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**Gpr88 deletion impacts motivational control without overt disruptions to striatal dopamine**

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Running title: Gpr88 impacts motivation independent to dopamine

Keywords: GPR88, orphan GPCR, motivation, striatum, dopamine, touchscreen
Abstract

**Background:** Disrupted motivational control is a common—but poorly treated—feature of psychiatric disorders, arising via aberrant mesolimbic dopaminergic signalling. GPR88 is an orphan GPCR highly expressed in the striatum and therefore well-placed to modulate disrupted signalling. While the phenotype of *Gpr88* knockout mice suggests a role in motivational pathways, it is unclear whether GPR88 is involved in reward valuation and/or effort-based decision making in a sex-dependent manner, and if this involves altered dopamine function.

**Methods:** In male and female *Gpr88* knockout mice, we used touchscreen-based progressive ratio, with and without reward devaluation, and effort-related choice tasks to assess motivation and cost/benefit decision making, respectively. To explore whether these motivational behaviours were related to alterations in the striatal dopamine system, we quantified expression of dopamine-related genes and/or proteins, and used $[^{18}\text{F}]$DOPA PET and GTPγ[$^{35}\text{S}$] binding to assess pre- and postsynaptic dopamine function, respectively. **Results:** We show that male and female *Gpr88* knockout mice display greater motivational drive than wild-type mice, which was maintained following reward devaluation. Further, we show cost/benefit decision making is impaired in male, but not female, *Gpr88* knockout mice. Surprisingly, we found *Gpr88* deletion had no effect on striatal dopamine by any of the measures assessed.

**Conclusion:** Our results highlight that GPR88 regulates motivational control, but that disruption of such behaviours following *Gpr88* deletion occurs independently of gross perturbations to striatal dopamine at a gene, protein or functional level. This work provides further insights into GPR88 as a drug target for motivational disorders.


Introduction

Aberrant motivational control is a common feature of psychiatric disorders with symptoms ranging from avolition and apathy to compulsive reward-seeking (1–3). Current treatments for such symptoms are often ineffective, possess unwanted side effects or, in some instances, exacerbate motivational deficits. As such, there is a significant need for novel treatments that are effective without compliance-prohibitive side effects.

The striatum is a key integrator of cognitive, motor and limbic circuitry that collectively function to regulate motivated behaviours (4). Midbrain dopaminergic projections and glutamatergic projections from numerous cortical and subcortical areas converge onto striatal medium spiny neurons (MSNs) to form cortico-striatal-thalamic loops, critical brain circuits for controlling movement, habit formation and reward processing (5). Within the striatum, functional subdivisions are associated with distinct aspects of reward learning and decision making. The dorsolateral (or sensorimotor) striatum is responsible for stimulus-response associations and habitual behaviours, while the dorsomedial (or associative) striatum is important for response-outcome associations and goal-directed behaviour (5). Finally, the ventral (or limbic) striatum is implicated in motivation and outcome evaluation. Concerted activity across all functional subdivisions—particularly with respect to dopamine signalling—is required for intact motivational control, therefore striatal targets are well positioned to modulate various aspects of motivational dysfunction.

GPR88 is an orphan G protein-coupled receptor almost exclusively expressed in the striatum, on both D₁- and D₂-expressing MSNs (6). GPR88 expression is altered following use of drugs
of abuse, highlighting a potential role in regulating motivation and reward-related pathways (7,8). Indeed, \textit{Gpr88} knockout mice display increased alcohol-seeking and risk-taking behaviour, and increased appetitive motivation (9,10). However, it is unclear if GPR88 is involved in reward valuation and/or effort-based decision making in a sex-dependent manner, and if behavioural changes are due to maladaptations to the dopaminergic system. To address these questions, we probed the motivational phenotype of male and female \textit{Gpr88} knockout mice using the rodent touchscreen system, which offers a translational platform to measure cognitive behaviours with better alignment of preclinical and clinical test constructs and outcomes (11,12). We tested the effect of reward devaluation on progressive ratio breakpoint to assess the potential for altered reward valuation in \textit{Gpr88} knockout mice. We also assessed cost/benefit decision making in \textit{Gpr88} knockout mice in an effort-related choice task where animals were given the option of a low-effort/low-reward or high-effort/high-reward. Finally, we assessed the effect of \textit{Gpr88} deletion on the dopamine system at a gene, protein and functional level using RT-qPCR, western blotting, \textsuperscript{[\text{18}F]}DOPA PET imaging and GTP\textsubscript{γ}[\text{35}S] binding, respectively. Our work aims to clarify the role of GPR88 in motivation and reward-related behaviours and provide further validation of its utility as a target for dysfunctional motivational control in psychiatric disorders.
Material and Methods

Animals

Gpr88\textsuperscript{Cre/Cre} mice on a C57BL/6J background were obtained from Jackson Laboratories (13). Wild type (WT) and Gpr88\textsuperscript{Cre/Cre} mice were bred in-house from heterozygote crossing and used for all behavioural procedures, qRT-PCR, and western blot. Gpr88 CRISPR mice (Gpr88\textsuperscript{−/−}) were generated using CRISPR/Cas9 gene editing (Supplementary Methods) and then used for \textsuperscript{18}F]DOPA PET after being validated against behavioural data from Gpr88\textsuperscript{Cre/Cre} mice (Supplementary Figure 1). Mice had access to water and food \textit{ad libitum} and housed in a 12-hour light/dark cycle at constant temperature and humidity. All experiments were approved by The Florey Institute of Neuroscience and Mental Health Animal Ethics Committee (16-034-FINMH, 18-132-FINMH) or by the Monash University Animal Ethics Committee (17661).

The animals used for each experiment are as follows:

Cohort 1: Gpr88\textsuperscript{Cre/Cre} mice approximately 11 weeks of age at the start of touchscreen operant training (Gpr88\textsuperscript{Cre/Cre} n=12 males, n=11 females; WT littermates n=11 males, n=10 females);

Progressive Ratio, Effort-Related Choice

Cohort 2: Gpr88\textsuperscript{Cre/Cre} mice approximately 11 weeks of age at the start of touchscreen operant training (Gpr88\textsuperscript{Cre/Cre} n=12 male, n=12 female; WT littermates n=12 male, n=12 female);

Progressive Ratio with devaluation, RT-qPCR

Cohort 3: Gpr88\textsuperscript{Cre/Cre} mice 12-40 weeks (Gpr88\textsuperscript{Cre/Cre} 6 male, 5 female; WT littermates n=6 male, n=5 female); western blotting

Cohort 4: Gpr88\textsuperscript{−/−} mice 8-16 weeks (Gpr88\textsuperscript{−/−} n=7 male, WT littermates n=7 male); \textsuperscript{18}F]DOPA PET

Cohort 5: Gpr88\textsuperscript{−/−} mice 12-40 weeks (Gpr88\textsuperscript{−/−} n=6 males, n=5 females; WT n=6 males, n=4 females); striatal GTP\gamma[S] binding
**Touchscreen apparatus**

The touchscreen automated system (Campden Instruments Ltd., Cambridge UK) was used as previously described (Supplementary Methods)(14,15).

**Behavioural procedures**

For all behavioural experiments, mice were housed under a reversed light-dark cycle condition to allow testing during the active phase. Briefly, animals were food restricted to 85% of their free feeding body weight. For two days immediately prior to the beginning of operant training, mice were exposed to a small amount of the liquid reward in their home cages to prevent neophobia. Further information in Supplementary Methods. Touchscreen training and tasks protocols were adapted from (14).

*Touchscreen operant training*

Operant training was conducted as previously described (Supplementary Methods)(14).

*Progressive Ratio (PR)*

Once mice completed touchscreen operant training, they were tested on the progressive ratio schedule as previously described (14). The number of screen touches required for reward delivery now increased linearly by 4 (progressive ratio (PR) 4: 1, 5, 9, 13, 17 touches etc.) or by 8 (PR8: 1, 9, 17 touches etc.) on each trial. The session ended after 60 mins or if no screen response or magazine entry was detected for 5 mins. Mice were tested on the progressive ratio schedule for four consecutive days to ensure all mice reached and maintained baseline performance.
Progressive ratio with devaluation

After touchscreen operant training, mice from Cohort 2 underwent progressive ratio training (four days PR4, baseline). For devaluation with chow and strawberry milk, animals were individually housed and given free access to chow or milk for 30 minutes prior to the PR test session. Chow/milk was weighed before and after the 30 minutes to determine the amount each mouse had consumed. Animals were tested on PR4 for three consecutive days for each devaluation procedure, with a baseline PR4 session between interventions. Mice were then given access to chow ad libitum until free-feeding weights were established, before being tested again on PR4 for four consecutive days.

Effort related choice (ERC)

Mice were tested in the effort-related choice task as previously described (14). Animals were tested on fixed ratio schedules of 16, 32, and 5 for four consecutive sessions as above, however three pre-weighed pellets of standard rodent chow were now available during the session, having been randomly placed on the floor of the touchscreen testing chamber prior to each session. Mice therefore had the option to consume freely available chow or complete trials to receive a strawberry milk reward. Each session ended at the first of 60 minutes or completion of 30 trials. Mice were then immediately removed and uneaten chow was weighed to calculate the amount consumed. As session length varied between animals, chow consumption was indexed as grams per hour.
**Behavioural measures**

All touchscreen data was recorded using the ABET recording software (Lafayette Instrument Co, IN, USA). For progressive ratio, the main measure of interest was the animals’ breakpoint, which is the number of touches made in the last successfully completed trial. For effort related choice, the main measure was the number of trials completed and amount of chow consumed during the testing session.

**[¹⁸F]DOPA PET**

All scanning was performed at Monash Biomedical Imaging. Mice from Cohort 4 were used in the experiments (Supplementary Methods).

**Striatal GTPγ[^35S] binding**

Striatal membranes were prepared from Cohort 5 and GTPγ[^35S] binding was assessed following addition of pramipexole (Supplementary Methods).

**Quantitative real time-PCR (qRT-PCR)**

Brain tissue of naive and Cohort 2 mice was collected and expression of dopamine-related genes was quantified by qRT-PCR (Supplementary Methods).

**Western blotting**

Striatal brain tissue from Cohort 3 was collected and expression of dopamine-related proteins was quantified by western blotting (Supplementary Methods).
**Statistical Analysis**

Statistical analysis was performed using Prism version 7 or 8 (GraphPad, CA, USA). Repeated measures analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were used to analyse behavioural experiments. Student's t-test and ANOVA were used for RT-qPCR and western blot analysis. When appropriate, post hoc analysis was done using the Tukey multiple comparisons test, with a significance level set at P<0.05.

**Results**

*Gpr88 deletion increases motivation for a palatable reward*

Progressive ratio tasks have been used in both animals and humans to assess a subject’s ability to maintain responding for a reward as response requirements increase. The breakpoint, or the number of responses at which the subject stops responding, provides a measure of motivation encompassing the reinforcing properties of the reward and the point at which effort outweighs the benefit of obtaining that reward. We used a touchscreen-based progressive ratio task to assess motivation in Gpr88Cre/Cre mice, where the number of touches required to elicit a strawberry milk reward increased linearly by 4 (PR4) or 8 (PR8) each trial. We found that there was no effect of sex on average breakpoint over the test sessions (Supplementary Figure 2A), therefore male and female data were combined. Gpr88Cre/Cre mice had a significantly higher breakpoint than WT littermates at both reinforcement schedules (Figure 1), indicating greater motivation for a palatable reward. This finding reflects previous reports of increased reward-seeking behaviour in Gpr88 knockout mice (9,10).

*Gpr88Cre/Cre mice show increased motivation despite devaluation of the reward*

GPR88 has a reported role in feeding and metabolism (16), therefore the increased progressive ratio breakpoint observed in Gpr88Cre/Cre mice may be explained by a metabolic, rather than
motivational, phenotype. To investigate the dependence of food intake on the progressive ratio breakpoint measure, we tested a separate cohort of Gpr88\textsuperscript{Cre/Cre} mice on PR4 following reward devaluation, where animals were given free access to either chow or milk prior to touchscreen testing, and under free-feeding conditions. Again, we found no main effect of sex on breakpoint therefore male and female data were combined (Supplementary Figure 2B). In accordance with our earlier observations, Gpr88\textsuperscript{Cre/Cre} mice still displayed a significantly higher breakpoint than WT mice (Figure 2A). Compared to testing under food restricted conditions, devaluation (chow and strawberry milk) or free-feeding prior to PR4 sessions, decreased breakpoint across both Gpr88\textsuperscript{Cre/Cre} and WT mice as expected (Figure 2A). Despite this, Gpr88\textsuperscript{Cre/Cre} mice retained a significantly higher breakpoint than WT mice in all conditions tested (Figure 2A). Importantly, there was no significant difference in the amount of chow or milk consumed by Gpr88\textsuperscript{Cre/Cre} and WT mice prior to PR4 sessions (Figure 2B). It is noteworthy that devaluation procedures shifted levels of responding in Gpr88 knockout mice at a similar rate to WT animals, suggesting that while reward valuation processes are disrupted, they are not abolished by Gpr88 deletion. Furthermore, we found no genotype effect on IR beam breaks during habituation sessions (Supplementary Figure 3), suggesting the previously reported hyperactive phenotype of Gpr88 knockout mice (13) is not present in this context and therefore unlikely to contribute to the motivational phenotype. Together this suggests that Gpr88 plays a role in regulating motivational processing, and loss of GPR88 increases responding for a palatable reward independently of whether energy requirements are met, and does not impact mechanisms of satiety.

**Effort-related decision making is impaired in male, but not female, Gpr88\textsuperscript{Cre/Cre} mice**

Cost/benefit analysis is a critical component that drives motivated behaviour: individuals evaluate estimated costs (i.e. effort) against the estimated value of an expected reward to
optimise action selection, dysfunction of which is associated with negative symptoms of psychiatric disorders like schizophrenia (17). To next evaluate cost/benefit decision making in $Gpr88^{Cre/Cre}$ mice, we used a touchscreen-based effort-related choice paradigm in which animals could either make operant touches to receive the strawberry milk reward, or consume chow that was freely available. The expectation is that as the required effort to obtain the more preferred reward choice (strawberry milk) increases, animals will instead choose to consume the low effort choice (standard chow) (14). Here, we observed sex-dependent effects (Figure 3): at fixed ratio schedules of 16 (FR16) and 32 (FR32), male $Gpr88^{Cre/Cre}$ mice completed a greater number of trials than male WT mice, reaching significance at FR16. Interestingly, no genotype effect was observed in female mice. When the effort required for the reward was reduced to a very low level by using a FR5 schedule, all groups completed the majority of trials without significant differences between genotype or sex. As session duration varied between animals, we corrected chow consumption for time spent in the chamber. Somewhat surprisingly, despite the increased number of trials completed, male $Gpr88^{Cre/Cre}$ mice consumed the same amount of chow during testing as female $Gpr88^{Cre/Cre}$ and WT animals.

**Gpr88 deletion does not alter mRNA or protein levels of dopamine-related targets**

To determine whether the increased motivational phenotype in $Gpr88^{Cre/Cre}$ mice was associated with transcriptional changes to dopamine-related genes, we quantified expression of those involved in dopamine synthesis ($Th$, $Ddc$), signalling ($Drd1$, $Drd2$, $Drd3$, $Ppp1r1b$), transport ($Slc6a3$, $Slc18a1$, $Slc18a2$), and metabolism ($Comt$, $Maoa$, $Maob$) in the dorsal and ventral striatum using qRT-PCR (Table 1; Figure 4A, B).
In addition, we investigated genes reported to be differentially expressed in Gpr88 knockout mice (Cartpt, Rgs4)(13,16) and hypothalamic tissue, where no changes in dopamine-related genes were expected (Figure 4C). Gpr88 and Grm8 were included as positive and negative controls, respectively. Expression of Gpr88 was significantly reduced in both dorsal and ventral striatal regions, and the hypothalamus (Figure 4; multiple Mann-Whitney test with Holm-Šídák's correction for multiple comparisons; dorsal striatum $P<0.0001$, ventral striatum $P=0.007$, hypothalamus $P=0.0002$).

No significant changes were found for any of the dopamine-related genes investigated. However, Rgs4 was significantly lower in the dorsal, but not ventral striatum, suggesting that a previous finding of striatal Rgs4 downregulation in Gpr88$^{Cre/Cre}$ mice is driven by changes in the dorsal region (Figure 4; multiple Mann-Whitney test with Holm-Šídák's correction for multiple comparisons; dorsal striatum $P<0.0001$, ventral striatum $P=0.807$; (13)).

Given that mRNA expression is not always indicative of protein expression, we further investigated enzymes and transporters involved in mesolimbic dopamine signalling in whole striatal tissue. In particular, we quantified levels of tyrosine hydroxylase (TH), amino acid decarboxylase (AADC), dopamine transporter (DAT), and monoamine oxidase A and B (MAO-A, MAO-B) by western blotting. Consistent with results from qRT-PCR, we found no changes in expression of dopamine-related proteins in Gpr88$^{Cre/Cre}$ mice, with respect to WT mice (Figure 5, Supplementary Figure 4; RM two-way ANOVA, genotype $P=0.948$).
Striatal dopamine synthesis capacity and dopamine D₂/D₃ receptor function are unchanged in Gpr88⁻/⁻ mice

While no genotype-dependent differences were found in the expression of dopamine-related genes and proteins, an obvious limitation to these techniques is that they do not provide functional information. In order to address this, we investigated dopamine synthesis capacity using [¹⁸F]DOPA PET and dopamine D₂/D₃ receptor function using GTPγ[³⁵S] binding.

[¹⁸F]DOPA PET is commonly used in clinical studies and provides a composite measure of presynaptic dopamine function. Striatum and cerebellum uptake of [¹⁸F]DOPA was corrected for bodyweight and radiotracer dose, and specific striatal uptake was calculated by subtracting the cerebellum time course from striatal time course (18). Specific striatal [¹⁸F]DOPA uptake was unchanged in Gpr88⁻/⁻ mice (Figure 6A). Similarly, dopamine synthesis capacity, indexed as Kᵢ₆₅, was not significantly different between WT and Gpr88⁻/⁻ mice (Figure 6B). The Kᵢ₆₅ values obtained are lower than previously reported, likely due to slight variations in experimental processes (19).

Having established that presynaptic dopamine content is unchanged, we studied post-synaptic dopamine D₂/D₃ receptor function in striatal membranes prepared from WT and Gpr88⁻/⁻ mice by GTPγ[³⁵S] binding. We found there was no effect of sex on GTPγ[³⁵S] binding, therefore male and female data were combined. The dopamine D₂/D₃ receptor agonist, pramipexole, stimulated GTPγ[³⁵S] binding in a concentration-dependent and biphasic manner with potencies for the two phases of approximately 100 nM and 5 μM in membranes from both WT and Gpr88⁻/⁻ mice (likely reflecting multiple Gαᵢ/o coupled receptor subtypes being activated by pramipexole in the native preparation; Figure 6C). Notably, there was no significant effect of genotype on pramipexole potencies.
Discussion

In this study, we investigated the effect of Gpr88 deletion on motivational behaviour and associated changes to the striatal dopamine system. We report that male and female Gpr88Cre/Cre mice display increased motivation for a palatable reward, which is maintained following reward devaluation and not driven by the metabolic phenotype previously reported in Gpr88−/− mice. Interestingly, we found that Gpr88 deletion affects cost/benefit decision making in a sex-dependent manner, whereby male, but not female, Gpr88Cre/Cre mice display a high-effort bias. Given the observed behavioural phenotypes are sensitive to manipulation by dopaminergic drugs, we hypothesised that changes in motivation may be driven by underlying changes to striatal dopamine, but somewhat surprisingly found no gross alterations. Together this work delineates the effect of Gpr88 deletion on motivation and reward-related pathways, but highlights the disruption of these behaviours occurs independently of major perturbations to striatal dopamine at a gene, protein or functional level.

Dopamine signalling in the striatum is heavily implicated in reward and motivation-related pathways. In progressive ratio tasks, both D1 and D2 antagonists reduce breakpoint, while inhibiting dopamine reuptake or increasing dopamine release increases breakpoint (20–22). Despite the clear effect of Gpr88 deletion on increasing the breakpoint in the progressive ratio task, we did not identify any changes to striatal dopamine at a gene, protein or functional level. Notwithstanding, there is some evidence to suggest that GPR88 deletion may indirectly potentiate dopamine signalling. First, Gpr88 deletion increases excitability of both D1 and D2 GABAergic MSNs, which account for ~95% of the striatal neuronal population (13). MSNs form reciprocal connections with midbrain dopaminergic neurons, and project via the direct D1-expressing striatonigral pathway, or the indirect D2-expressing striatopallidal pathway.
These pathways are embedded in broader cortico-striatal-thalamo-cortical loops that regulate a range of behaviours, including motivation (24,25). While activity of dopaminergic projections to the striatum may remain unchanged in Gpr88 knockout mice, supported by normal dopamine synthesis capacity (Figure 6B) and postsynaptic dopamine receptor function (Figure 6C), it is possible that increased excitability of MSNs leads to downstream potentiation of dopamine signalling.

Second, we confirm previous reports that Gpr88 deletion downregulates striatal expression of RGS4, and found this was specific to the dorsal striatum (Figure 4A)(13). RGS proteins are critical regulators of GPCR function, effectively switching off signalling following receptor activation (26). RGS4 acts at G\textsubscript{i}-coupled receptors (27), suggesting Gpr88 deletion may selectively increase the magnitude and duration of D\textsubscript{2} receptor signalling, therefore leading to an imbalance in D\textsubscript{1} vs D\textsubscript{2} signalling pathways (28). It should be noted that RGS4 is expected to have minimal effect on D\textsubscript{2/3} GTP\gamma\textsubscript{S} binding due to the use of membrane preparations. Given that both D\textsubscript{1} and D\textsubscript{2} receptor antagonists reduce breakpoint in progressive ratio tasks, it is likely that a balance in D\textsubscript{1}/D\textsubscript{2} receptor signalling, rather than signalling at either receptor per se, is critical for appropriate motivational control (20). This balance in D\textsubscript{1}/D\textsubscript{2} receptor signalling is not exclusively important for regulating motivation, but has been shown for a number of behaviours including working memory and locomotor activity (29,30). Indeed, Gpr88 knockout animals show increased sensitivity to the locomotor-inducing effects of amphetamine, suggesting an imbalance of striatal dopamine D\textsubscript{1}/D\textsubscript{2} receptor signalling may be at play (31).

Although our results show Gpr88 deletion may indirectly potentiate dopamine signalling to regulate motivational control, a number of caveats follow. First, it is not possible to rule out
phasic state-dependent alterations in striatal dopamine release, which is important for encoding aspects of reward value (37), and was not able to be captured by the functional measures used in the present study. However, given that GPR88 is not expressed in dopaminergic neurons, it is perhaps more likely that MSNs represent the main locus of dysfunction in Gpr88 knockout mice. While we did not investigate postsynaptic D1 function in Gpr88 knockout mice, our findings of decreased RGS4 expression and studies of conditional Gpr88 deletion in D1 or D2 MSNs (32) suggest that the greatest effect is in D2 MSNs. It should also be noted that GPR88 is expressed, albeit to a lesser degree, in extra-striatal regions such as the amygdala, thalamus and hypothalamus (33) therefore disruptions involving these regions may also contribute to the motivational phenotype of Gpr88 knockout mice. Finally, there may be an additional layer of neurodevelopmental effects in the constitutive Gpr88 knockout that could be contributing to the behavioural phenotypes observed.

While the progressive ratio task provides a basic measure of reward valuation, the effort-related choice task provides better insight into cost/benefit decision making given the competing choice between a low effort/low reward or high effort/high reward. In this task, male, but not female, Gpr88Cre/Cre mice had a higher breakpoint than WT mice indicating a high effort/high reward bias (Figure 2A). Increased synaptic dopamine and antagonism of the adenosine A2A receptor have been shown to shift preference towards the high effort reward (22,34,35). Interestingly, these pharmacological manipulations simultaneously decrease intake of the low effort reward, whereas male Gpr88Cre/Cre mice consume the same amount of chow as wildtype animals (Figure 2B). This shows that both male and female Gpr88 knockout mice engage with the low effort/low reward choice at the same level, while preference for the high effort/high reward is selectively disrupted in male Gpr88 knockout mice. The mechanisms underlying these sex-dependent impairment of cost/benefit decision making in Gpr88Cre/Cre mice is largely
unclear, but may be related to the metabolic phenotype of \( Gpr88 \) knockout mice - with which males display a more pronounced phenotype (16).

An important consideration in our work, and related approaches, is that we used free-operant behavioural paradigms of reinforcement learning that involve food restriction, which affects motivation in its own right (Figure 2A). In the case of \( Gpr88 \) knockout mice, the interplay between motivation and energy requirements is of particular interest as GPR88 is expressed in the hypothalamus and has an established role in feeding, body composition and energy expenditure (16). Lau et al. report that \( Gpr88 \) knockout mice have reduced spontaneous food intake and energy expenditure, with respect to WT mice, without changes to body weight gain or physical activity (16). Surprisingly, we observed no significant differences in food intake between \( Gpr88^{Coe/Coe} \) and WT mice (Figure 2B; 3B), but found that female, but not male, \( Gpr88^{Cre/Cre} \) mice had a consistently lower body weight than WT mice both before and during behavioural procedures (Supplementary Figure 5). Together with reports of reduced body weight in male \( Gpr88 \) knockout mice, this suggests genotype effects on weight may be sensitive to environmental factors (36). Indeed, \( Gpr88 \) deletion reportedly increases fasting-induced food intake under a high-fat, but not normal chow diet, highlighting a complex role of GPR88 in the maintenance of energy homeostasis. We found that the increased motivation in \( Gpr88 \) knockout mice occurred independently of food intake, however it is unclear exactly how the combination of food restriction and strawberry milk reinforcement interacts with energy homeostasis in \( Gpr88 \) knockout mice, and therefore how it may influence appetitive motivation.

Collectively, we identified that GPR88 regulates motivational control of behaviour, but that disruption of these behaviours following \( Gpr88 \) deletion occurs independently of gross
perturbations to striatal dopamine at a gene, protein or functional level. Our study provides further insights for targeting GPR88 to address motivational and mood symptoms in neuropsychiatric disorders. While only speculative at this stage, our findings suggest a GPR88-specific antagonist may alleviate mood symptoms without the side effects associated with overt manipulation of dopaminergic pathways.
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Disclosures

CMLC is a full-time employee of Les Laboratoires Servier. All other authors report no biomedical financial interests or potential conflicts of interest.
References


Table 1.

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<tr>
<td>Ppp1r1b</td>
<td>Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa</td>
<td>Integrator of striatal neurotransmission</td>
</tr>
<tr>
<td>Rgs4</td>
<td>Regulator of G protein signalling 4</td>
<td>Inhibits signal transduction</td>
</tr>
<tr>
<td>Grm8</td>
<td>Metabotropic glutamate metabotropic receptor 8</td>
<td>Negative control</td>
</tr>
<tr>
<td>Gpr88</td>
<td>G protein-coupled receptor 88</td>
<td>Positive control</td>
</tr>
</tbody>
</table>
Table Legend

Table 1. Description of genes and proteins quantified by qRT-PCT and/or western blotting.

Figure Legend

Figure 1. Gpr88 deletion increases motivation towards a palatable reward at progressive ratio schedules of (A) 4 (determined by Mann-Whitney test, $U=107.5$; $P=0.0012$) and (B) 8 (determined by unpaired t test, $t=2.411$, $df=42$; $P=0.0204$). **$P<0.01$, *$P<0.05$; $n=21-23$ (WT males $n=11$, females $n=10$; Gpr88Cre/Cre males $n=12$, females $n=11$). Individual data points presented with mean ± SEM. PR4, progressive ratio 4; PR8, progressive ratio 8.

Figure 2. (A) Gpr88Cre/Cre mice show increased motivation compared to WT mice following reward devaluation with chow ($t=5.607$, $df=45.89$), milk ($t=4.120$, $df=42.49$) and free-feeding ($t=5.794$, $df=40.99$). ***$P<0.001$, ****$P<0.0001$ determined by RM two-way ANOVA with Geisser-Greenhouse correction and Šídák’s multiple comparisons test. Main effect of Devaluation, $F (2.099, 96.54) = 130.6$; $P<0.0001$; Genotype, $F (1, 46) = 42.46$; $P<0.0001$; Devaluation x Genotype, $F (3, 138) = 3.39$; $P=0.0199$. (B) Consumption of chow and milk prior to testing does not significantly differ between Gpr88 knockout and WT mice. RM two-way ANOVA with Šídák’s multiple comparisons test; chow ($t=0.06545$, $df=92$; $P=0.997$), milk ($t=1.842$, $df=92$; $P=0.133$). $n=24$ (WT males $n=12$, females $n=12$; Gpr88Cre/Cre males $n=12$, females $n=12$). Individual data points presented with mean ± SEM. FF, free feeding.
Figure 3. (A) Effort-related decision making is impaired in male, but not female, Gpr88<sup>Cre/Cre</sup> mice when effort is high (RM two-way ANOVA with Geisser-Greenhouse correction and Tukey’s multiple comparisons test, Schedule x Group, $F(6, 80) = 3.064, P=0.0095, *P<0.05$) (B) despite equal consumption of chow across all fixed ratio schedules (RM two-way ANOVA, Schedule x Group, $F(6, 80) = 0.9428, P=0.4694$). Individual data points presented with mean ± SEM; n=10-12.

Figure 4. Expression of dopamine related genes is unchanged in Gpr88<sup>Cre/Cre</sup> mice in both the (A) dorsal and (B) ventral striatum, and (C) hypothalamus. **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ determined by multiple Mann-Whitney tests with Holm-Šidák's correction for multiple comparisons; dorsal striatum and hypothalamus n=13-14; ventral striatum n=8-9. Individual data points presented with mean ± SEM.

Figure 5. Striatal expression of dopamine-related proteins is unchanged in Gpr88<sup>Cre/Cre</sup> mice (multiple Mann-Whitney tests with Holm-Šidák's correction for multiple comparisons; AADC $U=51, P=0.9256$; DAT $U=58, P=0.9407$; MAO-A $U=50, P=0.9407$; MAO-B $U=49, P=0.9257$, TH $U=47, P=0.9165$; n=11). Individual data points presented with mean ± SEM.

Figure 6. (A) Gpr88 deletion does not affect striatal dopamine synthesis capacity (unmatched t-test, $t=0.8822, df=12, P=0.395; n=7$) or (B) striatal uptake of [18F]DOPA (RM two-way ANOVA with Geisser-Greenhouse correction, Genotype, $F(1, 12) = 0.6396, P=0.439; n=7$). (C) Striatal dopamine D<sub>2</sub>/D<sub>3</sub> receptor function is unchanged in Gpr88<sup>−/−</sup> mice (F-test, $P=0.75; n=10-11$). Data presented as mean ± SEM.