Effort-related decision making in humanized COMT mice: effects of Val^{158}Met polymorphisms and possible implications for negative symptoms in humans

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Abstract

Catechol-o-methyltransferase (COMT) is an enzyme that metabolizes catecholamines, and is crucial for clearance of dopamine (DA) in prefrontal cortex. Val^{158}Met polymorphism, which causes a valine (Val) to methionine (Met) substitution at codon 158, is reported to be associated with human psychopathologies in some studies. The Val/Val variant of the enzyme results in higher dopamine metabolism, which results in reduced dopamine transmission. Thus, it is important to investigate the relation between Val^{158}Met polymorphisms using rodent models of psychiatric symptoms, including negative symptoms such as motivational dysfunction. In the present study, humanized COMT transgenic mice with two genotype groups (Val/Val (Val) and Met/Met (Met) homozygotes) and wild-type (WT) mice from the S129 background were tested using a touchscreen effort-based choice paradigm. Mice were trained to choose between delivery of a preferred liquid diet that reinforced panel pressing on various fixed ratio (FR) schedules (high-effort alternative), vs. intake of pellets concurrently available in the chamber (low-effort alternative). Panel pressing requirements were controlled by varying the FR levels (FR1, 2, 4, 8, 16) in ascending and descending sequences across weeks of testing. All mice were able to acquire the initial touchscreen operant training, and there was an inverse relationship between the number of reinforcers delivered by panel pressing and pellet intake across different FR levels. There was a significant group x FR level interaction in the ascending limb, with panel presses in the Val group being significantly lower than the WT group in FR1-8, and lower than Met in FR4. These findings indicate that the humanized Val allele in mice modulates FR/pellet-choice performance, as marked by lower levels of panel pressing in the Val group when the ratio requirement was moderately high. These studies may contribute to the understanding of the role of COMT polymorphisms in negative symptoms such as motivational dysfunctions in schizophrenic patients.

Key words: motivation; operant; choice; catecholamine; metabolism; schizophrenia; depression

Introduction

Catechol-o-methyltransferase (COMT) is an enzyme that metabolizes catecholamines including dopamine (DA), norepinephrine, and epinephrine. COMT proteins are widely distributed in peripheral tissues and are found to be an intracellular enzyme particularly in the cerebral cortices and striatum (Myohanen et al., 2010). In these brain areas, neurons rather than glia are the main cell populations expressing COMT mRNA (Hong et al., 1998; Matsumoto et al., 2003; Tunbridge et al., 2007). The human COMT gene is located on chromosome 22, band q11.2, and it codes for two isoforms: a longer membrane-bound (MB-COMT, 1.5 kb) and a shorter soluble (S-COMT, 1.3 kb) protein (Mannisto & Kaakkola, 1999; Tunbridge et al., 2006). MB-COMT is the main form in the brain, and it has a greater affinity for DA than does S-COMT form (Lotta et al, 1995). Thus, MB-COMT is primarily involved in the inactivation of synaptic DA on the surface of pre- and post-synaptic neurons (Chen et al., 2011). COMT plays a more prominent role in regulating DA in prefrontal cortex (PFC) than striatum; more than 60% of DA is metabolized by COMT in prefrontal cortex, but no more than 15% in striatum (Karoum et al., 1994; Matsumoto et al., 2003). Consistent with this, alterations in COMT gene expression have been shown to be associated with disorders mediated by cortical DA (Tunbridge et al., 2006).

The human COMT gene Val^{158}Met polymorphism is a single nucleotide polymorphism (SNP; rs4680) that causes a valine (Val) to methionine (Met) substitution at codon 158 (Lachman et al., 1996). This SNP alters the thermostability of COMT enzyme and produces a trimodal distribution of COMT activity in PFC, in which the Val/Val (Val) homozygote has a greater COMT activity than the Val/Met heterozygote. Thus, the Val/Val form leads to higher metabolism and lower cortical DA levels, whereas the Met/Met (Met) homozygote displays reduced COMT activity and results in a higher cortical DA level (Lachmane et al., 1996; Chen et al., 2004; Billino et al., 2016). COMT Val^{158}Met polymorphism has been suggested to be linked to many psychiatric disorders, though the results are somewhat mixed. Some evidence has shown that schizophrenia, which is associated with

changes in cortical DA transmission (Sullivan et al., 2003, van Os & Kapur, 2009; Howes et al., 2015), also is associated with COMT Val^{158}Met polymorphism (Tunbridge et al., 2006; Hosak, 2007; O'Tuathaigh et al., 2007; Witte & Floel, 2012). However, human studies using the Wisconsin Card Sorting Test, a cognitive task that is widely used to assess frontal executive functions, have yielded mixed results regarding to the relationship between COMT Val^{158}Met polymorphism and schizophrenia (meta-analysis review, Barnett et al., 2007). For example, schizophrenia patients with Val/Val homozygote performed significantly worse than Val/Met and Met/Met carriers on the Wisconsin Card Sorting Test (Egan et al., 2001; Wang et al., 2010; Wan et al., 2011; Al-Asmary et al., 2014). In contrast, negative results finding that the COMT gene has little or no significant general role in schizophrenia susceptibility were also reported (Karayiorgou et al., 1998; Kang et al., 2010a, b). These inconsistent results could possibly be due to lack of statistical power in some of the SNP association studies. Nevertheless, some studies have shown that the Val/Val homozygote is associated with negative symptoms of schizophrenia, as marked by higher negative symptom scores in Val carriers tested on a Negative Symptoms Scale (Wang et al., 2010; Pelayo-Teran et al., 2008, 2011; Mao et al., 2016). Such inconsistencies in the literature are typical of modest sample association studies and therefore, alternative experimental strategies are needed to assess the impact of COMT activity on neurobehavioral processes known to be associated with psychopathology. Genomewide association studies are beginning to reveal the variants that are highly associated with human schizophrenia, and as major consortia (e.g. Psychiatric Genomics Consortium) combine efforts to generate the phenomenal sample sizes required, additional variants continue to be revealed. Mouse models harboring human allelic variation provide a system in which one can examine, in vivo, against a controlled genetic background, the effects of genetic variation on neurobiological and behavioral processes.

Loss of motivation, anergia, and psychomotor slowing are some of the major negative symptoms seen in patients with schizophrenia, as well as in depression and Parkinson's disease

(Salamone et al., 2006, 2016a,b; Treadway et al., 2012; Markou et al., 2013; Chong et al., 2015: Moran et al., 2017; Culbreth et al., 2018a, b). Negative symptoms in schizophrenic patients were reported to be related to increased sedentary behavior (Abdul Rashid et al., 2019) and low effort bias in patients tested on effort-based decision-making tasks (Barch et al., 2014; Moran et al., 2017; Bergé et al., 2018; Cooper et al., 2019; Luther et al., 2019). In first-episode schizophrenics, reduced willingness to expend high levels of effort was associated with amotivation but not self-reported anhedonia (Chang et al., 2019), and other studies reported that reduced effort expenditure in schizophrenic patients was not simply dependent upon reduced reward value or impaired processing (Bergé et al., 2018; Cooper et al., 2019). Considerable evidence from preclinical studies indicates that mesolimbic and prefrontal cortex DA, as well as related neural systems, play a key role in effort-related aspects of motivation (Salamone et al., 1991, 2016a, b, 2018a, b; Schweimer et al. 2005; Schweimer and Hauber 2006; Salamone and Correa, 2012; Mai et al., 2012). DA antagonism or depletion produces a low effort bias in rats tested on effort-based choice tasks (Salamone et al., 1991, 1994; Floresco et al., 2008; Salamone and Correa, 2012; Mai et al., 2012; Nunes et al., 2013; Hosking et al., 2015; Yohn et al., 2015; Salamone et al., 2015, 2016a; Bailey et al., 2020). The ability of DA antagonism or depletion of nucleus accumbens DA to reduce selection of high-effort instrumental behaviors reinforced by food is not dependent upon change in appetite, food preference, primary food motivation or reinforcement (Salamone et al.,1991; Nunes et al., 2013; Yohn et al., 2015). In addition to mesolimbic DA, prefrontal cortex DA also is involved in effort-related processes, as impaired transmission in prefrontal cortex has been shown to lead to a low effort bias (Schweimer et al. 2005; Schweimer and Hauber 2006; Munster et al. 2020). While the vast majority of papers reporting on the neural circuitry regulating effort-based choice have been performed in rats, there is now an emerging literature focusing on effort-based decision making in mice (Cagniard et al. 2006; Pardo et al., 2012; Trifilieff et al., 2013; Robles and Johnson, 2017; Fila et al., 2018; Yang et al., 2020; Bailey et al., 2020; DeBrosse et al. 2020; Correa et al. 2020). Moreover, despite evidence

showing that human COMT Val^{158}Met polymorphism mediates cortical DA in a trimodal manner, the role of COMT Val and Met variants on effort-related aspects of motivated behavior in rodent models remains unclear.

The present studies assessed the roles of Val and Met alleles on an effort-related decision-making task in a transgenic mouse model. By utilizing previously developed touchscreen operant procedures (Yang et al., 2019), two lines of COMT mice carrying either Val or Met homozygote (129S1/SvImJ background (abbreviated 129S1)), as well as 129S1 wild-type controls (WT), were tested in the study. These transgenic lines of mice are genetically identical except for the Val and Met alleles, which enables a strict comparison of the Val and Met variants *in vivo* (Risbrough et al., 2014). In the touchscreen paradigm, COMT mice were trained to choose between rearing up to press an elevated panel on the touchscreen on various fixed ratio (FR) schedules for highly preferred food (strawberry milkshake) vs. approaching and consuming concurrently available but less preferred choice food (high carbohydrate pellets). The current studies first assessed the effects of Val and Met genotypes on the ability to acquire various phases of the effort-related choice task, and then examined the effects of Val and Met mice on panel presses and choice food intake with various FR requirements (FR1, 2, 4, 8, 16). Based on the documented effects of higher COMT activity in Val homozygote on metabolizing cortical DA, and studies showing that prefrontal DA was involved in effort-based decision making, it was hypothesized that Val mice would show effort-related motivational impairments (i.e. decreased panel pressing and increased choice food intake) in the effort-based choice task.

Materials & Methods

Animals

Male COMT mice (The Jackson Laboratory) carrying either human COMT^{158} valine (Val/Val, 129-Comt$^{tm1(COMT)Xzho}$/J, N = 12; https://www.jax.org/strain/027990) or methionine homozygotes (Met/Met, 129-Comt$^{tm2(COMT)Xzho}$/J, N = 11; https://www.jax.org/strain/027993), and wild-type animals with a 129S1/SvlmJ background (WT, N = 12) were used in the present experiment. The Val and Met groups are genetically identical except for the Val or Met allele. Due to the known sex-differences in modulating COMT activity (Harrison & Tunbridge, 2008; Risbrough et al., 2014), only male mice were used in this study to eliminate potential sex-related confounding. Mice were housed individually in a vivarium maintained at 23˚C with a 12-h light/dark cycle (lights on at 07:00). These COMT mice were previously subjected to a modified pre-pulse inhibition paradigm (see review, Fitch et al., 2008) for auditory testing (see Supplementary Material). Mice weighed 24-32 g (4-5 months old) at the beginning of the study, and were food restricted to 85% of their free-feeding body weight for initial touchscreen training, after which modest growth was allowed throughout the experiment. Mice were weighed daily and fed supplemental chow to maintain weight, and water was available *ad libitum* in their home cages. All animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee, and followed NIH guidelines.

Touchscreen procedure training

Behavioral sessions (30-min sessions, 5 days/week) were conducted in Bussey-Saksida touchscreen chambers (Campden Instruments Led, Loughborough, UK). Each completed response (i.e. panel pressing) was reinforced with strawberry nutrition shake (Ensure Plus, Abbott Nutrition, Columbus, OH). The initial training procedure was conducted as described in detail in previous study (Yang et al., 2019). In brief, mice were individually exposed to 1.0 mL strawberry shake in their home cages for two days, and then were subjected to 30-min initial magazine training sessions for

three days. During these sessions, the feeder delivered 20.0 μL of the reinforcer automatically every 30 seconds, or 60.0 μL if one response to a white square panel (4.57 x 4.57 cm, 1.52 cm from the floor) on a black background screen was made. Following the initial magazine training, mice were trained to press the panel in a continuous reinforcement (i.e. FR1) schedule for two weeks. Subsequently, the panel was raised to the center of the touchscreen (6.1 cm from the floor) so that the mice had to rear up to respond. After four weeks of training on FR1 with the raised panel, the mice were exposed to a dish of fifteen high carbohydrate pellets (45 mg, Bio-Serv, Frenchtown, NJ) for two days in the home cages and were then tested on the concurrent FR/choice procedure. In this task, along with FR1 schedule for panel pressing, a dish of weighed pellets was used as a concurrent choice food and was placed in each chamber. At the end of the session, mice were removed immediately from the chambers, and pellet intake was determined by weighing the remaining pellets including spillage. In order to characterize the initial acquisition period of each phase of the touchscreen procedures, the numbers of panel pressing made in first five days of each of the training phases (Figure 1, i.e. FR1 with lower panel, FR1 with raised panel, FR1/choice) were analyzed.

Mice were trained in the FR1/choice procedure at least five weeks until they attained stable baseline levels of panel pressing and pellet intake. After this initial training, mice were then tested on a FR1/choice schedule for an additional week, and then were shifted to FR/choice with increasing FR requirements (FR 2, 4, 8, and 16; i.e., the ascending limb) with one FR schedule used each week. The number of FR requirement represents how many panel presses are needed to obtain one reinforcement delivery. Therefore, the cost for obtaining the milkshake reinforcer increased with higher FR requirements, whereas the choice food remained concurrently available. Following the week of FR16/choice schedule in the ascending limb, mice were shifted to a descending limb which started from FR 16, 8, 4, 2, back to 1, to see if there were any carryover effects or contrasts. Each of the concurrent FR/choice schedules was tested for one week (five daily sessions). For all the FR schedules except for FR 1, a 200 ms inter-response-interval was applied between each panel press.

The Bussey-Saksida boxes have live video available, and our laboratory has watched several hours of video during performance of the choice task. The animals have to rear to press the raised panel area, and it was never observed that the mice were able to eat out of the dish on the floor and press the raised panel at the same time.

Statistical analyses

The main dependent variables of interest were total number of panel presses and gram quantity of pellet intake during 30-min sessions. Data were separately analyzed for the ascending and descending FR sequences with repeated measures of analysis of variance (ANOVA) designs with genotype group as a between-groups factor and day (during acquisition) or FR levels (weeks with various FR levels) as within-groups factors. To determine the differences between conditions, post hoc comparisons were used if the group main effect was significant, or simple main effect analyses were conducted if the two-way interaction was significant (Keppel, 1991). Pearson correlation analysis was used to determine the relationship between number of reinforcers delivered by panel pressing and pellet intake. All data were presented as mean ± SEM, and significance level was set at $\alpha = 0.05$. SPSS 23.0 for Windows was used as a statistical analyses program.

Results

Acquisition

Mice were trained sequentially on the FR1 schedule with the lower panel target, then the FR1 with the raised panel target, and then with FR1/choice (Figure 1), and their ability to acquire these procedures was measured by the number of panel presses in the first week (five days) of each phase. During FR1 acquisition with the lower panel target (Figure 2A), a two-way ANOVA revealed a significant day effect ($F(4, 128 = 42.972$, $p < 0.001$) and significant group x day interaction ($F(8, 128) = 3.226$, $p < 0.01$), but no group effect ($p = 0.436$). The simple main effect analyses revealed the Val group had significantly more panel presses than the control group at Days 4 and 5 (both $p < 0.05$), and the control and Met groups differed on Day 4 ($p < 0.05$). These results demonstrated that the humanized COMT transgenic mice were capable of acquiring panel pressing as an instrumental behavior in the touchscreen paradigm, and in fact, the Val group showed higher performance compared to control group at the end of the first week of training. During the acquisition of the raised panel phase (Figure 2B), there was a significant day effect ($F(4, 128) = 32.777$, $p < 0.001$), but no significant group x day interaction ($p = 0.166$) and no significant interaction of the linear trends ($p=0.326$). There was no significant group effect ($p = 0.103$), though some animals in the Val group did show a tendency towards lower levels of panel pressing with the raised panel throughout the first 5 days of testing with the raised panel (Figure 2B), while the Met group only tended to show low pressing on the first two days. Lastly, in the phase during which the concurrent food pellets were introduced (Figure 2C & 2D), there was a significant day effect ($F(4, 128) = 5.717$, $p < 0.001$) and a significant group effect ($F(2, 32) = 4.465$, $p < 0.05$), but no significant group x day interaction ($p = 0.949$) in the panel presses. For pellet intake (Figure 2D), there was a significant day effect ($F(4, 128) = 30.763$, $p < 0.001$) and a subtle but not significant group effect ($p = 0.053$), and no group x day interaction ($p = 0.645$). Post hoc analyses of the group effect revealed that the Val group made significant fewer panel presses than the control group ($p < 0.05$), and despite the fact that the overall

group effect did not reach significance with pellet intake, analyses were performed indicating that there was a marginally significant effect of the Val group consuming more pellets compared to the WT group ($p = 0.050$). Additional analyses examined the initial transition from FR1 without concurrent food to FR1/choice, in which pellets were concurrently available (Figure 3). This figure expresses performance on the first day of FR1/pellet choice as a percentage of the last day of FR1 alone training. There was an overall significant difference between groups ($F(2,32) = 3.612$, $P < 0.05$), and paired comparisons showed that the Val group significantly differed from the 129S1 control group ($p < 0.05$).

Changes in FR levels

Figure 4 shows an overview of panel presses (Figure 4A) and pellet intake (Figure 4B) in various FR levels in the ascending and descending limbs of the study. Six mice were excluded from analyses in the descending limb (one in WT, three in Val, two in Met group) due to the fact that they were not able to re-baseline in the descending limb (panel presses < 10 in all FR levels), and therefore two limbs were separately analyzed.

In the ascending limb, there was a significant main effect of FR level in panel presses (Figure 4A, $F(4, 124) = 15.96$, $p < 0.001$) and a significant group x FR level interaction ($F(8, 124) = 2.76$, $p < 0.01$), and the group main effect approached significance ($p = 0.051$). To examine the further differences between groups and the sources of interaction, three different two-way ANOVAs (WT vs. Val, WT vs. Met, and Val vs. Met) were conducted. Results of WT control vs. Val revealed significant group main effect ($F(1, 21) = 7.649$, $p < 0.05$), FR level main effect ($F(4, 84) = 10.624$, $p < 0.001$), and group x FR level interaction ($F(4, 84) = 5.309$, $p < 0.01$). Moreover, post hoc analyses showed that the Val group pressed significant less than WT group in FR1, FR2, (both $p < 0.01$), FR4, FR8, and FR16 ($p < 0.05$). For the WT control vs. Met analysis, there was a significant FR level main effect ($F(4, 80) = 13.239$, $p < 0.001$), but no group effect ($p = 0.426$) or group x FR level

interaction ($p = 0.601$). Lastly, the results of Val vs. Met showed that there was a significant FR level main effect ($F(4, 84) = 8.211$, $p < 0.001$) and group x FR level interaction ($F(4, 84) = 3.686$, $p < 0.01$), and a trend towards a main effect ($p = 0.08$). Planned comparisons revealed that the Val group pressed significantly less than the Met group on the FR4 schedule ($p < 0.05$). Furthermore, an orthogonal analysis of trend revealed a significant interaction of the quadratic trends for the Val vs. Met comparison ($F(1, 21) = 4.503$, $p < 0.05$), which demonstrates that the quadratic function shown across FR levels (i.e., the tendency to increase from FR1 to FR8, and decrease at FR16) differed between the Val and Met groups. While the Met group showed a strong inverted-U shaped trend across FR levels, the Val group was relatively flat in this regard (Figure 4A). For the pellet intake in the ascending limb (Figure 4B), there was a significant FR level main effect ($F(4, 124) = 41.812$, $p < 0.001$), but no group effect ($p = 0.159$) or group x FR level interaction ($p = 0.539$).

For the descending limb, a two-way ANOVA revealed a significant FR level main effect ($F(4, 100) = 8.679$, $p < 0.001$) in panel presses (Figure 4A). There was a tendency towards a significant group effect ($p = 0.087$), and there was no group x FR level interaction ($p = 0.271$). For pellet intake (Figure 4B), there was significant FR level main effect ($F(4, 100) = 8.407$, $p < 0.001$) and a group x FR level interaction ($F(8, 100) = 2.075$, $p < 0.05$) but no group effect ($p = 0.288$). The simple main effect analyses revealed no significant difference in pellet intake between any two of the groups across all FR levels. It is likely that the analysis of the descending limb was substantially affected by the loss of animals who failed to respond after exposure to FR16 on the ascending limb.

In order to examine the relationship between performance on the high-effort alternative (i.e. panel pressing) and low-effort alternative (i.e. pellet intake) across FR levels, the number of panel presses was transformed into number of reinforcers delivered, which equals the total panel presses for that ratio divided by the FR requirement. Figure 5A shows the relationship between number of reinforcers delivered (black solid lines) and pellet intake (red dashed lines) across various FR levels in all three groups. Correlation analyses revealed a significant negative correlation between number

of reinforcers delivered and pellet intake in both ascending ($r = -0.882$, $p < 0.001$, Figure 5B) and descending limbs ($r = -0.752$, $p < 0.01$, Figure 5C). This inverse relationship demonstrates that mice tended to shift their preference from the high-effort alternative toward low-effort alternative as the work requirement (i.e. FR requirement) increased. When each group was analyzed separately, this relationship was strongest in the control group in both the ascending ($r = -0.914$, $p < 0.05$, $R^2=0.836$) and descending limbs ($r = -0.962$, $p < 0.01$, $R^2=0.925$), and the proportion of variance accounted for was lower in the Met (ascending: $r = -0.775$, $p = 0.124$, $R^2= 0.601$; descending $r = -0.769$, $p = 0.129$, $R^2=0.591$) and Val (ascending: $r = -0.798$, $p = 0.105$, $R^2=0.637$; descending $r = -0.865$, $p = 0.058$, $R^2=0.748$) groups.

Discussion

Rodent models that combine both behavioral and transgenic approaches are useful for understanding molecular processes that regulate behavior, and ultimately could be useful for providing insights into the development of clinical interventions for psychopathology. The human COMT Val^{158}Met gene polymorphism has been shown to be associated with dysfunctions that are cortically mediated and DA related, such as motivation deficits that are commonly seen in schizophrenia and mood disorders (Tunbridge et al., 2006), but results are often mixed due to human genetic heterogeneity. In contrast, the use of a humanized COMT mouse model is important because it enables neuroscientists to strictly compare distinct role of Val and Met variants *in vivo* (Risbrough et al., 2014) in an animal model. The present experiments studied humanized COMT mice that were assessed using a previously established touchscreen choice paradigm (Yang et al., 2020), and the results showed that all transgenic COMT mice exhibited the ability to acquire the panel pressing. Moreover, the Met carriers were generally unimpaired on various aspects of effort-based choice compared to 129S1 control mice. In contrast, Val carriers showed significantly decreased numbers of panel presses with slight tendency towards increased intake of concurrently available choice food compared with the 129S1 control mice, indicating a potential link between the Val allele and motivational impairments related to exertion of physical effort. It is unlikely that the pattern of effects shown by Val mice were due to a reduction in reinforcement value or sensitivity, as previous research has shown that devaluation of food reinforcement in mice tested on this task strongly decreases both panel pressing and pellet intake (Yang et al., 2020). Furthermore, recent studies in mice demonstrated that DA antagonism and modulation of striatal DA D2 receptor signaling had predominant effects on effort-based decision making and not value-based decision making (Fila et al., 2018; Bailey et al., 2020).

The present study is the first to investigate the roles of COMT Val and Met alleles in effort-related aspects of motivated behavior. Similar to the performance of CD1 mice in previous work that

established the touchscreen effort-based choice paradigm (Yang et al., 2020), the 129S1 control mice were able to acquire the task at each phase, and in the FR/choice tests they showed substantial levels of panel pressing up to FR8. With the FR16 schedule, there was a decrease in panel pressing in the 129S1 control mice, indicating that in the presence of the alternative food source (pellets), these mice showed "ratio strain", and decreased panel pressing relative to the FR8 schedule. Overall, the behavioral results showed that across FR level there was a reciprocal relationship between reinforcers obtained through panel pressing and intake of the concurrently available pellets (Figure 5), which is consistent with the concept from behavioral economics that the food pellets were able to act as a substitute for the milkshake used to reinforce panel pressing. Moreover, COMT Val and Met mice showed no initial acquisition impairment compared with 129S1 control mice, and actually they made significantly more panel presses than control mice during the last few days of initial FR1 acquisition when the panel target was low (Figure 2A). The intact acquisition ability seen in our COMT mice is consistent with a large number of studies reporting that mice with alterations in COMT activity showed no impairments across several learning and cognitive functions (Gogos et al., 1998; Hassio et al., 2003; Papaleo et al., 2008; Tammimaki et al., 2010; Simpson et al., 2014). Moreover, these results during initial acquisition of panel pressing are mirrored by parallel studies in which the Met and Val mice were able to acquire an auditory processing task, and in fact showed enhanced performance compared to the 129S1 controls (see Supplementary Material). These findings indicate that the impairments observed in Val mice on effort-based choice were unlikely to be due to a broad or general impairment in learning and cognitive function, though it is possible that impairments in cognitive flexibility specifically related to ratio performance contributed to the difficulties that Val animals had in adjusting to the increasing ratio requirements (but see also Nogueira et al. 2020, who reported improved motor learning in people with the Val/Val genotype).

In acquiring the FR1 task with the raised panel target, and also in terms FR1/pellet choice performance under various FR/choice schedules, the Met group had some animals with lower

responding than controls, but showed no significant impairments. The only tendency towards an impairment in the Met group was in the initial transition from FR1 alone to FR1/chow feeding choice, on the very first day (Figure 3). These findings indicate that introduction of the humanized COMT gene in itself did not produce widespread impairments on acquisition or performance of the various aspects of the panel pressing task. Moreover, in view of research indicating that animals with the Met genotype have decreased COMT activity, it is interesting to note that recent evidence indicates that the COMT inhibitor tolcapone did not alter effort-related choice in mice (DeBrosse et al. 2020). In contrast, the Val group showed impairments compared to the 129S1 control group at virtually every level of performance. On the first day of FR1/choice, the Val group was significantly impaired relative to the 129S1 control group in terms of the transition from the last day of FR1 performance without concurrently available pellets to the first day in which pellets were also available in the chamber (Figure 3). Putting an alternative food in the chamber produced a small reduction (approximately 35%) in panel pressing in the 129S1 group, but a much larger one (approximately 65%) in the Val group. In addition, the Val group showed significantly fewer panel presses and a slightly increased tendency to consume pellets compared with the 129S1 controls during the ascending sequence of FR1, 2, 4, through FR8, suggesting that Val mice were more sensitive to the increased physical effort requirement, and therefore had reduced preference for panel pressing compared to the concurrently available food pellets. Furthermore, Val mice were not only impaired relative to 129S1 control mice, but they also significantly differed from the Met mice. In analyzing the overall significant group x FR level interaction during the ascending sequence, it was determined that a major source of the interaction was the difference between Val and Met groups. With this specific comparison, there was a Val/Met group x FR level interaction, and a significant interaction of the quadratic trends. The trend analysis demonstrated that while Met mice showed a strong quadratic trend (i.e., increasing panel pressing from FR1 to FR8, but a decrease at FR16), the Val animals showed a significantly different trend across the FR levels. Moreover, the Val group

showed significantly fewer panel presses at the FR4 level compared to the Met group. Thus, although 129S1 and Met mice showed a strong tendency to be activated by the challenge of a higher work requirement on the FR schedules, at least up to FR4 and FR8, the Val mice did not respond well to this challenge, and essentially showed flat responding across all the FR schedules. This low effort bias in Val mice, characterized by the reduced preference for the high effort/high reward option compared to the low effort/low reward option and a failure to respond to increased effort requirements, can be interpreted as consistent with the role of DA in mediating elasticity of demand for food (Aberman & Salamone, 1999; Salamone et al. 2017). Elasticity of demand is a behavioral economics term that refers to how sensitive or responsive consumers are to price changes of a product, and evidence has shown that animals with impaired DA transmission show increased selection of low cost substitutes that are concurrently present (see review, Salamone et al., 2017). Therefore, it is possible that the low effort bias observed in Val mice is due to a reduction of prefrontal DA level induced by higher COMT enzyme activity in Val carriers, which is consistent with previous studies of prefrontal DA dysfunction in rats (Schweimer et al., 2005; Schweimer and Hauber, 2006; Munster et al., 2020), as well as and other research involving systemic DA antagonists, and depletions of subcortical DA (Salamone et al., 1991, 1994; Floresco et al., 2008a; Salamone and Correa 2012; Mai et al., 2012; Nunes et al., 2013; Hosking et al., 2015; Yohn et al., 2015; Salamone et al., 2015, 2016a).

It should be noted that COMT metabolizes not only DA but also other catecholamines such as norepinephrine (Napolitano et al., 1995; Tunbridge et al., 2006). It is thus possible that norepinephrine was also involved in the suppression of panel presses and increased choice food intake seen in COMT Val mice. However, it is unlikely that this is the case. Previous studies have suggested that administration of norepinephrine uptake inhibitors desipramine and atomoxetine had no effects on effort-based choice, and actually exacerbated motivational impairments at high doses, in rats tested on the FR5/chow-feeding and progressive ratio/chow-feeding tasks (Yohn et al., 2015,

2016). Although one could suggest that Val mice had reductions in norepinephrine transmission that mediated some of the behavioral effects seen in the present studies, evidence from COMT-knockout mouse models reported that COMT gene disruption increased cortical DA levels, but had no effects on either norepinephrine or 5-hydroxytryptamine (Gogos et al., 1998). Additionally, a microdialysis study indicated that administration of the COMT inhibitor tolcapone significantly potentiated the increase of extracellular DA levels, but had no effects on norepinephrine, in rat medial prefrontal cortex (Tunbridge et al., 2004). In sum, the results of these studies suggest that the motivational effects seen in COMT Val and Met mice in the present work can potentially be attributed to changes in cortical levels of DA. Nevertheless, future research that involves neurochemical measurements of catecholamines, as well as pharmacological interventions such as DA agonists and antagonists, would be needed to determine the precise mechanisms underlying the low effort bias seen in humanized COMT Val mice. Furthermore, additional behavioral testing related to other aspects of reinforcer processing need to be conducted.

Another important aspect of behavioral performance that is regulated by DA is motor function. It could be possible that the suppression of panel pressing and increase of choice food intake observed in the Val mice was due to subtle motor impairments induced by transgenic humanized COMT gene. Although locomotor activity was not assessed, Val and Met groups exhibited intact motor functions, such as eating and drinking, in the present study. In the first FR1 alone task, neither COMT group showed significant decreases in number of panel presses compared with 129S1 group (Figure 2A), and in fact tended to show higher responding. The Val group showed initial sensitivity to making an alternative food source available in the chamber (Figure 3), and also showed significant decreases in panel presses in various FR/choice schedules (Figure 4), which is an indication of a low effort bias mediated by DA as discussed above. Future research should study the performance of Val animals when there is no alternative food source available, to determine if ratio strain is still evident. Nevertheless, several studies have reported that transgenic mice with either COMT overexpression or

disruption did not show motor impairments on a battery of tests including open field, rotorod, and Morris water maze (Haasio et al., 2003; Susuki et al., 2009; Tammimaki et al., 2010; Simpson et al., 2016). On the other hand, Solis et al. (2017) reported that increased COMT expression would contribute to abnormal motor functions related to L-DOPA-induced dyskinesia (Solis et al., 2017). Despite the lack of evidence suggesting that alterations of COMT activity would suppress eating, one could argue that subtle changes in rearing could lead to suppression of panel presses. However, in this context, it should be emphasized that the present work is focused on physical effort as opposed to cognitive effort (see Hosking et al., 2015), and rearing was specifically used to provide a physical effort challenge related to panel pressing. Although we did not assess rearing behavior in the current study, Risbrough et al. (2014) showed that there was no difference in rearing between Val vs. Met male mice. Moreover, it should be noted that foraging behaviors, including rearing and effort exertion in lever or panel pressing, are not simply motor responses. Considerable evidence has suggested substantial overlap between motor functions and motivation in terms of underlying neural and molecular mechanisms, with DA systems mediating the overlap between these two constructs (see review, Salamone et al., 2017). Clinical evidence also highlights the overlap between motivational and motor function; for example, depressed people commonly show both psychomotor slowing and reductions in locomotion (Todder et al. 2009). To address this debate, future studies are required to determine the specific role of COMT Val^{158}Met polymorphism in the suppression of touchscreen panel pressing.

Although the COMT gene and its SNPs have been extensively studied, little is known about the dissociative roles of COMT Met and Val alleles in mediating behavior and cognitive functions *in vivo* (Risbrough et al., 2014). Developing effort-related touchscreen paradigms and using transgenic COMT mouse models in the current studies has provided additional novel findings that highlight the role of COMT Val and Met variants in regulating motivated behavior, and may contribute to a better understand the genetic basis of psychopathology. Accordingly, future directions of research should

involve investigation of male-female differences (Gogos et al., 1998; Harrison & Tunbridge, 2008; Risbrough et al., 2014), as well as examination of the effects of COMT inhibitors and DA related agents (Huotari et al., 2004; Tunbridge et al., 2004; Papaleo et al., 2008), which would be valuable for further understanding of the role of COMT in effort-based aspects of motivation.

In conclusion, the present studies illustrate the potential relation between the COMT Val allele and motivational dysfunctions, and provide an important finding in relation to the role of COMT enzyme activity and motivation. Optimal motivation and other cognitive functions are critically dependent on the particular levels of cortical DA and stimulation of DA receptors (Floresco, 2013), which can be affected by COMT enzyme activity. The present work has potential significance for understanding the role of COMT Val^{158}Met gene polymorphism in motivation symptoms that are seen in patients with schizophrenia, depression, and other disorders, and may ultimately contribute to facilitating the development of clinical interventions and treatments. Future studies should focus on the performance of Val and Met mice on tasks involving other functions of prefrontal cortex, such as executive function (e.g. Garner et al. 2006). In summary, these studies demonstrated that testing humanized COMT mice in the touchscreen paradigm is a useful animal model for assessment of underlying genetic modulation of motivated behavior, and illustrates the possible relation between COMT polymorphisms and motivational dysfunctions in human psychopathologies.

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Figure 1

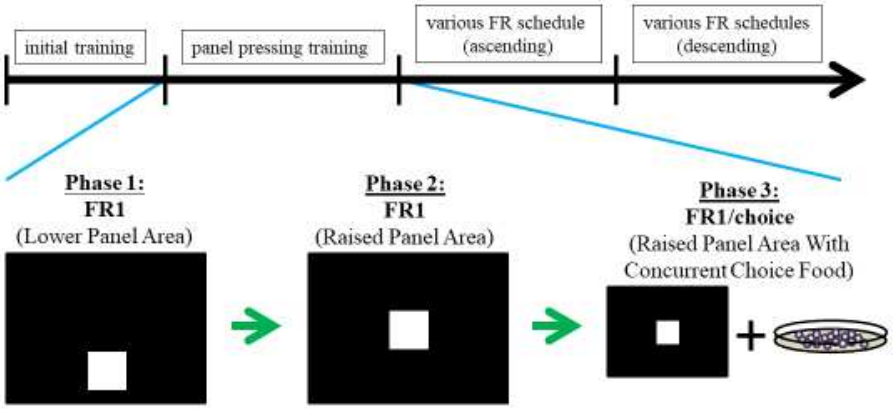

Figure 1. Timeline and schematic representations of panel area presented on the touchscreen in the mouse touchscreen procedures. Mice were initially trained in FR1 with lower panel area, and then were shifted to FR1 with raised panel area. After which, a dish of weighed pellets was introduced as a concurrent choice food (FR1/choice task)

Figure 2

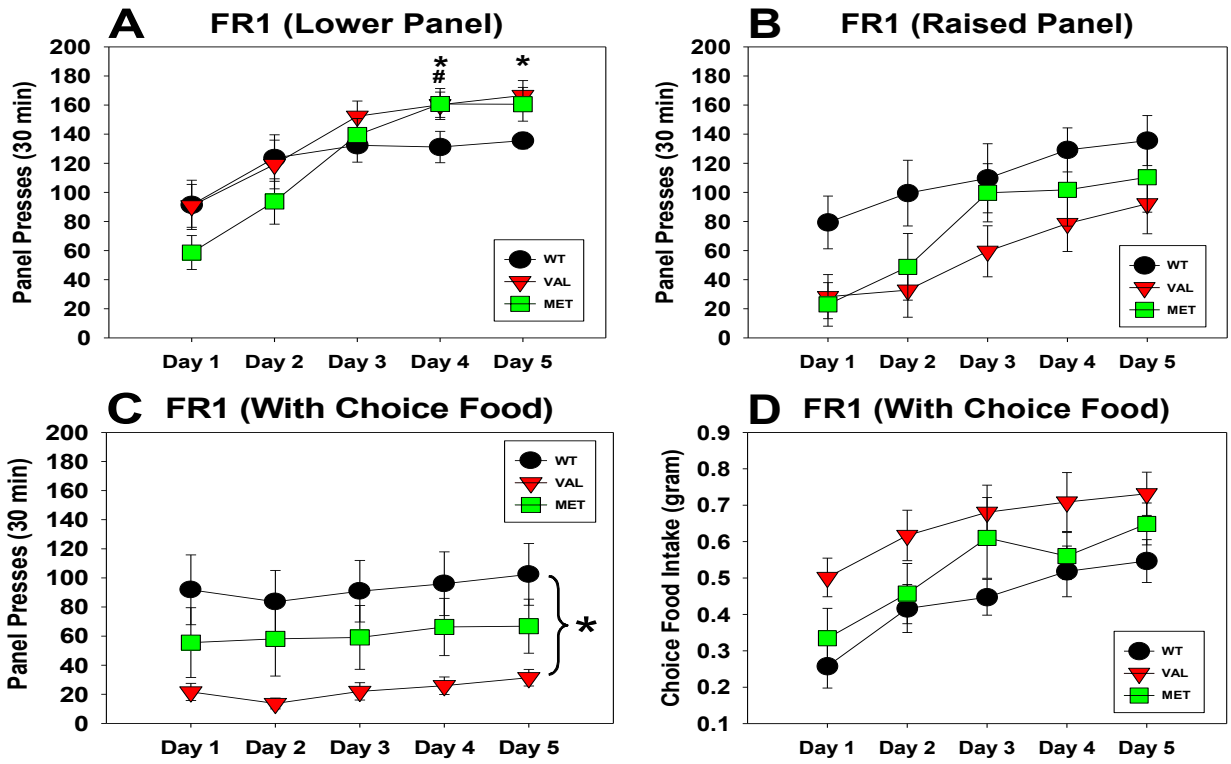

Figure 2. The ability of COMT mice to acquire the touchscreen procedures. All mice were sequentially trained in FR1 with lower panel (**A**), FR1 with raised panel (**B**), FR1/choice with raised panel (**C** and **D**). **A – C** panel presses and **D** concurrently available pellets were measured in first five days in each phase. * $p < 0.05$, significant difference between valine (VAL) group and wild type (WT) group; # $p < 0.05$, significant difference between methionine (MET) group and WT group

Figure 3

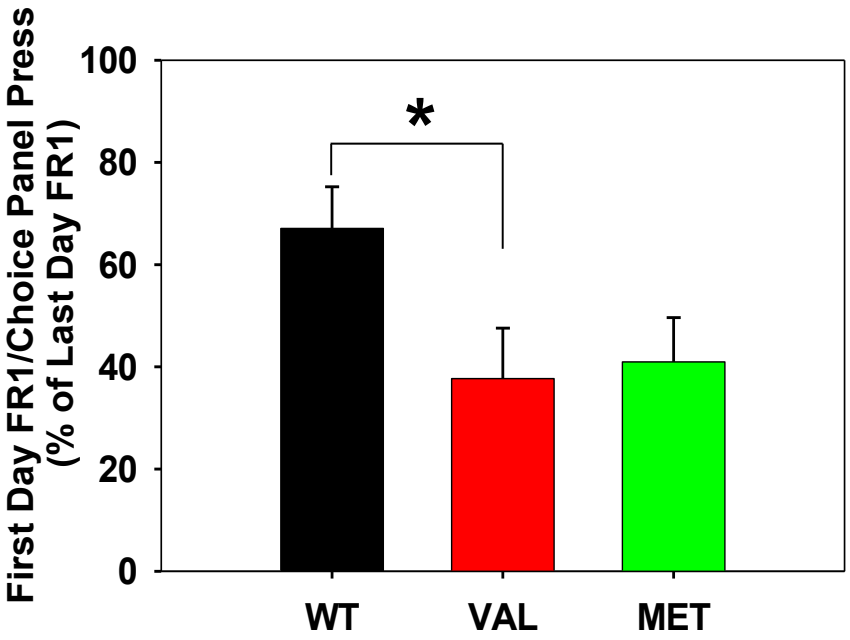

Figure 3. Performance of COMT mice on the first day of FR1/pellet choice as a percentage of the last day of FR1 alone training. * $p < 0.05$, significant difference between VAL group and WT group

Figure 4

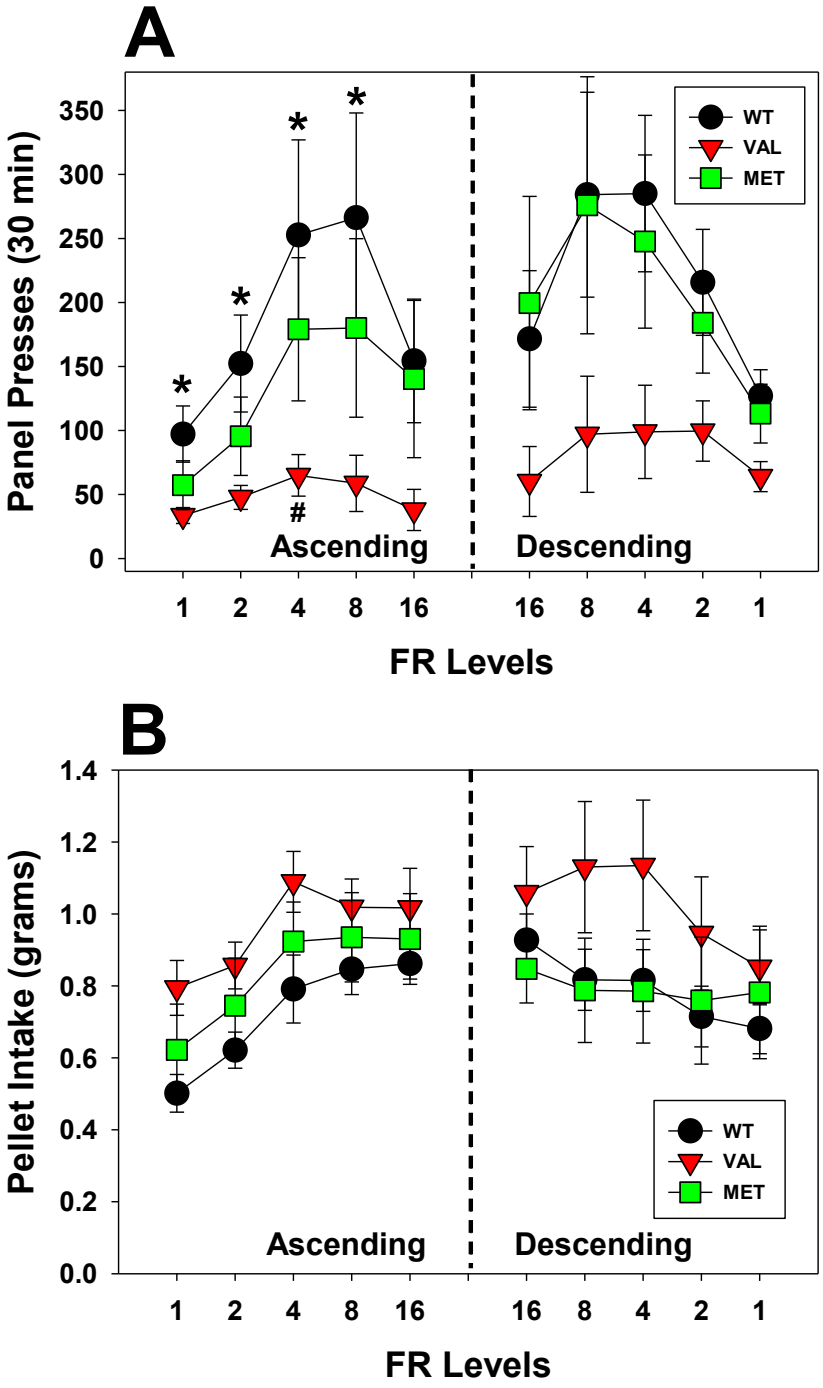

Figure 4. The effects of VAL and MET variants on panel presses (**A)** and intake of concurrently available food pellets (**B)** across various FR requirements (ascending limb: FR1, 2, 4, 8, 16; and descending limb: FR16, 8, 4, 2, 1). * $p < 0.05$, significant difference between VAL group and WT group; # $p < 0.05$, significant difference between VAL and MET group

Figure 5

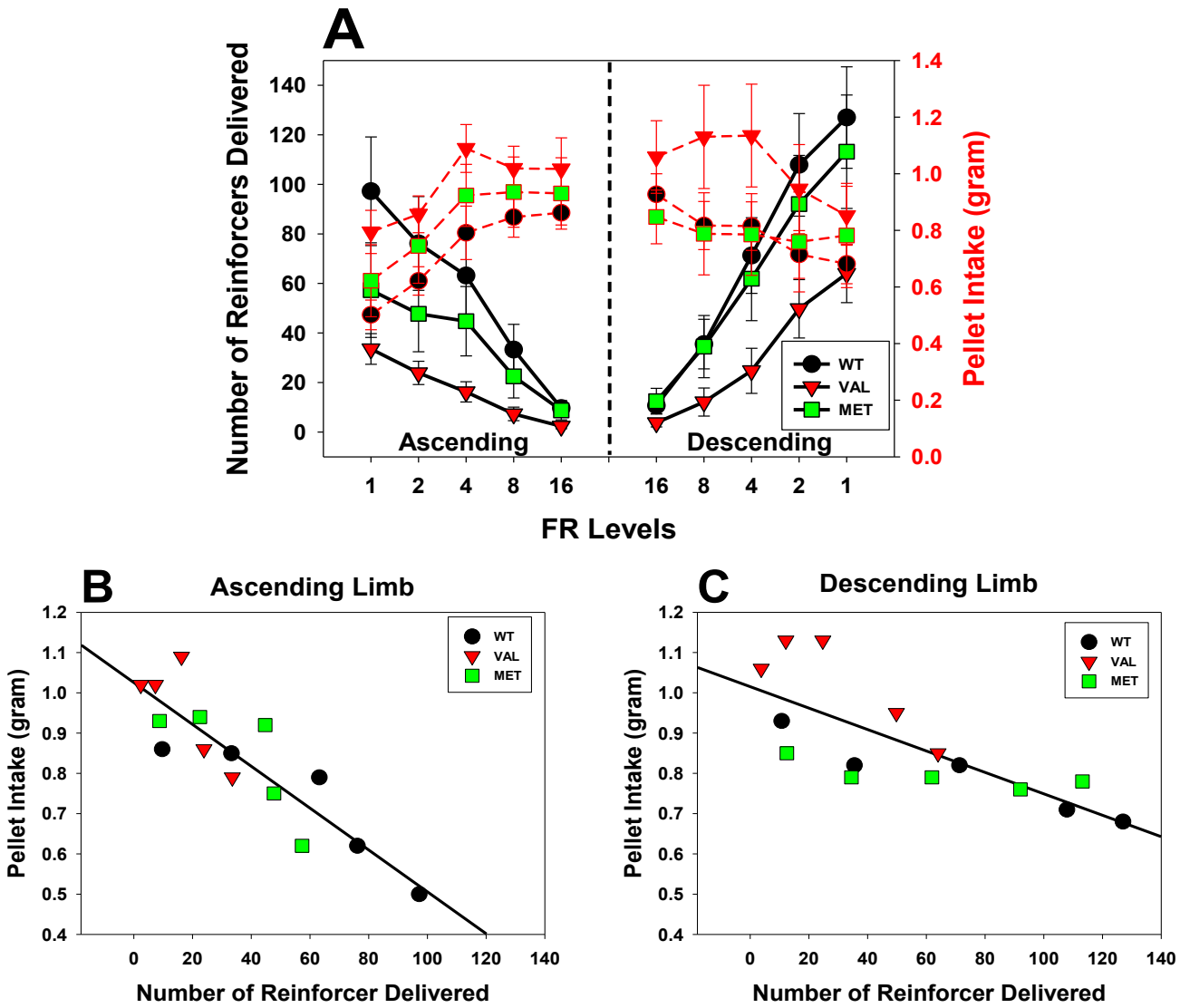

Figure 5. The relationship between engaging in the high-effort alternative (i.e. panel pressing) and low-effort alternative (i.e. pellet intake) in the effort-related touchscreen choice paradigm. **A.** There was an inverse relationship between number of reinforcers delivered from panel pressing (left y-axis; black solid lines) and gram quantity of food pellet intake (right y-axis; red dashed lines) across various FR levels in all three groups. **B – C.** Scatterplots and regression lines showing inverse relationship between number of reinforcers delivered and pellet intake in ascending limb ($r = -0.882$, $p < 0.001$) and descending limb ($r = -0.752$, $p < 0.01$).