performance deficit during the reversal learning phase of the task.

**Reward Evaluation -Gambling task**

In this study, *Gpr88* KO mice were tested in a translational touchscreen-based equivalent of the Iowa gambling task, which is used clinically to assess risk/reward assessment. Both genotype groups completed comparable levels of trials across the initial 'Free choice' 10 test sessions, implying that there are no discernible differences in motivation levels between WT and *Gpr88* KO mice in this test. However, response latencies were slightly slower for *Gpr88* KO mice, although reductions in time were noted for both genotypes as the animals became more competent across days in the task. Most measures yielded a significant difference according to sex, with males tending to show a slightly enhanced performance in terms of selection of advantageous choices. Similar sex difference effects have been reported in humans [66]. However, this measure did not interact with genotype, and so does not represent a confound.

While both genotype groups demonstrated a clear preference for the optimum contingency choice (P2), *Gpr88* KO mice selected more risky choices (P4) at the expense of more advantageous, lower risk options (P1). This confirmed the initial prediction, that lack of GPR88 would perturb the processing of risk/reward contingencies. Corticostriatal networks are well-known to play a powerful role in risk-related decision making and reward experience [67,68]. The ventral striatum (nucleus accumbens) in particular is involved in monitoring of reward, and dopaminergic dysfunction in this region is strongly

linked to abnormal risk-taking behaviour, pathological gambling and drug addiction. Dopamine acts in the nucleus accumbens to encode reward prediction and process information about reward value [69].

The finding that *Gpr88* KO mice select less advantageous choices implies that GPR88 in ventral striatal efferent neurones is involved in the reward-monitoring process. Rainwater et al [46] have reported that GPR88 KO mice are unable to distinguish between high reward and low reward-associated choices, indicating a profound inability to process risk/reward contingencies effectively. Our results are slightly different, in that *Gpr88* KO mice do indeed adopt a relatively effective strategy when exposed to four differing risk/reward contingencies. It is rather that the strategy that they adopt is rather more risky, and less optimal, than that adopted by WT mice. Rodent data support dopaminergic involvement in reward monitoring. Mice lacking the dopamine transporter, and hence with increased levels of synaptic dopamine, show a tendency towards suboptimal choice selection, (and also increased premature responding), in a rodent version of the IGT [70]. In a rat lever-press version of the IGT, amphetamine impairs selection of optimal choices, increasing preference for the second best option (P1) at the expense of the best option (P2) [71]. We have recently confirmed this finding in the mouse touchscreen IGT [72].

A further clear finding from this test set is the occurrence of increased premature responding in the *Gpr88* KO mice. Across the initial 10 days of free choice training *Gpr88* KO mice consistently committed more premature responses compared to wild types. This finding is unlikely to be related to overall motivational levels as both groups returned similar levels of completed trials. Increased premature responding is seen with amphetamine and other dopaminergic compounds in other operant tasks such as the 5-Choice serial reaction time task and has been linked to altered prefrontal- ventral striatal interactions (see [73]). Once again, this provides support for a hyperdopaminergic phenotype of GPR88KO mice

In summary the data demonstrate that GPR88 is involved in the processing of reward, and assessment of the cost/benefit of risky choices. Together these data suggest an action of GPR88 in OFC and anterior cingulate cortico-striatal-thalamic loops which are implicated in reward evaluation and cognitive control of these processes [9,12,15].

The clinical implications of this are not completely clear, but it may suggest a phenotype relevant to several disorders including schizophrenia (where patients also show impaired reward assessment, impulsivity, and deficits in the IGT)[74,75], attention deficit hyperactivity disorder (ADHD) (since ADHD patients show impaired reward assessment, impulsivity, and deficits in the Iowa gambling task - IGT)[76-78], or Parkinson's disease (where patients show altered reward processing which is affected by dopaminergic medication) [79,80].

Concluding comments

In conclusion, our findings of an involvement of GPR88 receptors in working memory, reversal learning, motivation and reward processing demonstrate the importance of GPR88 on striatal efferent pathways, above and beyond the classical 'motor' cortico-striatal-thalamic loop, to those loops involving the OFC, PFC and anterior cingulate cortices. It is interesting that the cognitive dysfunction in many respects phenocopies the effects of a hyperdopaminergic state, despite the evidence for increased dopamine function in this strain being somewhat equivocal. Taken together, these data demonstrate that GPR88 KO mice are a useful model to evaluate novel targets for a range of cortico-striatal mediated behaviours relevant, not only to schizophrenia, but also to disorders such as Parkinson's disease[81] and a range of other neuropsychiatric disorders[82]. ~~Finally, the present observations also suggest that GPR88 receptors justify further exploration as potential targets for the~~

~~treatment of the cognitive, mood and other symptoms of schizophrenia, Parkinsons and other neuropsychiatric disorders~~

Further studies are needed to better understand the pathophysiological and therapeutic significance of GPR88 in the mood, motor and cognitive symptoms of schizophrenia and other CNS disorders such as Parkinson's disease. Such investigations would benefit both from the identification of putative endogenous ligands of GPR88 receptors as well as the availability of GPR88-specific agonist and antagonists as pharmacological tools. However, despite reports on several promising agents like 2-PCCA, RTI-13951-33, hydroxyphenylglycine and hydroxyphenylglycinol derivatives, no highly selective and brain-penetrant with suitable for clinical development have as yet been described [83-87]. Moreover, selective antagonists are likewise eagerly awaited, although gene knockout and knockdown approaches have proven instructive for evaluating the function of GPR88 [26,47,49]; and antisense oligonucleotide strategies might be adopted for the regional neutralization of GPR88 in disorders where it is thought to be overactive.

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There are no conflicts of interest.

**Data availability.** The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Figure Legends**

**Figure 1** shows the stimulus used for the current pairwise discrimination task rewarded stimulus = S+, punished stimulus = S-

**Figure 2** Reward contingencies for each of 4 choices available during the gambling task.

**Figure 3** Gpr88 KO mice show working memory deficits in the N-back task. Results (% correct responses) at each N-back (N) level are shown as the mean ± SEM (n=10/group) over the 16 sessions of testing. ### p<0.0001 genotype main effect (ANOVA); *** pp<0.0001; **p<0.01 vs corresponding WT group, same "N"

**Figure 4** Gpr88 KO mice show improved motivation-like behaviour in a Progressive Ratio task compared to WT control mice. Breakpoint ratio (A), locomotion (B), session time (C) and shuttle latency (D) during the progressive ratio task, including the final 3 days of extinction trials in WT and Gpr88 KO mice. Results shown are mean ± SEM. ### p<0.001, ANOVA main effect of genotype.

**Figure 5** Performance of WT control and Gpr88 KO mice in a paired associate learning and reversal learning task. A) Percentage correct rates over 12 days of initial discrimination learning. * ANOVA (days 1-3) effect of genotype B) Percentage correct rates in reversal phase of task, across all six 3-day test blocks. * p<0.05, ANOVA genotype effect. Where results approached significance, the relevant p value is shown. Results shown as mean ±SEM.

**Figure 6** Gpr88 KO mice show altered risk/reward preference in the mouse Iowa Gambling Task, exhibiting a reduced preference for the 2nd best option (P1) and an elevated preference for the riskiest, and overall least advantageous option (P4). **A-D:** contingency choices across the 4 contingency options, for P1(A), P2 (B), P3 (C) and P4 (D), for each day over the 10 days of free choice testing. ** $p<0.01$, *** $p<0.001$, ANOVA main effect of genotype. Data shown as mean ±SEM; n=9/group.

**Figure 7** Performance of WT control and Gpr88 KO mice in the mouse IOWA Gambling Task. Advantageous choices (A) and premature response (B) over the 10 days of free choice testing, for WT and GPR88 KO mice. Data shown as mean ±SEM; n=9/group**.** *** ANOVA main effect of genotype $p<0.001$, ** ANOVA main effect of genotype

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