A role for reward sensitivity in the serotonergic modulation of impulsivity

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Abstract

While the neural substrates of impulsive behavior are commonly studied in humans and preclinical models, the behavioral substrates which contribute to impulsivity are still understudied. Understanding the behavioral underpinnings of impulsive behavior will allow us to better model disorders of impulsive behavior in animals, and also help more clearly define the underlying neural circuits. Our goal here was to explore behavioral correlates and effectors of impulsive behavior, using a mouse model for disordered impulsivity, namely mice lacking the serotonin 1B receptor (5-HT_{1B}R). Our past work, along with others', implicates 5-HT_{1B}R in the regulation of impulsivity, specifically, impulsive action. In mice, the absence of 5-HT_{1B}R expression in adulthood results in a reduced ability to wait or withhold responses. We report here, that in addition to increased impulsive action, mice lacking expression of 5-HT_{1B}R show increased goal-directed responding and motivation, with no differences in extinction, development of habitual behavior, or impulsive choice measured in a delay discounting paradigm. Interestingly, mice lacking 5-HT_{1B}R also show increased hedonic responses to sweet rewards. Finally, using a newly developed paradigm, we report that increasing reward value increases impulsive action on a trial-by-trial basis, showing how changing reward value can directly influence impulsive behavior. Taken together, these data support the hypothesis that the effects of 5-HT_{1B}R on impulsive action reflect enhanced reward sensitivity, and point to potential neural and phenotypic causes for clinically-relevant increases in impulsivity.

Introduction

Impulsivity is a multi-dimensional behavioral construct that is dysregulated in a number of psychiatric disorders [1-3]. Individual dimensions of impulsive behavior, for example impulsive action and choice, are likely mediated by different behavioral/cognitive processes with different neurobiological substrates [3-6]. Consistent with this view, processes such as reward sensitivity, compulsivity, motivation, attention deficits, novelty-seeking, and anxiety have all been associated with impulsivity [7-13]. Some of these relationships have been determined using trait-level behavioral measures in humans (particularly in psychiatric populations), and others have looked at the relatedness in preclinical models. Understanding how these behavioral and emotional states are associated with different components of impulsive behavior will lead to an understanding of the behavioral/cognitive scaffolding and associated underlying neural circuits which lead to impulsive behavior.

Our previous work has examined how different dimensions of impulsivity relate to locomotor activity and motivation in mice [4]. Specifically, we showed that impulsive action (withholding or delaying responses) was independent, behaviorally and biologically, from impulsive choice (discounting future or risky options). Factor analysis of a large number of mice tested in behavioral assays of impulsive action (go/no-go and differential reinforcement of low-rate responding) and impulsive choice (delay discounting and probability discounting) showed that there were unique sources of variability underlying these two dimensions of impulsivity. Furthermore, using a mouse model of disordered impulsive action [14], we showed that there were dissociable biological determinants of impulsive action and choice. Specifically, we and others have shown that an absence of the serotonin 1B receptors (5-HT_{1B} R) results in deficits in impulsive action but not impulsive choice [4,15,16]. Our goal in the present study was to better specify the behavioral/cognitive processes that contribute to impulsivity.

Here, we explored the effect of 5-HT_{1B}R on potential substrates of impulsivity including goal-directed responding, motivation, habitual-like responding, and reward sensitivity. While each of these can be conceptualized as unique behavioral phenotypes with distinct neural substrates, our goal was to investigate how alterations in impulsive action could potentially be subserved by altering these processes. By assessing these phenotypes in a mouse model for pathological impulsivity (absence of 5-HT_{1B}R), we were able to test the hypothesis that the effects of serotonin on impulsivity are be mediated by one or more of these behavioral or cognitive mechanisms. Since the behavioral effect of the absence of 5-HT_{1B}R is limited to the impulsive action, rather the impulsive choice domain, this model is a good system to reveal how associated phenotypes are related to different domains of impulsivity [5]. Coming to a better understanding of the specific neural *and* behavioral substrates of different dimensions of impulsivity will help us understand how these components combine to generate dysregulated impulsivity in psychiatric disorders.

Methods

Mice

Animals were bred in the Center for Comparative Medicine at Dartmouth College, or in the Department of Comparative Medicine at the New York State Psychiatric Institute. All mice were weaned at postnatal day (PN) 21 into cages of 2-5 same sex littermates on a 12:12 light-dark cycle, and maintained on ad lib chow until experimental operant behavioral testing began at 10-14 weeks. The floxed tetO1B mouse model was used to generate groups of mice lacking expression of 5-HT_{1B} through crosses to a BActin-tTS mouse line (tetO1B+/+ females crossed to tetO1B+/+::BActin-tTS+ males), as previously reported [14]. In the variable-value Go/No-Go paradigm, only tetO1B+/+ mice were used. In all other studies, tetO1B::BActin-tTS+ mice and their littermate controls- tetO1B::BActin-tTS- mice were used. For the adult rescue groups, tetO1B::BActin-tTS+ mice were fed chow with doxycycline (40mg/kg, BioServ) beginning at PN60. One group of male (N= 23) and female (N=18) mice were used in goal-directed behavior, extinction, concurrent choice, and satiety-induced devaluation experiments. A subset of these animals were used in impulsivity assays (N=12 tetO1B::BActin-tTS-; N=8 tetO1B::BActin-tTS+) with no adult rescue group. A separate group of mice (N=19), all female [n=10 control (6 tetO1B+/+ and 4 tetO1B+/+::βactin-tTS-) and n=9 tetO1B+/+::βactin-tTS+] were used to test reward sensitivity by evaporated milk consumption. A naïve group of mice (males N=6, females N=5) was used to reward sensitivity in the lickometer (N=6 tetO1B+/+::BActin-tTS-, N=5 tetO1B+/+::BActin-tTS+). Finally, another naïve group of 12 tetO1B+/+ mice (males N=7, females N=5) was used in the variable value Go/No-Go paradigm.

Operant Behavioral Apparatus

Operant studies were conducted in eight identical chambers (Med Associates, St. Albans, VT) individually enclosed in ventilated isolation boxes. Each operant chamber consisted of stainless steel modular walls, and stainless steel bar floors. Each chamber contained a noseport receptacle for the delivery of liquid reward by a dipper (0.02ml cup volume), with head entry detected by an infrared beam break detector. On either side of the noseport, the chamber contained two ultra-sensitive retractable stainless steel levers placed 2.2 cm above the chamber floor. In paradigms in which only one of the two levers was used, the lever was counterbalanced across mice and remained the same throughout all paradigms. There were LEDs located above each lever, and a houselight and speaker located on the upper portion of the wall opposite the levers. A computer equipped with MED-PC IV (Med Associates Inc., St Albans, VT) computer software delivered stimuli and collected behavioral data.

Operant Behavioral Training

Operant training and testing were run 5-7 days a week. Mice were maintained at approximately 90% of their free-feeding weight. Water was provided ad libitum throughout the experiment. All mice were first trained to retrieve an evaporated milk reward through head entry into the receptacle, and then trained to press one of the two retractable levers to receive the evaporated milk reward on a continuous reinforcement (CRF) schedule. Daily sessions ended when mice received a maximum of 60 rewards, or after 60 minutes elapsed if the maximum had not been reached. Mice were trained until

the criterion of 55 lever presses in a 60 minute session was reached. The mice were then trained on a random ratio (RR) schedule of escalating effort requirements (3 days of RR5, 3 days of RR10, 3 days of RR20).

Progressive Ratios of Responding

Following random ratio testing, mice were tested on a progressive ratio (PR) schedule for three consecutive days. A PRx2 schedule was used in which the number of lever presses required to receive a reward doubled following each reward. The session ended following either 2 hours, or a 3 minute period in which no lever presses were recorded [17]. The total number of lever presses summed over the session. The total number of lever presses rather than break point was used in the factor analysis to provide a continuous rather than categorical variable. One mouse was excluded from analysis due to technical problems with the operant box.

Extinction Testing

Mice were exposed to an RR20 schedule of reinforcement for 3 days, before being tested in three consecutive days of extinction training. Mice were placed in the operant box with the lever extended, however rewards were not administered. Lever presses and head entries were recorded for the duration of the 60 minute extinction sessions.

Concurrent Choice

Following 3 days of RR20 schedule of reinforcement, mice were placed in the operant box on each of two days with either freely available chow pellets or freely available evaporated milk in a cell culture dish. The lever of the operant box was also extended, and was rewarding the mice with evaporated milk on a RR20 schedule. These chow and milk conditions were counterbalanced across mice over the two days separated by a no choice RR20 schedule day. Chow pellets and the dish of evaporated milk were weighed before and after the test session. Lever presses and head entries were recorded during the 60 minute session.

Satiety-induced devaluation

Following 3 days of RR20 schedule of reinforcement, mice were prefed either chow or evaporated milk on each of two days, counterbalanced across mice. Mice were placed individually in a holding cage similar to their home cage for 1h, and were free to consume an unlimited amount of either chow or evaporated milk presented in a cell culture dish. Chow pellets, the dish of evaporated milk, and the mice were weighed before and after the hour-long prefeeding session. Mice were then placed in the operant box and allowed to lever press for a RR20 schedule of reinforcement. Lever presses and head entries were recorded during the 60 minute session.

Go/No-Go

Mice were trained and tested as previously described [14]. Briefly, following training on Go Trials, mice were presented with 7 daily sessions consisting of 30 discrete Go trials and 30 No Go trials which were pseudo-randomly presented across blocks of 10 trials with a variable ITI averaging 45s. In No-Go trials, the lever was presented simultaneously with 2 cues (the house lights turning off, and a small LED light above the lever turning on). A lever press during the 5 second trial caused the lever to retract, the house lights to turn on, the LED light to turn off and a new ITI to begin without any reward for that trial.

A lack of presses during the 5 sec trial resulted in a reward presentation. The impulsivity index was calculated by subtracting the proportion of correct no go trials from the proportion of correct go trials.

Delay Discounting

Mice were trained and tested as previously described [4]. Briefly, following training mice were presented with two levers for which presses resulted in either small or large (3x volume) rewards. The large reward was assigned to the lever which was initially least preferred by the mice, and remained consistent throughout the paradigm. Each daily session began with 10 forced choice trials (five on each lever randomly distributed) to ensure a minimum experience with each lever in each session, before presentation of 20 experimental choice trials. Mice were trained on 10 sessions with no delays on either lever. Subsequently, a delay was introduced after the large reward lever was pressed, before the reward was presented. There was no delay for the small reward and time delays for the large reward (0, 2, 4, 6, 8, or 10 s) were presented in separate sessions with 3 days for each time delay, in ascending delay order. Data were used from the last session of each time delay to allow for learning of the new contingency.

Reward consumption

Prior to testing in this paradigm, mice were previously exposed to evaporated milk in both consumption tests and 13 weeks of operant behavioral testing under food restriction (as described above) rewarded with 100% evaporated milk in a variety of reinforcement paradigms (data not shown). For the reward testing, mice were placed individually in a cage and given 5 minute free access to a small cell culture dish (Falcon, 35mmx10mm) with varying concentrations of evaporated milk in a separate clean cage identical to their home cage. Milk concentration was varied across 5 days of testing, with 33%, 66%, 100%, 66%, and 33% on each day respectively (data was only analyzed for first 3 days because of anchoring effects on the descending concentration presentation). Mice were weighed immediately before and after testing to determine milk consumption during the session. Change in mouse weight was used to assay consumption.

Lickometer

A Davis Rig 16-bottle Lickometer (Med Associates MED-DAV-160M) was used to test the effect of genotype on reward sensitivity to various concentrations of sucrose in sated and fed conditions as described previously [18]. Mice were water restricted for 5 days of initial training, in which mice were allowed to freely drink water from the spout in the behavioral testing apparatus. Subsequently, mice were maintained on *ad libitum* water, and exposed daily to sucrose in the testing chamber. Licking behavior was recorded for 30 minutes in each of 4 conditions: sated (ad lib food and water) or hungry (18h food deprived with ad lib water); and 2% or 10% sucrose. Each condition was run twice on consecutive days, and data averaged across the two days. Number of licks over the whole session, and lick rate for the first 2 minutes were analyzed.

Variable Value Go/No-Go Paradigm

To assess the effect of reward value on impulsive action, we developed a novel paradigm based on the Go/No-Go Test of impulsive action. Mice were trained as described in Operant Behavioral Training, except CRF training took place with both levers extended such that pressing either lever provided reward. All mice initially sampled each lever. Training continued for 6 days, by which point all mice had

formed stable and strongly biased lever preferences, which was determined based on average percentage of presses during the final three days of CRF training (range: 77% to 100%). In order to cause a reversal of their preference, the less preferred lever was then rewarded with three times the amount of evaporated milk reward compared to the more preferred lever, which remained at 0.02ml evaporated milk. In order to deliver the larger, 0.06ml reward, the dipper was activated three times in short succession, as previously described. In these reversal sessions, mice were presented with 10 forced choice trials (5 per lever) in which only one lever was extended until the lever was pressed (requiring them to sample each lever), followed by 20 choice trials in which both levers were presented. After 13 sessions, mice were choosing the high reward lever 62 ± 4 % of the time. Next mice were exposed to 5 sessions in which only Go trials were presented on each lever. 60 trials were presented in each session, with 30 trials presented on each of the large and small reward levers randomly in blocks of 10 trials. In all trials, the lever extended for 5 sec. A press within 5 seconds initiated reward delivery, and lever retraction ("Successful Go Trial"). Otherwise, the lever retracted after 5 seconds and no reward was delivered ("Unsuccessful Go Trial"). Finally, mice were exposed to 8 sessions in which No-Go trials were added such that there were 16 Go and 16 No-Go trials on each lever (64 total trials/session). In No-Go trials, the lever was presented simultaneously with 2 cues (the house lights turning off, and a small LED light above the lever turning on). A lever press during the 5 second trial caused the lever to retract, the house lights to turn on, the LED light to turn off and a new ITI to begin without any reward for that trial ("Unsuccessful No-Go Trial"). A lack of presses during the 5 sec trial resulted in a reward presentation ("Successful No-Go Trial"). Impulsivity index was calculated for small and large reward levers by subtracting the proportion of Successful No-Go trials from the proportion of Successful Go trials.

Statistical Analysis

Group effects were evaluated using analysis of variance (ANOVA), with post hoc Fisher's PLSD in StatView (SAS Software, Cary, NC) or SPSS (IBM, Armonk, NY). One way ANOVAs were used to assess the effects of 5-HT_{1B}R (control, no expression, rescued expression) on RR, concurrent choice, and devaluation. Repeated measures ANOVAs were used to assess the effects of 5-HT_{1B}R on progressive ratio, extinction, impulsivity and reward sensitivity; and the effect of reward value on impulsivity in the variable value go/no-go paradigm. Two way ANOVAs were also used to assess the interaction of sex with these variables. There were no significant effects of sex on the behaviors measured, and therefore the sexes are combined for all analyses presented.

Results

Mice lacking 5-HT_{1B} receptor expression showed increased lever pressing on random ratio and progressive ratio schedules, which was reversed by adult rescue of receptor expression. Specifically, an absence of 5-HT_{1B}R throughout life resulted in increased number of presses on RR5 and RR20 schedules (Fig 1A; $F_{2, 40}$=5.6, p<0.01 for RR5; $F_{2, 40}$=3.5, p<0.05 for RR20), with adult rescue of receptor expression returning behavior to the intact phenotype (ps<0.05 for control and adult rescue versus 5-HT_{1B}KO, ps>0.05 for control vs adult rescue). The absence of 5-HT_{1B}R also increases motivation as measured by a

progressive ratio schedule. Here, tested over three consecutive days, there was a significant effect of 5-HT_{1B} R expression on lever pressing over the three days ($F_{2, 37}$=3.4, p<0.05). In the three consecutive days of PRx2 schedule, all mice decreased their presses over days ($F_{2, 76}$=46.7, p<0.001 for main effect of day; $F_{4,74}$=5.50, p<0.001 for genotype x day interaction). Interestingly, the effect of 5-HT_{1B} on lever pressing was only present on the first day ($F_{2, 37}$=6.0, p<0.01), and not on the second or third days ($F_{2,37}$<1.5, p>0.05).

One interpretation of the increased responding in the PR is that 5-HT_{1B}R KO mice show slower extinction since persistence in the PR involves an increasing number of non-reinforced responses within each session. To test this idea, we directly measured extinction of lever pressing behavior in non-rewarded sessions following RR20 training. Over three days of extinction sessions, all mice decreased lever pressing (Fig 1C; $F_{2, 76}$=137.4, p<0.001), however, there were no effects of 5-HT_{1B}R expression on number of lever presses ($F_{2, 38}$=0.4, p>0.05 for main effect; $F_{4, 76}$=0.3, p>0.05 for interaction). To account for the higher baseline lever pressing in 5-HT_{1B}R KO mice, lever pressing was normalized to baseline lever pressing behavior, and still there were no differences in extinction rates between groups ($F_{2,38}$=1.4, p>0,05; Fig 1D).

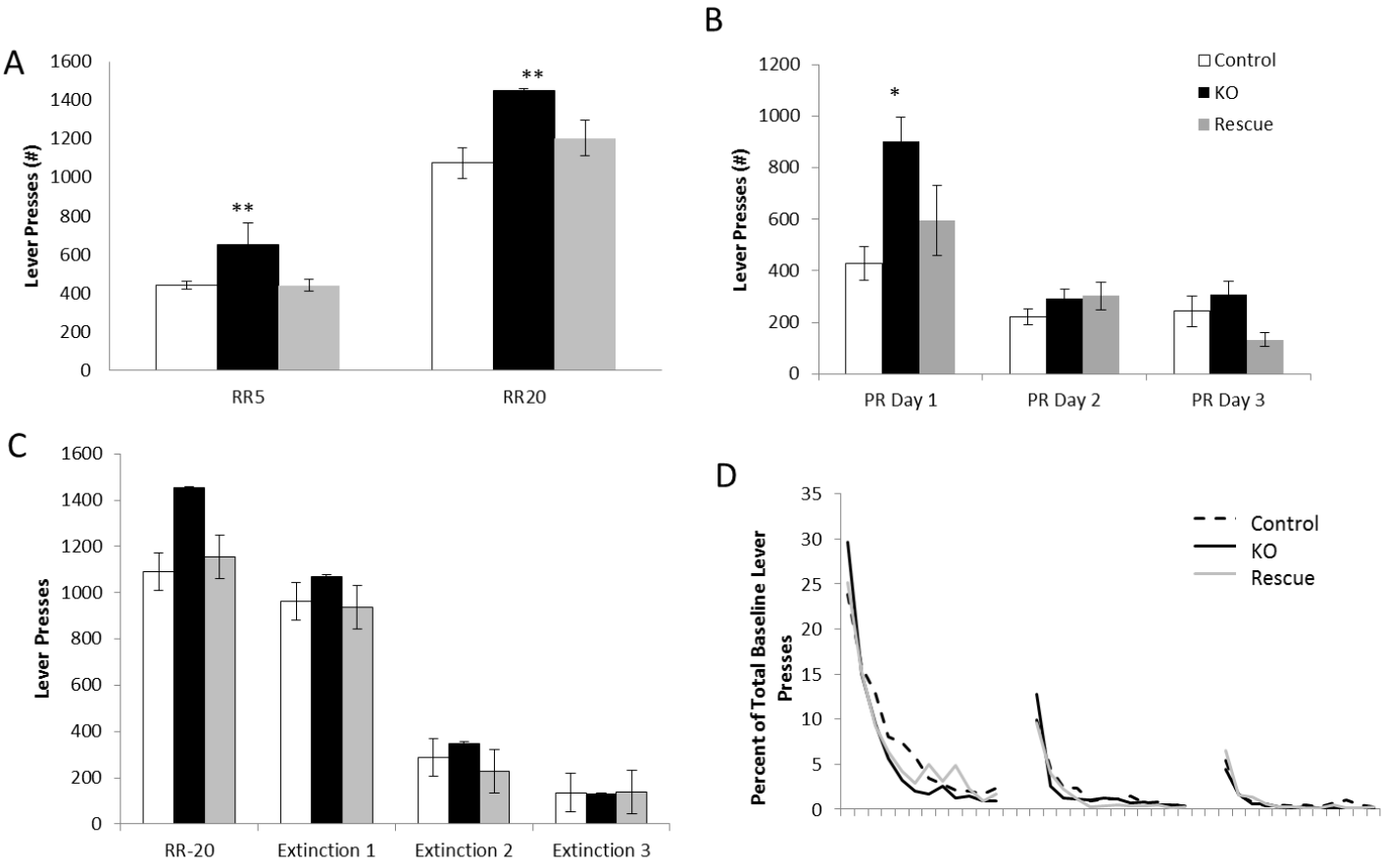

Figure 1. Lack of serotonin 1B receptor increases motivation. (A) Number of lever presses are shown during random ratio 5 and 20 schedules of reinforcement. **, p<0.05 compared to Control and Rescue groups. (B) Number of lever presses are shown for a progressive ratio x 2 schedule of reinforcement, presented over three consecutive days. *, p<0.05 compared to Control group. C) Lever presses shown during 3 extinction sessions, compared to the previous RR20 session. D) Percentage of presses from RR20 baseline, during 3 sessions of extinction trials, binned by 5 minutes. All data shown are group means +/- SEM.

Another interpretation for the increased persistence seen in the PR is that 5-HT_{1B}R expression influences effort-based decision making. We used a concurrent choice task to test goal-directed decision making, in which mice were provided with a choice between freely available reward or lever pressing on an RR20 schedule for evaporated milk. While all mice decreased their lever pressing behavior when the freely available option was evaporated milk, mice lacking 5-HT_{1B} R expression pressed significantly more compared to controls even when normalized to their baseline responding ($F_{2, 38}$=16.7, p<0.001; Fig 2A). In other words, mice lacking 5-HT_{1B} R expression continued pressing at 54% of their baseline rate despite concurrent access to freely available evaporated milk in the operant chamber, while control mice and mice with adult rescue of receptor expression reduced their pressing to 17% and 25% of their baseline rates, respectively. All mice continued to lever press at high rates for evaporated milk when the freely available option was chow, with no group differences in responding ($F_{2, 38}$=0.7, p>0.05; Fig 2A). There were no group differences in the consumption of the freely available reward ($F_{2,38}$=1.3, p>0.05; Fig 2B). Taken together, these results suggest that 5-HT_{1B}R expression might be affecting the representation of the outcome value that guides goal-directed action.

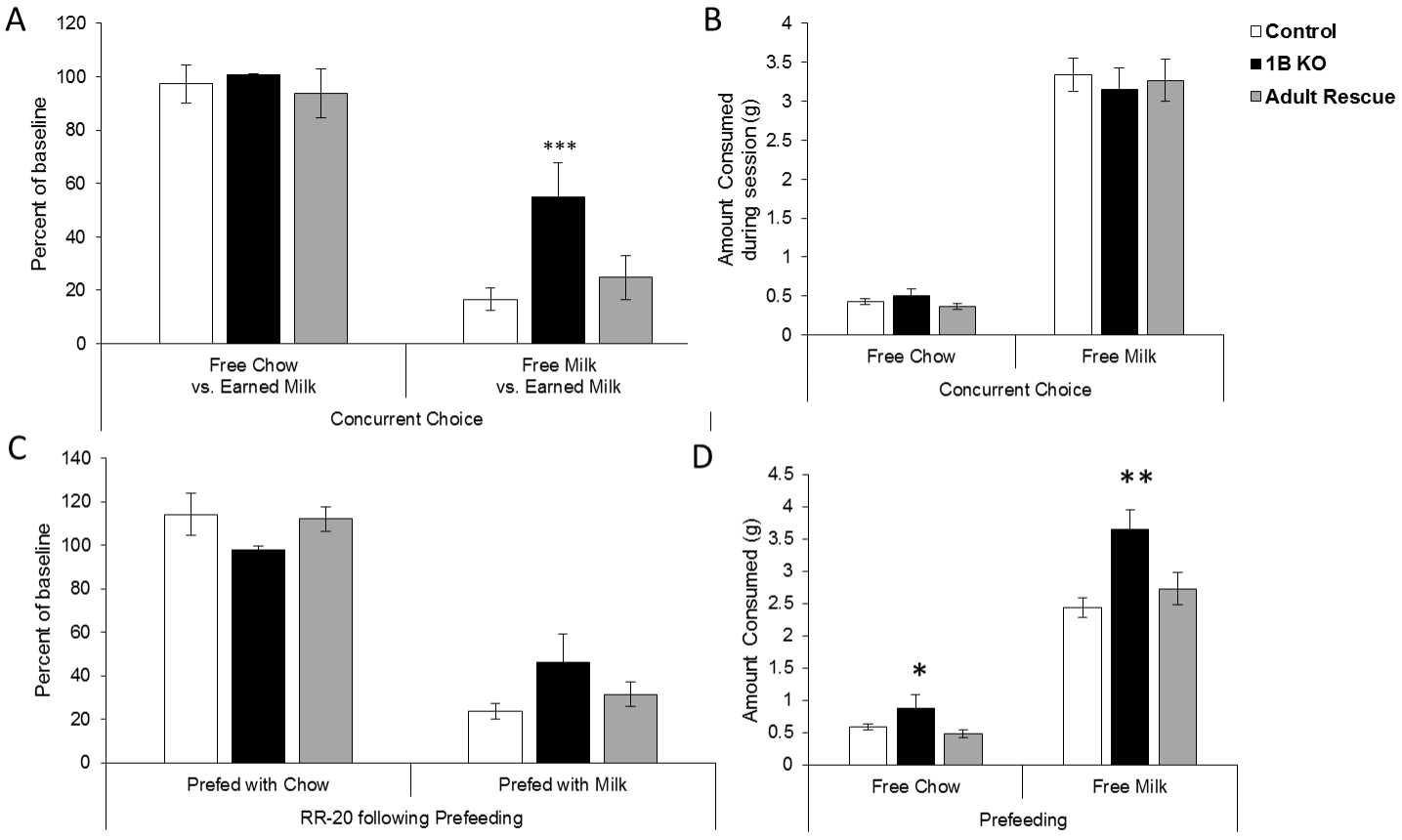

Figure 2. Effects of 5-HT1B on habitual and effort-based responding. A) Lever presses are shown as a percentage of a total presses from a baseline random ratio 20 schedule in conditions in which Chow or Evaporated milk were presented as free alternatives to lever pressing for Evaporated milk. ***, p<0.001 compared to Controls and Adult Rescue mice. B) The amount of free alternative Chow or Evaporated milk that was consumed during the operant session is shown. C) Lever presses are shown as a percentage of a total presses from a baseline random ratio 20 schedule in conditions in which mice were prefed chow or evaporated milk before the operant test session. D) The amount of chow or evaporated milk that was consumed during the prefeeding session prior to operant session is shown. *, p=0.043 for 1B KO vs Adult Rescue; **, p<0.01. All data shown are group means +/- SEM.

Another possibility for the increased responding in the PR is that mice lacking 5-HT_{1B} R might respond more habitually and be less guided by the outcome of their actions. To assess the relative contribution of habitual-like behavior, we assessed the effect of 5-HT_{1B} R expression on goal-directed behavior following satiety-induced devaluation of the reward. As expected, all mice reduced responding when pre-fed with evaporated milk reward, but not when pre-fed with chow, suggesting that the overall behavior of these mice on this RR20 schedule was goal-directed, rather than habitual (Fig 2C). Furthermore, there were no significant effects of 5-HT_{1B}R expression on the number of lever presses in either pre-fed condition ($F_{2, 38}$=0.6, p>0.05 for chow; $F_{2, 38}$=3.0, p=0.064 for milk), suggesting that the effect of 5-HT_{1B} expression on the increased lever pressing behavior in the concurrent choice paradigm is not a function of increased habitual-like responding. Interestingly, there were group differences in the pre-operant test consumption of both chow ($F_{2, 38}$=3.38, p=0.045) and milk ($F_{2, 38}$=7.26, p<0.01; Fig 2D). While the increased feeding may represent some increased feeding drive, it may also represent an increased reward sensitivity in the food deprived state, given that the effect size of the increased consumption is larger in the milk compared to the food condition ($F_{2, 38}$=5.14, p<0.05; group x condition interaction).

We hypothesized that an exaggerated representation of outcome value could arise from a difference in hedonic reactions to the reward. In order to test the effect of 5-HT_{1B}R on hedonic value, two tests of reward sensitivity were performed. The first showed that mice lacking 5-HT_{1B}R expression have increased reward consumption of (Fig 3A). All mice showed increased consumption of evaporated milk as the concentration increased ($F_{2,34}$= 15.23, p<0.001), mice lacking 5-HT_{1B}R expression showed increased consumption of evaporated milk compared to controls ($F_{1,17}$=5.92, p<0.05). This increase was significant at the intermediate 66% concentration (p<0.01). There was a significant interaction of concentration and consumption suggesting that the mice lacking the 5-HT_{1B}R showed a greater sensitivity to reward value ($F_{2,34}$= 4.42, p<0.05).

To more directly measure hedonic reactions, we examined the lick rate to different concentrations of sucrose [19,20]. Mice lacking 5-HT_{1B}R expression showed increases in hedonic value. In a food restricted condition, mice lacking 5-HT_{1B}R expression had more total licks for sucrose than controls ($F_{1,9}$=10.71; p<0.01), at both 2% and 10% sucrose concentrations (p<0.01 and p<0.05, respectively; Fig 3B). In the sated condition, there was also an increase in licking in mice lacking 5-HT_{1B}R ($F_{1,9}$=8.76; p<0.05), however this was only significant for 10% sucrose (p<0.01; Fig 3C). Importantly, there was no increased licking in the lowest hedonic condition tested (sated with 2% sucrose; p>0.05) suggesting that the increased licking in the absence of 5-HT_{1B}R represents an increase in the hedonic response to the reward. Lick rate was also increased in mice lacking 5-HT_{1B} R (Fig 3D,E). Specifically, mice that lack 5-HT_{1B}R expression showed increased lick rate in the sated conditions ($F_{1,9}$=11.87, p<0.01) to both 2% (p<0.05) and 10% sucrose (p<0.01). This suggests that the effect is driven by baseline hedonic value rather than a feeding restriction-related effect. Both genotypes showed increased lick rates to 10% sucrose compared to 2%, but mice lacking 5-HT_{1B}R showed greater increases compared to controls ($F_{1,9}$=7.45 , p<0.02 for genotype x condition interaction).

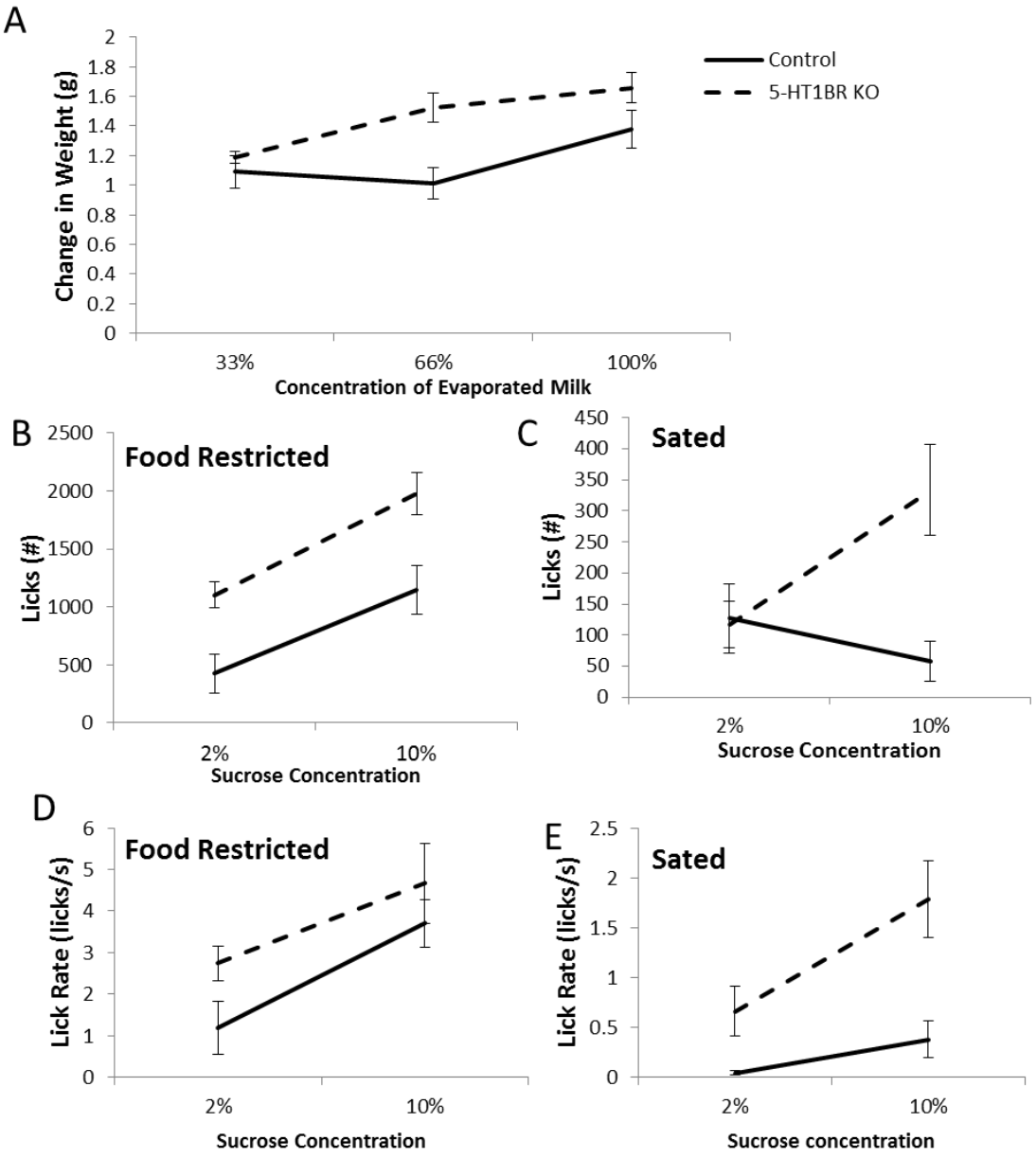

Figure 3. Serotonin 1B influences hedonic value. A) Increases in body weight are shown following 5 minute consumption of evaporated milk. B, C) Total number of licks to a spout delivering sucrose is shown in food restricted (B) and sated (C) conditions to 2% and 10% sucrose. All data are shown as group means +/- SEM. D, E) Lick rate in the first 2 minutes of the session for food restricted (D) and sated (E) conditions to 2% and 10% sucrose.

We conducted two experiments to explore how 5-HT_{1B}R expression might affect impulsivity through the modulation of reward value. First, we replicated the finding that mice lacking the 5-HT_{1B} receptor show increased impulsive action as measured in the Go/No-Go task. As previously reported, mice lacking the 5-HT_{1B} R showed an increased impulsivity (Fig 4A), compared to littermate control mice ($F_{1, 18}$=6.96, $p<0.05$). Additionally, control mice showed a faster reduction in their impulsivity over days ($F_{18,72}$=3.33, $p<0.01$ for interaction). In a delay discounting test of impulsive choice, as reported previously, mice

lacking 5-HT_{1B} R expression did not show deficits in impulsivity (Fig 4B). Specifically, although there was an effect of genotype on preference for the large reward ($F_{1, 18}$=5.84, p<0.05), and a decrease for preference for the large reward as the delay lengthens ($F_{4, 72}$=59.13, p<0.001), there was no interaction between genotype and delay ($F_{18,72}$=1.60, p>0.05), suggesting that there is no genotype difference in discounting. Interestingly, the overall increase in preference for the large reward (across all delays) in mice lacking 5-HT_{1B}R is consistent with the hypothesis that 5-HT_{1B} influences reward valuation.

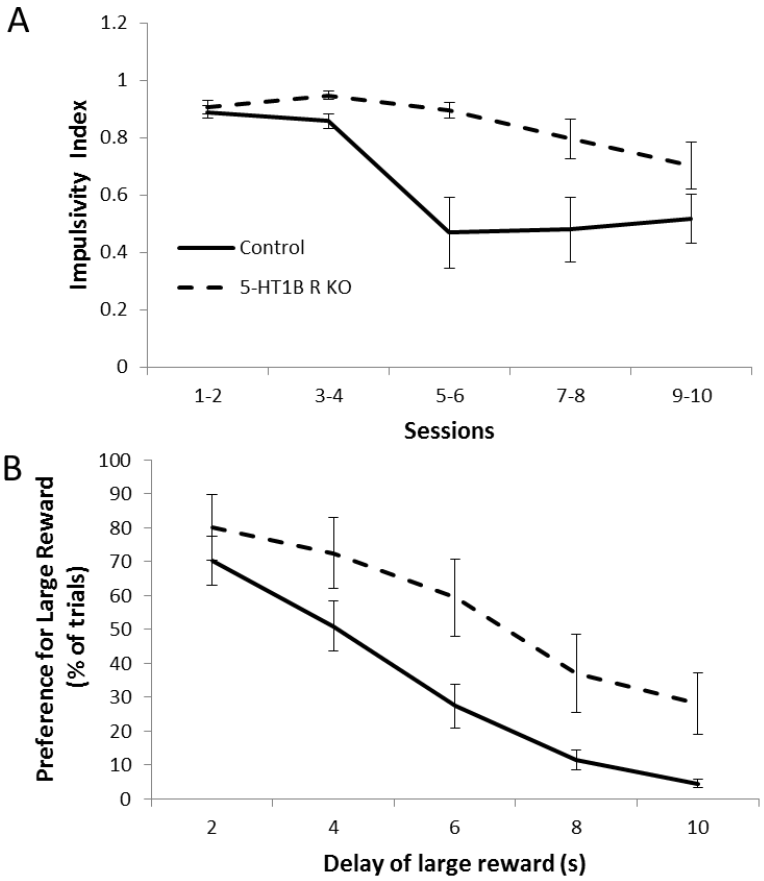

Figure 4. Lack of serotonin 1B receptor expression increases impulsive action but not impulsive choice. (A) Impulsivity index calculated as the number of successful Go trials minus the number of successful No Go trials is shown as a measure of impulsive action over 10 sessions. (B) Data from a delayed discounting paradigm is shown as the percentage of trials on which the large (delayed) reward was chosen, represented over delays ranging from 2 to 8 seconds. Data is shown as means +/- SEM.

Next, we directly examined if reward value could impact impulsive action. We developed a novel paradigm in which we varied reward value in a Go/No-Go paradigm within a single session. Thus, we can compare impulsivity between trials in which large or small rewards were expected. Mice were more impulsive on large reward trials compared to small reward trials, as measured by impulsivity index (Fig 5A; $F_{1,11}$=19.1, p<0.001). As expected, all mice showed decreased impulsivity over the 10 days of testing ($F_{9,99}$=7.0, p<0.001). The response latency measure also supported the finding that mice were more impulsive on large reward trials, with faster response times on large reward trials (Fig 5B; $F_{1,11}$=12.3,

p<0.01). Specifically, they showed decreased latencies to respond on Go trials (t_{11}=3.39, p<0.01), and incorrect No-Go trials (t_{11}=3.36, p<0.01). Importantly, this difference in speed of responding was not present before the impulsivity task was introduced ($F_{1,11}$=1.32, p>0.05), suggesting that the latency isn't a general readout of preference for a large reward.

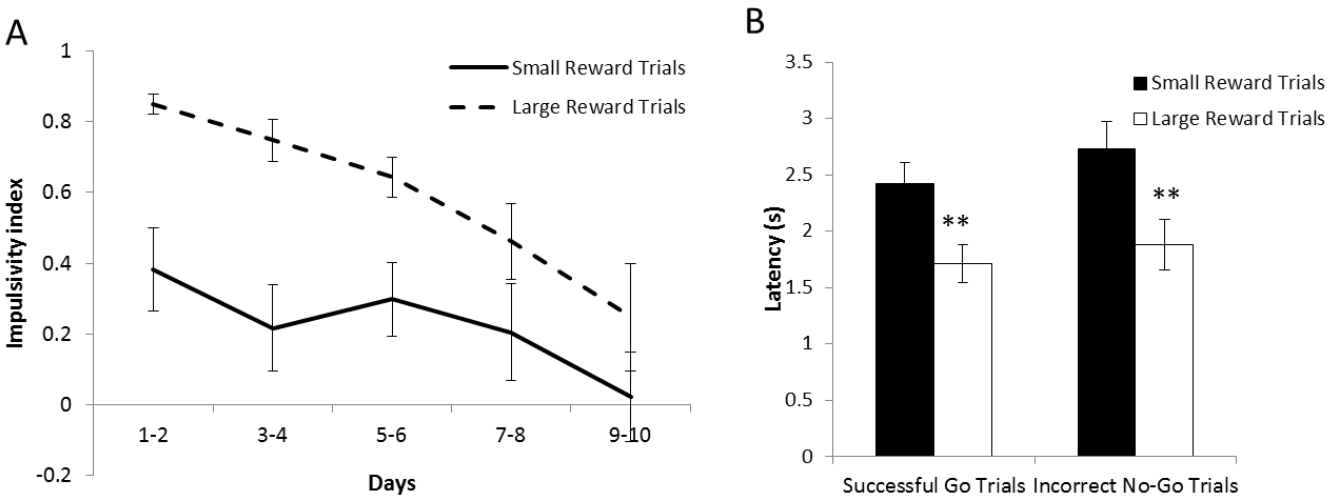

Figure 5. Reward value influences impulsive action. (A) Impulsivity index calculated as the proportion of successful Go trials minus the proportion of successful No Go trials is shown as a measure of impulsive action (1.0 is the highest impulsivity that a mouse can display) over 10 days presented in 2-day bins. (B) Latency to press the lever is shown for Successful Go Trials and for Incorrect No-Go trials during Small and Large reward trials. **, p<0.01. All data are shown as group means +/- SEM.

Discussion

Overall, our data shows that 5-HT_{1B}R expression influences goal-directed behavior, motivation, and reward sensitivity, along with the previously reported effects on impulsive action, but not impulsive choice. Specifically, an absence of 5-HT_{1B}R expression results in greater responding on random ratio and progressive ratio tasks. This increase in goal-directed responding is sensitive to extinction and devaluation at the same rates seen in control mice. Interestingly, mice lacking 5-HT_{1B}R expression show increases in hedonic responses to evaporated milk and sucrose solutions. Taken together with effects of 5-HT_{1B}R on impulsive action, these data point to the possibility that the effects of 5-HT_{1B}R on hedonic value may underlie the effects on goal-directed behavior, motivation, and also impulsive action.

Previous studies in humans and animal models have examined the relationship between hedonic value and impulsivity [13,21,22]. In humans, increased hedonic value measured with varying sweet concentrations is associated with increases in impulsive choice (assessed in a delay discounting task), but not impulsive action (measured in a go/no-go paradigm) [13]. In rats, increased sucrose-seeking is associated with increased impulsive action (measured in the 5-choice serial reaction time task) [23]. Also, rats bred for high sucrose consumption displayed higher levels of impulsive action (on the go/no-go task) when responding for cocaine [22], and higher levels of impulsive choice (on the delay discounting task) [24]. However, a confound in the interpretation of many of these studies suggesting

associations between reward value and impulsivity arises from between-subjects designs measuring more trait-like phenotypes. This leaves open the possibility for another trait-level behavioral construct to mediate the association between reward value and impulsivity (e.g. learning about appetitive goal-directed behavioral contingencies). In order to test the causal association of higher valued incentive stimuli with increased impulsivity, we performed a within subject, within session experiment varying reward value, and measuring the resulting effects on impulsive action in the go/no-go task. The results in our novel Variable Value Go/No-Go paradigm show that increased reward value causes increased impulsive action as measured by a decrease in behavioral inhibition in no-go trials. This supports a causal role for reward value in impulsive action.

While we have shown that 5-HT_{1B}R influences both reward valuation and impulsive action, and that reward value impacts impulsive action, we have yet to show if all of the effects of 5-HT_{1B}R on impulsive action are mediated by the effects on reward sensitivity. There may be a component of the increased impulsive action seen in mice lacking 5-HT_{1B}Rs that is independent of the effects of 5-HT_{1B}R on reward sensitivity. One way to test this might involve normalizing reward value across all mice, and then testing behavior in the Go/No-Go paradigm using individualized reward values. This would mean that mice lacking 5-HT_{1B}R would receive smaller rewards relative to controls, in an attempt to equate value between genotypes. Therefore, any remaining increases in impulsivity seen in mice lacking 5-HT_{1B}R could be attributed to direct effects of 5-HT_{1B} on impulsivity independent of reward sensitivity.

It is interesting to note that 5-HT_{1B}R expression does not seem to influence impulsive choice, despite effects on reward valuation. While there are no significant effects of 5-HT_{1B}R expression on the discounting slope, there are reliable differences in overall preference for the large reward. Specifically, mice lacking 5-HT_{1B}R prefer the large reward more when there is no time delay. This suggests that reward sensitivity may underlie the difference in the intercept of the choice in the delay discounting paradigm, which may be independent of the discounting (impulsivity) read out by parallel but offset discounting curves. This suggests that reward sensitivity correlates with behavioral inhibition in an impulsive action paradigm, but not value discounting in an impulsive choice paradigm.

Overall, our studies investigate the neural and behavioral substrates of impulsivity, and propose a behavioral mechanism for the effect of serotonin signaling on impulsive action, namely through changes in reward sensitivity. Furthermore, we show that there is a causal effect of reward value on impulsive action in our novel Variable Value Go/No-Go paradigm which supports the possibility of increases in impulsivity resulting from changes in reward sensitivity. This is a valuable line of research for understanding factors that contribute to increases in impulsivity seen in clinical populations, and points to the utility of treatment strategies aimed at different subtypes of impulsivity, including those with disordered valuation of reward.

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Author Contributions

SSD, EL, VMM, and KN acquired and managed the data. SSD, EL, and KN conducted data analysis. SSD and KN wrote the first draft of the paper. SSD, KN, and PB designed the studies. All authors contributed to and approved the final version of the paper.

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Figure Legends

Figure 1. Lack of serotonin 1B receptor increases motivation. (A) Number of lever presses are shown during random ratio 5 and 20 schedules of reinforcement. **, p<0.05 compared to Control and Rescue groups. (B) Number of lever presses are shown for a progressive ratio x 2 schedule of reinforcement, presented over three consecutive days. *, p<0.05 compared to Control group. C) Lever presses shown during 3 extinction sessions, compared to the previous RR20 session. D) Percentage of presses from RR20 baseline, during 3 sessions of extinction trials, binned by 5 minutes. All data shown are group means +/- SEM.

Figure 2. Effects of 5-HT1B on habitual and effort-based responding. A) Lever presses are shown as a percentage of a total presses from a baseline random ratio 20 schedule in conditions in which Chow or Evaporated milk were presented as free alternatives to lever pressing for Evaporated milk. ***, p<0.001 compared to Controls and Adult Rescue mice. B) The amount of free alternative Chow or Evaporated milk that was consumed during the operant session is shown. C) Lever presses are shown as a percentage of a total presses from a baseline random ratio 20 schedule in conditions in which mice were prefed chow or evaporated milk before the operant test session. D) The amount of chow or evaporated milk that was consumed during the prefeeding session prior to operant session is shown. *, p=0.043 for 1B KO vs Adult Rescue; **, p<0.01. All data shown are group means +/- SEM.

Figure 3. Serotonin 1B influences hedonic value. A) Increases in body weight are shown following 5 minute consumption of evaporated milk. B, C) Total number of licks to a spout delivering sucrose is shown in food restricted (B) and sated (C) conditions to 2% and 10% sucrose. All data are shown as group means +/- SEM. D, E) Lick rate in the first 2 minutes of the session for food restricted (D) and sated (E) conditions to 2% and 10% sucrose.

Figure 4. Lack of serotonin 1B receptor expression increases impulsive action but not impulsive choice. (A) Impulsivity index calculated as the number of successful Go trials minus the number of successful No Go trials is shown as a measure of impulsive action over 10 sessions. (B) Data from a delayed discounting paradigm is shown as the percentage of trials on which the large (delayed) reward was chosen, represented over delays ranging from 2 to 8 seconds. Data is shown as means +/- SEM.

Figure 5. Reward value influences impulsive action. (A) Impulsivity index calculated as the proportion of successful Go trials minus the proportion of successful No Go trials is shown as a measure of impulsive action (1.0 is the highest impulsivity that a mouse can display) over 10 days presented in 2-day bins. (B) Latency to press the lever is shown for Successful Go Trials and for Incorrect No-Go trials during Small and Large reward trials. **, p<0.01. All data are shown as group means +/- SEM.