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Functional Coding Variation in the Presynaptic Dopamine Transporter Associated with Neuropsychiatric Disorders Drives Enhanced Motivation and Context-Dependent Impulsivity in Mice

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Abstract

Recent genetic analyses have provided evidence that clinical commonalities associated with different psychiatric diagnoses often have shared mechanistic underpinnings. The development of animal models expressing functional genetic variation attributed to multiple disorders offers a salient opportunity to capture molecular, circuit and behavioral alterations underlying this hypothesis. In keeping with studies suggesting dopaminergic contributions to attention-deficit hyperactivity disorder (ADHD), bipolar disorder (BPD) and autism spectrum disorder (ASD), subjects with these diagnoses have been found to express a rare, functional coding substitution in the dopamine (DA) transporter (DAT), Ala559Val. We developed DAT Val559 knock-in mice, as a construct valid model of dopaminergic alterations that drive multiple clinical phenotypes, and here evaluate the impact of lifelong expression of the variant on impulsivity and motivation, utilizing the 5-choice serial reaction time task (5-CSRTT) and Go/NoGo as well as tests of time estimation (peak interval analysis), reward salience (sucrose preference), and motivation (progressive ratio test). Our findings indicate that the DAT Val559 variant induces impulsivity behaviors that are dependent upon the reward context, with increased impulsive action observed when mice are required to delay responding for a reward, whereas mice are able to withhold responding if there is a probability of reward for a correct rejection. Utilizing peak interval and progressive ratio tests, we provide evidence that impulsivity is likely driven by an enhanced motivational phenotype that also may drive faster task acquisition in operant tasks. These data provide critical validation that DAT, and more generally, DA signaling perturbations can drive impulsivity that can manifest in

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specific contexts and not others, and may rely on motivational alterations which may also drive increased maladaptive reward seeking.

Keywords

Attention-Deficit Hyperactivity Disorder; Motivation; Impulsivity; Dopamine Transporter; Transgenic Model; Instrumental Learning

1. INTRODUCTION

Attention-Deficit/Hyperactivity Disorder (ADHD) is the most prevalent neurobehavioral disorder of childhood, affecting approximately 4–12% of school-aged children in the United States^{1–3} and risk for ADHD has been found to be significantly heritable¹. Consistent with these findings, polymorphisms in DA system genes, including those encoding dopamine (DA) receptors^{4–8}, DA metabolizing enzymes^{9, 10} and the DA transporter (DAT^{11–14}) have been associated with ADHD. In a search for functional coding variation in human DAT, we identified a Val substitution at Ala559 in two male siblings with ADHD^{15, 16}, a variation that had been previously identified in a female with Bipolar Disorder (BPD)¹⁷. Subsequently, the Val559 substitution was found in two unrelated male subjects with autism spectrum disorder (ASD)¹⁸. In studies examining functional alterations in transfected cells, the DAT Val559 variant failed to impact DA recognition or uptake, but instead, promoted DAT-dependent DA efflux¹⁵, which we hypothesized could establish tonically-elevated, extracellular DA levels *in vivo* and “short-circuit” vesicular DA release mechanisms. Our subsequent studies with DAT Val559 knock-in mice bore out both of these predictions, as *in vivo* microdialysis studies demonstrated elevated extrasynaptic DA levels and *ex vivo* slice studies revealed tonic activation of presynaptic D2 DA autoreceptors (D2ARs) leading to blunted depolarization-induced vesicular DA release¹⁹.

The availability of DAT Val559 mice represents a key opportunity to evaluate molecular, cellular and circuit level perturbations underlying the behavioral changes in ADHD through studies of a construct-valid animal model. In our initial studies^{19, 20}, we demonstrated that DAT Val559 mice display a stress-dependent hyperactivity (darting), as well as altered rearing behavior. More conspicuous was a blunting of locomotor and rearing enhancements produced by the psychostimulants used to treat ADHD, D-amphetamine and methylphenidate. Here, we extend these analyses to evaluate whether the DAT Val559 variant induces cognitive changes, with a particular focus on impulsivity, given the presence of the trait in our two ADHD probands as well as the maternal grandmother¹⁶. Through the use of complementary operant tasks, we were able to evaluate different facets of impulsivity as well as look at other behavioral perturbations associated with both the DA system and ADHD that could contribute to impulsive phenotypes, such as the ability to time events and reward valuation and motivation. We discuss our findings in the context of a model whereby DAT Val559 dependent DA efflux and tonic elevations in extracellular DA drive schedule-dependent impulsivity that may be driven by an enhanced motivational state for predictable rewards.

2. METHODS

2.1 Animals

All experiments were performed under a protocol approved by the Institutional Animal Care and Use Committees at Vanderbilt University and Florida Atlantic University. Homozygous DAT Val559 and wildtype (WT) littermate mice used in the study were bred from heterozygous breeders on the hybrid background used in our prior studies¹⁹ (75% 129S6 and 25% C57BL/6J). Males were evaluated in the present study owing to the bias toward male subjects for ADHD diagnoses²¹. Animals were housed on a 12:12 (L:D) cycle. Mice were tested during their active cycle, achieved by either raising animals on a reverse light cycle with lights on and off at 3 pm and 3 am, respectively, or by raising mice on a normal light cycle, with lights on and off at 7 am and 7 pm and then with transfer of mice to the reverse light cycle at 5 weeks of age, after weaning. Mice were approximately 6–7 weeks old when training for different behavioral assays commenced. For all operant conditioning tasks mice were placed on food restriction one week prior to the start of training. Mice were brought to approximately 85%–90% of their baseline weight (weighed every other day). On the fourth and fifth days (still under food restriction), mice were exposed to 33% Vanilla Ensure Original, the reward used for all the operant tasks, for one hour in the home cage. All operant tasks were performed under red light with operant chambers housed in sound attenuation boxes. All tests were conducted with chamber houselights turned off, unless specifically stated otherwise.

2.2 5-Choice Serial Reaction Time Task (5-CSRTT)

The 5 choice serial reaction time task (5-CSRTT) was utilized as it allows for a variety of traits to be measured including gross attention impairments (omissions), impulsivity issues (premature responses and preservative responses), and sustained attention (accuracy). Additionally, acquisition time of the task can be used as an indication of learning deficits that could result from difficulties in the above measures. 5-CSRTT performance can also be aligned with measures gathered from the continuous performance test, a common task used to assess and diagnose children with ADHD^{22, 23}. We implemented the 5-CSRTT using Bussey-Saksida Mouse Touch Screen Chambers (Lafayette, IN) with a mask forming five 4 cm × 4 cm square touch zones arranged horizontally. All testing occurred under red light. It should be noted that for both 5-CSRTT and Go/NoGo (section 2.3) illumination of touch zones was performed with 100% illumination levels. See Supplemental Figure 1 for training schematic and paradigm diagram. During the Habituation phase of training, mice were exposed to the testing chamber for 30 min during which a 30 µl liquid reward was delivered every 10 sec coinciding with illumination of the reward receptacle. The 10 sec reward delivery timer was reset by head entry into the reward receptacle. Mice had to collect 30 rewards on two consecutive days to move to the Initial Touch phase of training. During the Initial Touch phase of training, one of the five touch zones was randomly illuminated for a 30 sec interval. Mice received reward regardless of touching the screen but received 3X reward if touch occurred during illumination, accompanied by a tone and illumination of reward receptacle. Mice had to collect 30 rewards in 30 min in order to move on to the Must Touch phase of training. During the Must Touch phase, single touch panels were illuminated pseudo-randomly (could not appear in the same location more than 3X consecutively) in one

of the five locations. Illumination remained on until the correct location was touched. Blank touches (unlit locations) were not punished. Upon touching an illuminated panel, reward was delivered with concurrent tone and illumination of the reward receptacle. Upon head entry to receive reward, a five sec inter-trial interval (ITI) was initiated, followed by touch zone illumination to restart a trial. Mice had to collect 30 rewards in 60 min to move on to the Must Initiate phase of training. In the Must Initiate Phase, the session was initiated with delivery of a free reward. Once the reward was collected, a single touch zone was illuminated. Upon touch of this panel, reward was delivered concurrently with tone and reward receptacle illumination, followed by a 5 sec ITI. At this point, the reward receptacle was re-illuminated and mice were required to make a head entry into the reward receptacle to initiate a trial, at which time a single touch zone was illuminated to repeat the process. Mice had to collect 30 rewards in 60 min to move on to the Punish Incorrect phase.

In the Punish Incorrect phase, touch of an incorrect panel during stimulus presentation resulted in the stimulus being immediately turned off and the chamber being fully illuminated for a five sec time out. Subsequently, a five sec ITI was imposed before the receptacle light came on again for the initiation of a new trial. Mice had to complete 30 trials in 30 min at an accuracy of 80% correct before they could move on to the 5-choice evaluation phase. It is important to note that a hybrid advancement method²⁴ was used to progress mice through the different training phases, such that mice who reached the punish incorrect phase the fastest were then rested at that stage. Occasionally, additional training sessions were performed to ensure that these mice remained at criterion, though done sparingly to limit over-training effects. Once all mice successfully completed the punish incorrect phase, they were moved as a group to the 5-choice phase. During the 5-choice phase, mice learned to respond to the illuminated zone with a progressively shorter stimulus presentation duration (16s, 8s, 4s, and 2s). Mice had to initiate the trial and wait through a 5 sec delay before presentation of the stimulus. Responses had to occur either within the stimulus presentation or within a 5 sec window post-stimulus presentation (limited hold). Mice received a 5 sec timeout with the house light on if they responded during the 5 sec delay (before stimulus presentation but after trial initiation), made an incorrect response, or omitted a response (no response within stimulus duration plus 5 sec limited hold). If mice made a premature response the trial was reset. Mice were required to complete 80 trials in 60 min with at least 80% accuracy and less than 20% omissions for two days in a row before being moved to a shorter stimulus duration of the training series. All mice were brought to a baseline performance which consisted of trial initiation, 5 sec delay, 2 sec stimulus presentation, 5 sec limited hold (7 sec to make a response), reward delivery, and a 5 sec ITI. Once this was reached, the delay component of the task was manipulated to further explore impulsivity. A long delay session was performed in which the mice had to wait 15 sec between trial initiation and onset of stimulus. A separate set of trials was implemented where a 2, 5, 10, and 15 sec delays were intermixed randomly (20 trials of each). Measures collected for this assay included % correct, % omission, number of premature responses, number of perseverative responses, and session length.

2.3 Go/NoGo Task

As a secondary measure to look at a different aspect of impulsivity, we used the Go/No-Go task. This assay is distinct from the impulsivity measures obtained in the 5-CSRTT in that Go/No-Go requires the mouse to learn to distinguish different signals associated with operant response and behavioral restraint in order to obtain a reward, making it a measure of motor inhibition/suppression versus a measure of waiting capabilities^{25–27}. Mice were initially trained in the same manner as they were for 5-CSRTT up through the Punish Incorrect phase (section 2.2), except mice had two larger (7.6 cm × 7 cm) touchscreen windows to interact with instead of five. Once mice completed the Punish Incorrect phase they were initially moved directly into Go/NoGo (60 trials, 20% No-Go trials). However, this was changed to Go-only trials upon failure of mice to complete the number of required trials and inability to acquire the No-Go behavior. Go-only trials consisted of trial initiation, 5 sec delay, and then the presentation of the stimulus in one of the two touchscreen windows for 3 sec with a 5 sec limited hold. The No-Go signal involved having both touchscreen windows lighting up together. Sessions consisted of 30 trials within 30 min. Mice received 8 training sessions on Go-only and were then moved to Go/NoGo, where mice were required to complete 30 trials within 30 min with 50% of the trials being No-Go trials. Mice were trained on Go/NoGo until stable performance rates were achieved (13 days). Mice were then tested in a single session where the delay between trial initiation and stimulus presentation was extended from 5 to 15 secs. The main dependent measures were omissions, premature responses, and correct rejections during No-Go trials (mice withheld responding).

2.4 Peak Interval Task

To evaluate the ability of mice to estimate time as one possible explanation for impulsivity, we implemented a Peak Interval Task. Animals were tested in Med-Associates (St. Albans, VT) operant chambers housed in sound attenuation boxes. For the first 2 days of training, mice were presented with a free reward and given 5 sec to consume the reward upon head entry. Reward was then presented every 10 sec after reward consumption, unless a nose-poke was made in any of the 3 nose-poke holes (backlit by LEDs), which elicited reward delivery (a Fixed Ratio 1-Fixed Time 10 secs schedule; FR1-FT10). Sessions lasted 30 min and mice could earn up to 30 rewards. Days 3 and 4 utilized an FR1-FT30 schedule. On day 5, mice were moved to continuous reinforcement. Under this protocol only the center back nose-poke hole was lit and mice had to nose-poke to get a reward. Mice were given 60 min to obtain 30 rewards. Once all mice could collect 30 rewards within 60 min, they were moved to either a fixed interval schedule of 5 sec or 15 sec. Importantly, mice that reached criterion for CRF at a faster pace were rested to prevent over-training, and then once all mice reached CRF criterion they were run for additional sessions as a group and then moved to a fixed interval schedule. The center nose-poke would illuminate, but it was only the first nose-poke after 5 or 15 sec of illumination that was rewarded. A 10 sec limited hold from the fixed interval mark was used. If mice made a nose-poke within that time, the nose-poke light went off, a click sounded, and the dipper was brought up. If no response was made within the limited hold, the nose-poke light went off and an omission was counted. Mice had 5 sec to consume the reward upon head entry into dipper. Trials were separated by a variable ITI that averaged 11 sec between reward collection or response omission and center nose-poke re-illumination. Mice were trained on FI 5 sec or FI 15 sec until their response latencies

stabilized. Mice were then moved as a group to Peak Interval testing. This task functioned very similarly to Fixed Interval except with the addition of probe trials. During the probe trial, the nose-poke hole was lit for 3X the length of the fixed interval time, however, nose-poke responses produced no reward, allowing for a response curve to be recorded. Gaussian curves generated from the responses in the probe trials were used to provide information on interval timing variability (peak spread), maximum response rate (peak height), and timing accuracy (x-axis location of peak).

2.5 Progressive Ratio

To evaluate motivation for reward, mice underwent 2 days of habituation to operant chambers with dipper training. Every 3 min of a 30 min session, mice received a free reward, but were able to obtain reward sooner with a nose-poke into the center hole opposite the dipper. The dipper remained raised until mice made a head entry into the food receptacle, and then animals had 10 sec to consume the reward. Mice were then moved to a continuous reinforcement (CRF) paradigm, where a nose-poke to the center hole on the opposite wall of the food receptacle was required for a reward. Nose-poke holes were also located to the left and right of the food receptacle and were used to measure non-specific nose-poke activity. Mice were required to make 50 correct responses in 45 min, and given 10 sec to drink their reward. After 2 consecutive days of successful completion, mice were trained for an additional week (6–7 training sessions) at 50 correct responses in 45 minutes, with 5 sec to drink their rewards. Mice were then run through a progressive ratio schedule as a group, requiring an increasing number of nose-pokes to obtain a reward. Sessions were ended after 60 min had elapsed or if the mouse was inactive for 5 minutes, whichever came first. Several measurements related to motivation are recorded, including the break point (the nose-poke requirement at which the mouse gives up), value met (last successful round of nose-pokes met before the mouse gives up), response rate, number of correct responses made, and number of rewards earned.

2.6 Sucrose Preference Test

To assess reward salience, we subjected mice to the Sucrose Preference test, singly housing mice with access to two bottles filled with water for a 2 day habituation, followed by substitution of water in 1 bottle with a 1% sucrose solution. On day 5, the sucrose solution was increased to 3%. Bottles were weighed daily and switched in placement. Sucrose consumption was calculated as the amount of sucrose solution consumed divided by total liquid consumption.

2.7 Statistical Analyses

Statistical analyses was done using GraphPad Prism 7 software package. Statistical significance was set at $P < 0.05$ for all experimental results. The type of statistical analysis used was determined independently for each experiment and is listed in the relevant figure caption. Outlier analysis was performed using the ROUT method with the false discovery rate set at 2%.

3. RESULTS

3.1 DAT Val559 mice acquire 5-CSRTT faster than WT littermates

To explore multiple cognitive capacities, as well as impulsivity, of the DAT Val559 mice, we first evaluated their performance on the 5-CSRTT. During task acquisition (9 Phases), we observed no deficits in task acquisition compared to WT littermates (Figure 1A; WT n=18, Val559, n=19). Instead, DAT Val559 mice progressed more quickly through both the early training phases (habituation-punish incorrect) and during the stimulus reduction phases than WT animals. Although accuracy assessed in the full 5-CSRTT was largely equivalent, we detected a difference on day 1 of testing (16 sec stimulus duration), which may also suggest that retention of the rules of the test were more effectively stored or retrieved in DAT Val559 mice (Figure 1B).

3.2 DAT Val559 mice demonstrate waiting-dependent impulsivity in 5-CSRTT

Impulsivity can be assessed in the 5-CSRTT during training as well as when temporal variables are manipulated following training^{28, 29}. DAT Val559 mice exhibited no differences in premature responses compared to WT during training (Figure 1C), nor did they differ in other baseline performance measures (e.g. perseverative responses, % omissions, activity, and session length; Supplemental Figure 2A–D). Importantly, we observed no genotype difference in activity levels, as measured by beam breaks (Supplemental Figure 2C), an important consideration as both hyper- and hypo- locomotive behaviors could confound additional measures collected. Notably, when the delay between trial initiation and stimulus presentation was extended from the 5 sec delay used during training to a 15 sec delay, we detected a significant increase in premature responses in DAT Val559 mice (Figure 1D; WT n=15, Val559, n=19), indicative of a waiting impulsivity²⁵. Interestingly, when multiple delay times (2, 5, 10, and 15 secs) were randomized and delivered across a session, preventing a predictable use of time as a preparatory cue, DAT Val559 mice displayed fewer premature responses than WT animals (Figure 1E). When responses were analyzed for each of the delay values used, a trend was observed across all the tests, reaching statistical significance at the 15 sec delay (Figure 1F).

3.3 DAT Val559 mice display normal interval timing capacity with an increased response rate with increased waiting load

We hypothesized that the waiting impulsivity of DAT Val559 in the long delay condition in 5-CSRTT could be driven by a faster internal clock for interval timing. Agents and mutations that impact DA signaling, and DAT specifically^{30–33}, have been demonstrated to impact interval timing, the ability of animals to accurately report time between cue onset and an imposed response delay. To look at the ability to time intervals we implemented the Peak Interval paradigm (Figure 2A). We observed no significant difference between the estimated peak for delay timing whether at 5 sec or 15 sec between the DAT Val559 and WT littermates (Figure 2B and 2C; WT, n=10 and 9 respectively, VAL559, n=10 and 10 respectively). However, at the 15 sec interval DAT VAL559 demonstrated a significantly elevated response rate (Figure 2C).

3.4 Alterations in Go/NoGo reveal that DAT Val559 mice can withhold responding when a withheld response has a probability of being rewarded

Given our findings related to impulsivity in the 5-CSRTT, we decided to utilize another test commonly implemented to assess this trait, the Go/NoGo paradigm. In this test, we detected a difference in acquisition of the task with DAT Val559 progressing through habituation to the punish incorrect phase faster (Figure 3A; WT, n=8, Val559, n=12), though for this task this effect predominantly seems to be driven by differences in task performance on stage 1 (Supplemental Figure 3A & 3B). During the Go/NoGo sessions, when examined across sessions, DAT Val559 and WT mice initially demonstrated an equal percentage of omissions. By session 8, however, DAT Val559 mice demonstrated significantly more omissions than WT animals (Figure 3B). Increased omissions did not reflect an inferior recognition of the rules of the test as DAT Val559 were able to withhold responding during NoGo trials just as well as WT animals, if not better, as reflected by session correct rejections (Figure 3C). Moreover, no significant differences were detected across days for premature responding (Figure 3D). Next, in a single session, we extended the delay between trial initiation to stimulus presentation from 5 sec to 15 sec. This alteration produced no significant differences in percent omissions (Figure 3E). However, as with their superior performance during training, DAT Val559 mice made significantly more correct rejections than WT animals (Figure 3F) in the absence of significant premature responses (Figure 3G).

3.5 DAT Val559 mice have enhanced motivation

In prior studies, an increased response rate in the interval timing paradigm has been associated with increased motivation^{34–37}. To assess motivation more directly, we tested DAT Val559 and WT littermates for their willingness to sustain responses despite increasing demand, as assessed in the Progressive Ratio (PR) Test. Here, DAT Val559 mice exhibited clear evidence of enhanced motivation for reward, demonstrating a significantly increased average PR break point (Figure 4A; WT n = 22, Val559 n = 21), greater PR value met (Figure 4B), increased correct responses (Figure 4C), greater number of rewards collected (Figure 4D), and overall a significantly higher response rate (Figure 4E). No differences were detected in total number of head entries made, number of incorrect responses made, or total experimental session time (Supplemental Figure 4A–C). Importantly, there was no difference in sucrose preference (Figure 4F, n = 8 per genotype), indicating that the changes detected in motivational measures in the PR test are likely not driven by a change in reward preference.

4. DISCUSSION

Multiple rodent models have been advanced to gain insights into the mechanisms driving traits associated with ADHD^{38, 39}, including models featuring a disruption of DA signaling. Recently, we introduced the DAT Val559 mouse model as a construct valid model of disorders with DAergic dysfunction^{19, 20}. Although ADHD was the pathophysiological focus that led to our identification of the DAT Val559 variant, the mutation has been found in a female with BPD¹⁷, as well as two unrelated males with ASD¹⁸. Notably, ADHD is more prevalent in relatives of BPD subjects⁴⁰ and many individuals diagnosed with ASD meet clinical criteria for ADHD^{41, 42}, suggesting that the DAT Val559 mice may be most

properly considered to model DAergic alterations that can lead to distinct clinical trajectories depending on interacting genetic and environmental factors. While impulsivity is one of the core components of an ADHD diagnosis, and is present in both ASD and BPD, the source of that impulsivity and the type of impulsivity can vary greatly⁴³. Understanding the underlying driving force of impulsivity can guide future circuit-based evaluations of mechanism and possibly assist in the development of better treatments. Behavioral inhibition deficits can be categorized and tested for in a number of ways⁴³. One such type is impulsive action of pre-potent motor responses, which results in premature responding during a task that requires a set wait time before the motor response is appropriate (i.e. difficulty waiting). Here, we demonstrate that DAT Val559 have a waiting impulsivity, driven by a heightened motivational state.

Owing to elevated impulsivity traits in our two DAT Val559 expressing probands, as well as the maternal transmitting grandmother¹⁶, we initiated our testing efforts with the 5-CSRTT, a test that can reveal impairments in behavior ascribed to facets of attention, learning and impulsivity^{23, 24, 44}. DAT Val559 mice demonstrated no deficiencies in learning the 5-CSRTT. Indeed, these animals reached task criteria during training more rapidly than their WT littermates. Although initially surprising, these findings may relate to studies in humans reporting a stronger association of DAT polymorphisms with impulsivity versus inattention⁴⁵, as well as brain activity linked to inhibitory response control versus attentional orienting⁴⁶. When DAT Val559 mice were challenged to withhold responses for three times the length of the fixed delay imposed during training (5s to 15s), they demonstrated significant premature actions, suggestive of impulsivity. Interestingly, when tested with a variable delay, DAT Val559 animals demonstrated reduced premature responses that became increasingly evident as the delay was extended, demonstrating a differential effect of timing context on impulsive responding. These results are interesting with respect to recent findings that performance of subjects with ADHD is context dependent, being diminished relative to controls when simple two choice tests are used, versus a more complex five choice option⁴⁷. Additionally, studies done in amphetamine-treated rats have shown a connection between training context and waiting, such that performance improves on a response inhibition timing task in a variable timing climate, compared to an increase in premature responses when treated rats are tested in a fixed timing climate³². As DAT Val559 induces a state of chronic increased extracellular DA, we hypothesized that the DAT Val559 mice use timing cues differently.

Given the 5-CSRTT randomized delay paradigm may prevent mice from using interval time as a cue of when to expect the stimulus, we hypothesized, that increased premature responses by the DAT Val559 mice in the long delay paradigm may reflect perturbed time-estimation, underlying the appearance of impulsivity versus deficits in response inhibition *per se*. In support of this idea, significant evidence supports a contribution of DA signaling in interval timing^{30, 48–53}. For example, normal human subjects given amphetamine exhibit an increase in perceived elapsed time³³. These and other findings have led to suggestions that disruptions in the ability to perceive time intervals (e.g. sped up internal clocks and/or increased clock variability) may represent an ADHD endophenotype^{54–58}. However, DAT knockdown mice demonstrate an ability to estimate elapsed time correctly in the peak interval task, though they initiate responding sooner than WT animals and display a general

increase in overall response rate³⁵. To investigate this issue, we examined DAT Val559 mice and their WT littermates in the peak interval task, training mice to report the elapse of 5 or 15 secs, recapitulating the times used in 5-CSRTT. If time estimation was contributing to the differences observed in the 5-CSRTT, we hypothesized that we would observe genotype differences with the 15, but not the 5 sec interval, manifested as a leftward shift in the peak interval response curve of DAT Val559 mice. Instead, we failed to observe a genotype effect on time estimation at either time point, but instead detected an increase in response amplitude for the 15 sec interval by the DAT Val559 mice versus WT animals.

Increased response amplitude in the peak interval task has been suggested to reflect increased motivation^{35, 49}. Additionally, DA signaling has been directly implicated in response rate in the peak interval task, as overexpression of the DA D2 receptors (D2Rs) selectively in striatal medium spiny neurons delays the onset of response initiation and reduces response rates in the peak interval task^{37, 59}. Interestingly, the early start of response and the overall heightened responses seen in the peak interval task with DAT knockdown mice can be normalized by low doses of raclopride, a D2R antagonist, in a range thought to predominantly impact D2ARs³⁵. This is an intriguing with respect to the DAT Val559 model, as D2ARs are both constitutively activated by tonic DAT Val559-mediated DA efflux¹⁵ and drive efflux⁶⁰. Our ongoing studies seek to determine whether all, or subsets of DAergic projections are equivalently influenced in the DATVal559 model by functional and/or structural D2R/DAT interactions. In this regard, we previously documented D2AR-mediated physiological changes in substantia nigra DA neurons of DAT Val559 mice¹⁹, suggesting a reasonable orientation toward dorsal striatum.

Our peak interval studies indicate that DAT Val559 mice exhibit impulsive action when required to wait if trained on a fixed, predictable timing delay, independent of the ability of the animals to estimate elapsed time. This waiting impulsivity does not reflect blanket deficits in pre-potent motor inhibition as assessed in the Go/NoGo test. Thus, DAT Val559 mice had no trouble withholding behavioral responses on NoGo trials. In fact, as mice progressed through sequential sessions, we found that DAT Val559 mice actually trend towards being better at correct rejections than their WT littermates. Intriguingly, this performance appears to come at the cost of an increase in percentage of omitted responses, in contrast to the lack of a genotype difference in omissions in 5-CSRTT. Moreover, unlike our findings with the 5-CSRTT, we observed no increase in measures of impulsivity in Go/NoGo test when the delay before stimulus presentation was increased from 5 to 15 secs. In fact, the DAT Val559 mice made significantly more correct rejections than WT, arguing for better inhibitory control. These findings can be reconciled if the DAT Val559 variant instills an increased motivation/anticipatory drive rather than a frank deficit in motor inhibition. It may be that different motivational and/or response inhibition demands of the two tasks contribute to the distinct phenotypes of DAT Val559 mice on each task. Our studies of DAT Val559 mice in the progressive ratio task supports this idea, where increased motivation is suggested by a significant increase in break point, an increased number of values met before reaching a break point, an increased number of correct responses made and rewards earned, and a significant overall increase in response rate, relative to WT littermates.

We do not believe that this enhanced motivation is driven by a general increased hedonic value of rewards as no genotype differences were observed in sucrose consumption in the two-bottle choice test. Instead, we suspect that the increased willingness of DAT Val559 mice to expend effort for reward indicates an intrinsically elevated drive for reward. In contrast, DAT KO mice show a very strong increased sucrose preference that increases with exposure⁶¹, but do not show the same increase in motivational indices of progressive ratio tests⁶². These findings presumably reflect the differences in the genetic insult driving increased extracellular DA in the DAT Val559 model vs the DAT KO (e.g. DA leak versus constitutive loss of DA uptake) and the degree to which different DAergic pathways rely on the transporter to maintain extracellular and intracellular DA homeostasis.

Enhanced motivation for rewards in the DAT Val559 mice is at first blush difficult to align with reports of reduced reward motivation in ADHD subjects^{63, 64}, or reports of a lack of perturbed motivation altogether⁶⁵. Certainly, ADHD is not a homogeneous disorder and subjects with and without motivational changes likely arise from the distinct etiological mechanisms. Studies with construct-valid models such as the DAT Val559 mouse may thus assist in the dissection of subgroups defined not by overt behavioral categories but by underlying pathophysiology. In this regard, prior studies have demonstrated that increased levels of extracellular DA increase both motivation and risky/impulsive behaviors^{66, 67}. Motivational changes may also be reward-specific, accommodating reduced, normal or even elevated motivation for some rewards but not others⁶⁸. It has been argued that although intrinsic motivation may be low in ADHD subjects, they are more positively affected by the promise of an immediate extrinsic reward, leading to greater improvements in task performance compared to non-ADHD children^{69–71}. Thus, children with ADHD can improve their performance in measures such as error monitoring relative to their age-matched controls, in the context of specific extrinsic motivational conditions (i.e. performing a task for money or gifts rather than praise or a letter grade)⁶⁹. Additionally, receipt of rewards appears able to normalize impulsivity tendencies, suggesting a significant intersection between motivational and impulse control circuits^{72, 73}. An enhanced motivational drive for reward could also explain the increased performance of DAT Val559 mice in the Go/NoGo test where the mutants may be more motivated by the 50% chance of receiving a reward through behavioral inhibition, as compared to WT animals, yielding higher instances of correct rejections and low premature responses.

It seems reasonable to propose that the increased speed in task acquisition in both 5-CSRTT and the Go/NoGo task is driven by the increased motivation of the DAT Val559 mice. This idea nicely dovetails with the Reinforcement Sensitivity Theory from the human literature in ADHD, specifically the Behavioral Approach System (BAS), which hypothesizes that high BAS traits and resultant impulsivity are driven by an increased motivation for reward^{74, 75}. A high BAS trait index would be expected to allow for increased sensitivity to response-based reward and faster establishment between instrumental learning and reward, similar to the increased acquisition seen in the DAT Val559 with 5-CSRTT. Imaging studies involving BAS-related tasks have implicated several brain regions heavily involved in DA signaling including the dorsal and ventral striatum and orbital frontal cortices⁷⁴. Furthermore, children and adults with ADHD, specifically of the hyperactive/impulsive subtype, score higher on traits of BAS and display increased behavioral approach tendencies to reward associated

tasks^{76, 77}, bringing to mind the premature responses seen with the increased delay in 5-CRTT and the increased response rate in the peak interval task in DAT Val559 mice.

The idea that impulsivity in the clinical context may be for some subjects, at its core, a disorder of dysregulated motivation is important for the development of both behavioral and pharmacological interventions for disorders involving DA dysfunction including ADHD. Finally, given that the DAT Val559 variant has been identified in BPD and ASD, studies with the mouse model affords a unique opportunity to examine how various genetic and environmental factors shape the different clinical presentations that arise with changes arising from singular insults in DAergic signaling.

5. CONCLUSION

In conclusion, our studies reveal that DAT Val559 mice display several behavioral phenotypes with direct relevance to ADHD symptomology. As such, our work offers potential insights into the contribution that heritable alterations in DAT expression and function make to the pathophysiology of neurodevelopmental disorders. Our work predicts that molecular changes that impact tonic extracellular DA levels can enhance motivation for reward, which can emerge phenotypically as elevated impulsivity, effects separate from perturbations of intrinsic time estimation or deficits in attention. DAT Val559 mice may therefore prove useful as a construct valid model with which to elaborate the circuitry and signaling disruptions that drive clinical syndromes characterized by impulsivity as well as their treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- DAT Val559 mouse a relevant model for understanding ADHD and related disorders
- Model shows alterations in motivation, cognition, and impulsivity
- Impulsivity driven by increased reward motivation and changes with reward context

Figure 1. DAT VAL559 mice show alterations in 5-CSRTT acquisition and impulsivity

(A) DAT Val559 mice learn 5-choice serial reaction time task (5-CSRTT) faster than their WT littermate counterparts. (WT, $n=18$; Val559, $n=19$; Two-Way RM-ANOVA, time $P < 0.0001$, interaction $P < 0.0001$, genotype $P < 0.01$; post-hoc tests reveal $P < 0.05$ at day 6 and 27, $P < 0.01$ at day 9 and 26, and $P < 0.0001$ at Day 7 and 8). (B) DAT Val559, similar to their faster acquisition of the task, also display better accuracy on the 5-choice task on their first day experience of the full paradigm at a 16 sec stimulus duration (Two-Way RM-ANOVA, Time $P < 0.05$, interaction $P < 0.0001$, genotype $P < 0.05$; post-hoc tests reveal $P < 0.01$ at day 1 16 sec). (C) No genotype differences were observed for premature responses working down to and at baseline (WT, $n=18$; Val559, $n=19$; Two-Way RM-ANOVA). (D) DAT Val559 mice display a higher impulsivity than WT mice when the delay between the start of a trial and stimulus presentation is increased from 5 sec to 15 sec (two-tailed Student's t -test, premature responses $P < 0.05$ (WT, $n=15$; Val559, $n=19$)). (E) DAT Val559 mice display reduced impulsivity compared to WT mice when the delay between the start of a trial and stimulus presentation is variable/unpredictable (two-tailed Student's t -test, premature responses $P < 0.05$ (WT, $n=15$; Val559 = 19)). (F) Additionally, when premature responses were separated out based on the delay duration, DAT Val559 mice specifically show a reduction at the 15 sec duration (delay $P < 0.0001$, genotype $P < 0.05$, interaction $P < 0.05$; post-hoc reveals a genotype difference at the 15 sec delay, $P < 0.01$).

Figure 2. DAT Val559 mice have normal interval timing, but heightened response rates with longer wait times

(A) Training schematic for Peak Interval task. Mice were trained on a fixed interval of 5 sec or 15 sec (WT, $n=10$ and 9 per training group, VAL559, $n=10$ per training group). Mice were then tested on a probe trial for accuracy of response timing. (B) Both WT and VAL559 mice display the same response curves at the 5 sec timing (Two-Way RM-ANOVA; interaction $P > 0.05$, time $P > 0.05$, genotype $P > 0.05$). (C) However when the interval is increased to 15 sec, DAT Val559 display an increase in response rate amplitude (Two-Way RM ANOVA; interaction $P < 0.0001$, time $P < 0.0001$, genotype $P = 0.0557$).

Figure 3. DAT Val559 mice show alterations in the Go/NoGo task

(A) DAT Val559 mice acquire the Go/NoGo task differently than WT (WT, $n=8$, Val559, $n=12$; Two-Way RM-ANOVA, genotype $P < 0.05$, day $P < 0.0001$, interaction $P < 0.05$). (B) DAT Val559 mice progressively make more omissions (Two-Way RM-ANOVA, genotype $P < 0.05$, day $P < 0.0001$, interaction $P < 0.05$; post-hoc tests reveal $P < 0.05$ at day 8). (C) DAT Val559 mice trend towards making more correct rejections than WT (Two-Way RM-ANOVA, genotype $P = 0.07$, day $P < 0.0001$, interaction $P > 0.05$). (D) No difference was seen in premature responses between genotypes (Two-Way RM-ANOVA, Two-Way RM-ANOVA; interaction $P > 0.05$, time $P > 0.05$, genotype $P > 0.05$). (E) No genotype difference was present in percent omissions during long delay (two-tailed Student's t -test, $P > 0.05$). (F) DAT Val559 mice made more correct rejections during long delay (two-tailed Student's t -test, $P < 0.05$). (G) No genotype difference was seen in premature responses during long delay (two-tailed Student's t -test, $P > 0.05$).

Figure 4. DAT Val559 mice have enhanced motivation as seen in progressive ratio without alterations in sucrose preference

(A) DAT Val559 mice display a higher break point in the Progressive Ratio (PR) task compared to WT (two-tailed Student's *t*-test, PR Break $P < 0.04$ (WT, $n = 22$; Val559; $n = 21$)) and (B) PR met ($P < 0.05$). (C) DAT Val559 made more correct responses ($P < 0.05$) and (D) received more rewards ($P < 0.05$). (E) Higher response rates were also seen ($P < 0.05$). (F) Sucrose preference was calculated showing no statistical difference between genotype (Two-Way RM ANOVA; $P > 0.05$; $n = 8$ per genotype).