Disruptions in effort-based decision-making following acute optogenetic stimulation of ventral tegmental area dopamine cells

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Abstract

The dopamine system has been implicated in decision-making particularly when associated with effortful behavior. We examined acute optogenetic stimulation of dopamine cells in the ventral tegmental area (VTA) as mice engaged in an effort-based decision-making task. Tyrosine hydroxylase-Cre mice were injected with Cre-dependent ChR2 or control eYFP in VTA. While eYFP control mice showed effortful discounting, laser stimulation of dopamine cells in ChR2 animals disrupted effort-based decision-making by reducing choice towards the lever associated with a preferred outcome and greater effort, without affecting discrimination processes or non-specific motoric behaviors. These findings suggest increases in dopamine activity can disrupt effort-based decision-making.

The neurotransmitter dopamine has been implicated in a wide-range of learning and motivational processes including effort-based decision-making. At present, many studies investigating dopamine's role in this form of decision-making have found a dichotomous pattern of results in which receptor antagonism (Robles & Johnson, 2017; Bryce & Floresco, 2019) or depletion (Cousins & Salamone, 1994; Mingote et al., 2005) of dopamine results in a biasing of behaviors away from more preferred rewards associated with increased effort. Conversely, dopamine stimulation (Bardgett et al., 2009; Wardle et al., 2011) or transgenic overexpression of dopamine receptors (Trifilieff et al., 2013) tends to invigorate responding for outcomes associated with higher effort. While much has been learned, a limiting factor with these approaches is they lack temporal specificity, thus constraining resultant interpretations. We employed optogenetics to determine the effect of acute ventral tegmental area (VTA) dopamine stimulation at the timepoint of decision-making. Such an approach allows for a focus strictly on the role of dopamine in evaluating choices with different cost-benefit outcomes, without the potentially confounding factor of consistently altered dopamine signaling throughout the decision-making test.

Twenty-three mice expressing Cre recombinase under the control of the tyrosine-hydroxylase promoter were produced via outbreeding with wild-type C57BLJ mice (Jackson Laboratory, Bar Harbor, ME) under the auspices of the Michigan State University Institutional Animal Care and Use Committee. Mice were housed by sex, up to five per cage pre-surgery. At ten weeks of age, mice underwent stereotaxic surgery in which 0.5 µl of either AAV5-Ef1α-DIO-ChR2-eYFP (n = 10♂, n = 8♀) or AAV5-Ef1α-DIO-eYFP (n = 5♂, n = 3♀; Vector Biolabs, Malvern, PA) was virally infused unilaterally at the level of the VTA (AP -3.08, ML +/-0.6, DV -4.5) in a manner counter-balanced for hemisphere. Optic fiber ferrule tips (200µl core, 4.1mm; Thorlabs, Newton, NJ) were implanted dorsal to the injection site (AP -3.08, ML +/-0.6, DV -4.5) and affixed with dental acrylic (Lang Dental Manufacturing Co, Wheeling, IL). Mice were given four weeks post-surgery to recover and to allow for sufficient viral transfection, during which

time they were singly housed, which continued for the duration of the experiment in order to avoid damage to ferrule tips.

Following recovery, mice were food deprived to 90% of their free-feeding weight by limiting access to a single daily portion of lab chow. Subsequently, mice were trained in operant chambers (Med Associates, St Albans, VT) where in separate sessions, responses on one lever led to 50 µl delivery of a preferred high value reinforcer (e.g., left lever→orange-flavored 20% sucrose), whereas a different lever resulted in delivery of the lower value outcome (e.g., right lever→grape-flavored 5% sucrose). Apart from reversal testing, these lever contingencies remained fixed throughout and were counterbalanced across viral groups. Training continued until mice hit a criterion of 25 responses for each lever in a single session. Next, mice were trained on the effort-based decision-making task at which time they were tethered, however no optical stimulation occurred. These approximately 1hr sessions were divided into four blocks consisting of ten trials each and were conducted once per day for ten days. Within each block, mice first received four forced trials (two low effort, two high effort, randomly ordered) in which only one lever was extended. Subsequently, mice received six choice trials where both levers extended simultaneously. Across blocks, a single press on the low effort, low choice lever led to the delivery of the low value reinforcer (i.e., fixed-ratio 1; FR-1). Alternatively, responses on the high choice lever led to the delivery of the high value reinforcer, which gradually required more responses to acquire across blocks: block 1 = FR-1; block 2 = FR-5; block 3 = FR-20; block 4 = FR-40. For choice trials, the first response during block 1 trials led to the retraction of both levers, whereas during blocks 2-4 the first response on the high choice lever led to the retraction of the low choice lever. Each trial was separated by a fixed 60 s interval, and during the trials if mice failed to perform a lever response across a 60 s interval the trial was omitted.

After this training, mice underwent four separate test days (two stimulated; two non-stimulated) that were identical to effort-based decision-making training, with the exception that on the two stimulated test days (counterbalanced order), optogenetic stimulation (1 s, 473 nm, 5

ms pulses at 20Hz) of VTA dopamine began 5 s prior to each choice trial and was delivered via a waveform generator (Agilent Technologies, Santa Clara, CA) integrated into the Med Associates apparatus. During choice trials, the number of lever presses on the high and low effort levers were recorded along with omissions for each block. In order to control for any potentially confounding effects of dopamine stimulation on contingency learning, a small cohort of mice (n = 4) received additional training where response-outcome contingencies were reversed (i.e. low effort lever always yielded 20% sucrose under FR1, while high effort lever yielded 5% sucrose under increasing effortful action). Following ten sessions of training, these mice received optogenetic testing as described above though with the new response-outcome contingencies.

Once testing had concluded, subjects were sacrificed via transcardial perfusion with 4% paraformeldahyde (Sigma-Aldrich, St. Louis, MO). Brains were then extracted, sliced at 30 µm using a freezing microtome and stained using a mouse-anti-tyrosine-hydroxylase primary (Catalog# MAB318; MilliporeSigma) in addition to a rabbit-anti-GFP primary (Catalog# MAB318; MilliporeSigma) with donkey-anti-rabbit-488 (Catalog# A21206; Invitrogen) and donkey-anti-mouse-568 (Catalog# A10037; Invitrogen) corresponding to ChR2 and TH positive cells, respectively. Imaging of the VTA (Fig. 1a) was carried out using a Nikon A1 laser-scanning confocal microscope (Nikon Instruments Inc, Melville, NY). Viral targeting and co-localization were scored qualitatively using separate raters. At this time, n = 2 ♂ and n = 1♀ from group ChR2 were excluded from analysis due to poor targeting.

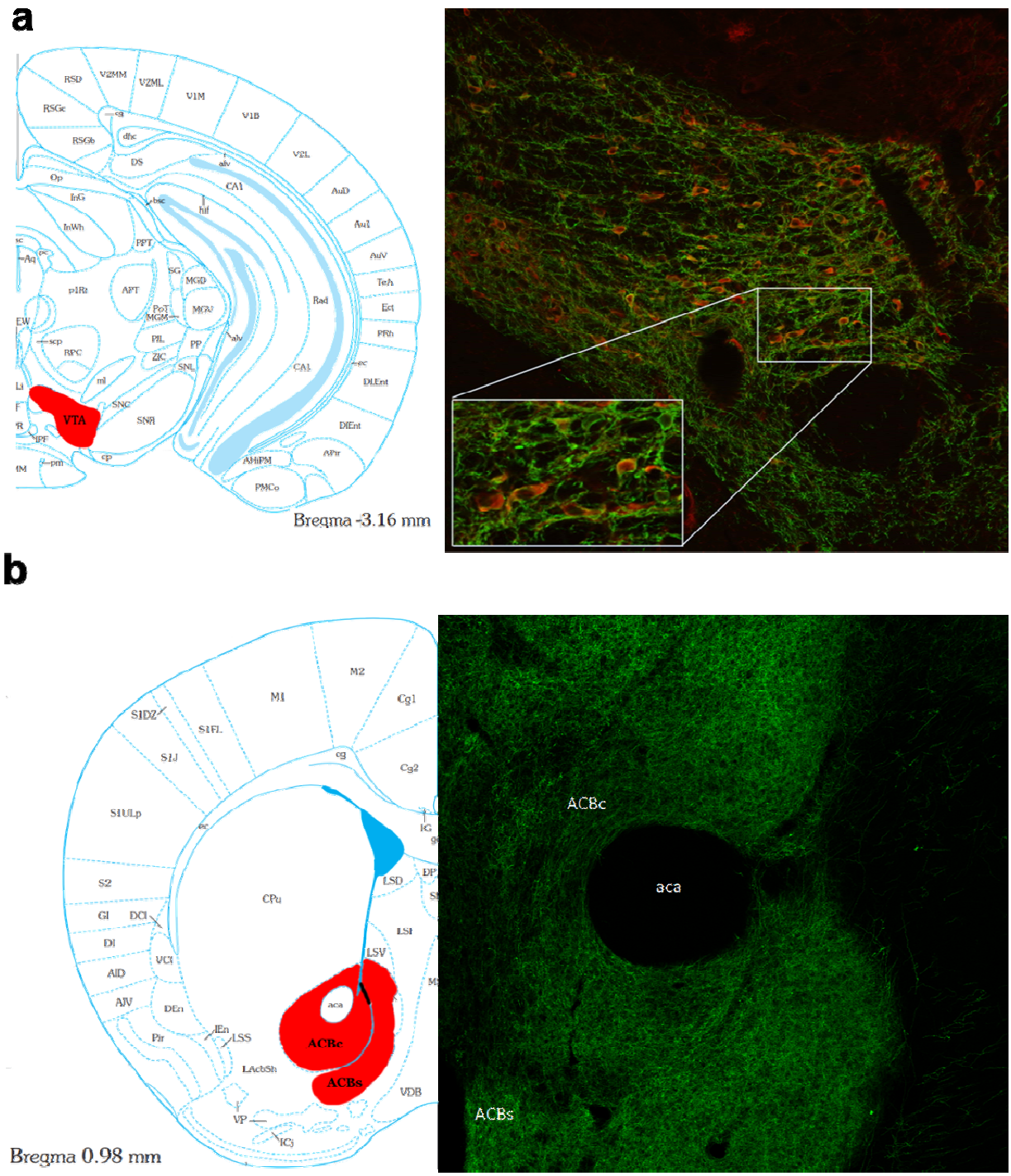

Figure 1.

For data analysis, for each trial block the percentage choice on the high choice lever was averaged for the two non-stimulated and stimulated test days. These data were subjected to three-way repeated measures ANOVA with a between-subjects variable of virus (ChR2 vs eYFP) and within-subjects variables of laser (stimulated vs non-stimulated test days), and block (1-4). Separate analyses for each viral group were carried out via a laser X block within-subject ANOVA, followed by post-hoc Bonferroni correction to control for multiple comparisons. Choice omission data were subjected to two-way virus X laser mixed ANOVA. Finally, due to a lack of variance in a number of the test blocks and non-uniformed distribution, analysis of the contingency-reversal data was conducted using Wilcoxon matched pairs test on the percentage choice on the low choice lever responses. The α level for significance was .05 and all analyses were conducted using Statistica (Statsoft, Tulsa, OK).

The data of primary interest are depicted in Figure 2. For the choice test data (Fig's 2a,b), analyses revealed a main effect of block ($F(3,60) = 12.54$, $p<0.0001$) and a significant laser X virus interaction ($F(1,20) = 5.16$, $p<0.05$). Subsequent analysis revealed for eYFP mice a main effect of block only ($F(3,21) = 8.38$, $p<0.001$). Post-hoc tests confirmed that laser stimulation had no effect on choice responding across any of the trial blocks (p's>0.05). In addition, for both stimulated ($p<0.0001$) and non-stimulated ($p<0.01$) test sessions, eYFP mice displayed a significant reduction in high choice lever responses on block 4 compared to block 1, indicating that mice discounted the 20% sucrose reinforcer as the amount of effort increased to FR-40 on the final block of trials. By contrast, in ChR2 mice a main effect of laser ($F(1,13) = 7.67$, $p=0.01$) and block ($F(3,39) = 3.86$, $p=0.01$) were revealed. Post-hoc comparisons revealed that in stimulated ($p=0.01$) but not non-stimulated ($p>0.05$) test sessions, ChR2 mice displayed a significant reduction in high choice lever on block 4 compared to block 1. Moreover, relative to corresponding non-stimulated trial blocks, laser stimulation reduced high choice responding at each block (p's≤0.02). This reduction could not be attributed to non-specific effects of laser stimulation on motoric action as the total number of choice omissions did not differ between the

groups during either stimulated or non-stimulated test sessions (Fig. 2c,d). Two-way virus X laser ANOVA confirmed this impression with no significant main effects or interactions between the variables (F's<1; p's>0.33). Finally, as the effects of optogenetic stimulation in ChR2 animals were present from the first block (Fig. 2a) we wanted to confirm that laser activation in this group did not disrupt the capacity of mice to encode and/or retrieve lever contingencies (Fig. 3). Analyses revealed that irrespective of dopamine VTA stimulation, mice continued to bias their choice performance towards the low choice lever, which at this stage of testing led to delivery of the preferred higher value reinforcer (largest Z-value; block 3, $Z = 1.60$, p=0.1).

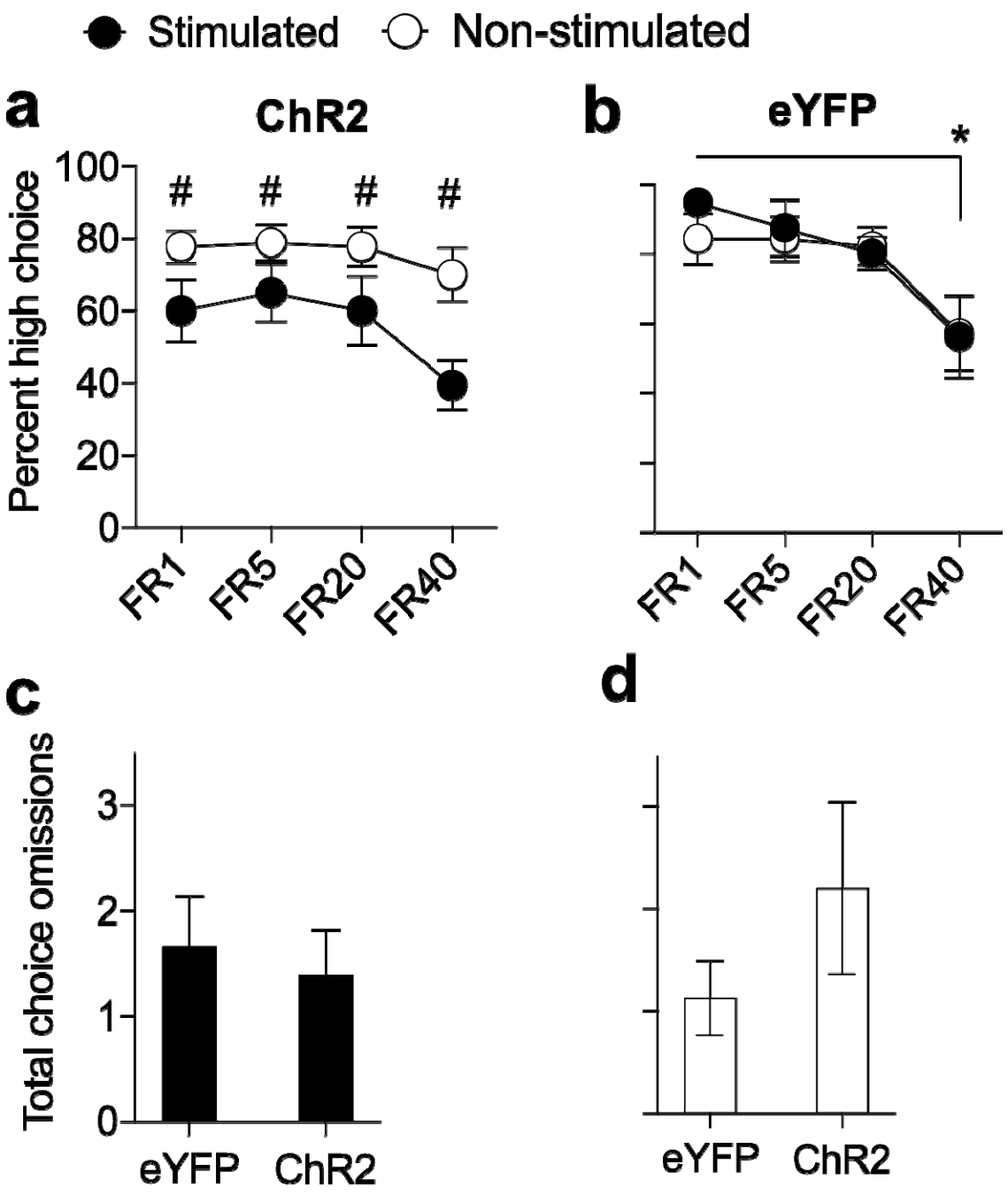
Stimulated
Non-stimulated
a
ChR2
Percent high choice
100
80
60
40
20
0
#
#
#
#
FR1
FR5
FR20
FR40
b
eYFP
*
FR1
FR5
FR20
FR40
c
Total choice omissions
3
2
1
0
eYFP
ChR2
d
eYFP
ChR2

Figure 2.

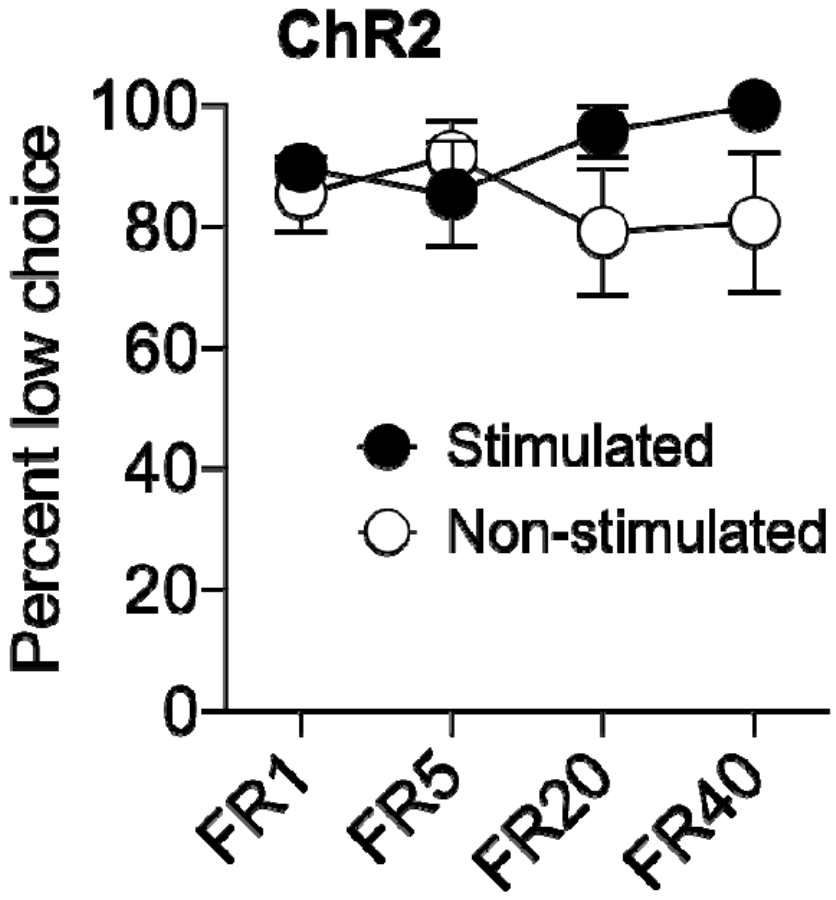
ChR2
Percent low choice
100
80
60
40
20
0
Stimulated
Non-stimulated
FR1
FR5
FR20
FR40

Figure 3.

Previous studies have shown that dopamine depletion or antagonism reduces performance following increases in the effort required to obtain reward (Robles & Johnson, 2017; Salamone et al., 1991; Bardgett et al., 2009; Nowend et al., 2001; Mingote et al., 2005), whereas facilitations in dopamine signaling bias performance towards effortful actions (Wardle et al., 2011; Bardgett et al., 2009; Trifilieff et al., 2013). These findings suggest dopamine plays an integral role in decision-making when options differ in their costs and are broadly consistent with the sensorimotor activational hypothesis (Salamone et al., 2007), which posits that dopamine promotes action generation and the prolongation of effortful behavior. However, a limitation of these studies is their reliance on perturbations of dopamine function over relatively protracted timeframes. In the current study, acute stimulation to dopamine cells in VTA was applied immediately and transiently prior to decision-making, which surprisingly attenuated performance towards the high effort action.

Although the vast majority of decision-making studies indicate increases in dopamine promote responding for more effortful actions, several findings suggest nuances in this relationship. For instance, peripheral injections of amphetamine in rats significantly decreased lever responding for preferred food pellets (Cousins et al., 1994) and reduced preference for a high value food outcome as the amount of effort required to obtain it increased (Floresco et al., 2008). Similarly, administration of the D2/D3 receptor agonist quinpirole in rats (Depoortere et al., 1996), or transgenic overexpression of post-synaptic striatal dopamine D2 (Drew et al., 2007) or D3 (Simpson et al., 2014) receptors produce progressive ratio deficits. Moreover, D2 receptor overexpression also reduced the willingness to work for palatable food in a cost-benefit decision-making task (Filla et al., 2018). More targeted pharmacological approaches in rats likewise reveal a similar pattern of performance, whereby administration of a D2/D3 receptor agonist (but not D3 agonism alone) in the nucleus accumbens reduced choice responding for the high effort lever during effort-based decision-making (Bryce & Floresco, 2019). In the current

study, VTA injections of ChR2 was trafficked to a number of targets including the nucleus accumbens (Fig. 1b) potentially suggesting mesostriatal modulation underlies the disruptions in effort-based decision-making. Moreover, our findings are consistent with a D2-dependent inverted-U-shaped influence in this circuit, where either pharmacological knockdown or stimulation produce disruptions in effort discounting (Bryce & Floresco, 2019).

Nevertheless, an important consideration in the current findings is that unlike past studies, acute stimulation of VTA dopamine cell activity reduced preference for the high value outcome even when effort costs were equated (i.e., during FR-1 block trials; Fig. 2a). This suggests optogenetic stimulation may have disrupted the reinforcing efficacy of the higher value reinforcer, and/or the animals' capacity to discriminate between lever contingencies. However, this interpretation is unlikely given that when mice were tested under conditions where the higher value outcome was continually paired with low effort, mice maintained their preference for the larger preferred reward across trial blocks independent of VTA stimulation (Fig. 3). In addition, our findings are unlikely to reflect gross changes in motoric output or motivation as omissions were comparable across stimulated and non-stimulated test days (Fig. 2b,c). This raises the intriguing question as to why acute stimulation disrupted choice performance when effort costs were equated. In addition to its activational effects, dopamine acts a value-based prediction error signal critical for reward learning (Schultz, 1997; Waelti et al., 2001; Bayer & Glimcher, 2005; Eshel et al., 2016; Steinberg et ., 2013; Pessiglione et al., 2006; Bayer et al., 2019). However, more recently studies suggest dopamine transients can be uncoupled from model-free reinforcement value signals to encode associative information that is computationally detailed in nature (e.g. its sensory features; Gardner et al., 2018; Sharpe et al., 2019). This in mind, the current findings also suggest that DA functions beyond a pure value-based signal, to include recall of past, and/or anticipation of detailed upcoming contingencies, which may bias performance towards actions that overall have the highest immediacy of reinforcer delivery. This could serve to direct actions towards the low effort lever where execution of a single lever press

consistently results in immediate reward delivery even under cost-benefit conditions where larger rewards may be available. Alternatively, the pattern of responding in our study may be an artifact of optogenetic stimulation of VTA dopamine transients, whereby endogenous phasic dopamine release that supports effortful action when produced by extension of the levers may have been attenuated as a result of the preceding optogenetic stimulation (e.g., via an insufficient refractory period). It would be of interest in future studies to deliver laser stimulation when the levers are extended to determine how this might influence choice responding.

While our study provides important insight into the role of VTA dopamine transients on effort-based decision-making, a number of caveats should be acknowledged. As suggested above, this study would benefit from optical stimulation at different time points during the choice test. In addition, VTA dopamine efferents target numerous striatal (nucleus accumbens core and shell), limbic (basolateral amygdala) and prefrontal (orbitofrontal cortex, anterior cingulate cortex) sites that are implicated in decision-making (Cousins et al., 1996; Winstanley et al., 2004; Floresco & Ghods-Sharifi, 2007; Hauber & Sommer, 2009; Walton et al., 2009). Thus, photostimulation of axonal terminals in these downstream targets will provide additional insight into the circuit level effects of dopamine stimulation on effortful discounting. It should also be noted that under non-stimulated conditions, ChR2 mice failed to show evidence of effortful discounting; therefore, it is unclear if the reduced responding in high choice responding in block 4 (i.e., FR-40) under stimulated conditions reflects enhanced discounting. Finally, a number of relevant measures including latencies for deliberation time were not recorded.

Effort-based decision-making impairments have been noted in numerous settings including in patients with depression, schizophrenia and autism (Treadway et al., 2012, 2015; Damiano et al., 2012; Green et al., 2015), along with obese individuals (Mata et al., 2017). These impairments may broadly reflect energy-related disruptions in behavioral output (e.g., anergia). Although reduced dopamine transmission has been generally attributed to underlie these disruptions, our findings add to a smaller yet important body of work indicating that

aberrant elevations in dopamine signaling may also impair cost/benefit decision-making. Moreover, they are consistent with contemporary accounts that indicate a more complex computational role for dopamine, which may include promoting action selection towards more immediate reward delivery.

References

Bardgett ME, Depenbrock M, Downs N, Points M, Green L. 2009. Dopamine modulates effort-based decision making in rats. Behavioral neuroscience 123: 242.

Bayer HM, Glimcher PW. 2005. Midbrain dopamine neurons encode a quantitative reward prediction error signal. Neuron 47: 129-141.

Bayer HM, Lau B, Glimcher PW. 2007. Statistics of midbrain dopamine neuron spike trains in the awake primate. Journal of Neurophysiology 98: 1428-1439.

Bryce CA, Floresco SB. 2019. Alterations in effort-related decision-making induced by stimulation of dopamine D 1, D 2, D 3, and corticotropin-releasing factor receptors in nucleus accumbens subregions. Psychopharmacology 236: 2699-2712.

Cousins MS, Salamone JD. 1994. Nucleus accumbens dopamine depletions in rats affect relative response allocation in a novel cost/benefit procedure. Pharmacology Biochemistry and Behavior 49: 85-91.

Cousins MS, Atherton A, Turner L, Salamone JD. 1996. Nucleus accumbens dopamine depletions alter relative response allocation in a T-maze cost/benefit task. Behavioural brain research 74: 189-197.

Damiano, C. R., Aloi, J., Treadway, M., Bodfish, J. W., & Dichter, G. S. 2012. Adults with autism spectrum disorders exhibit decreased sensitivity to reward parameters when making effort-based decisions. Journal of Neurodevelopmental Disorders, 4(1), 13.

Depoortere R, Perrault GH, Sanger DJ. 1996. Behavioural effects in the rat of the putative dopamine D 3 receptor agonist 7-OH-DPAT: Comparison with quinpirole and apomorphine. Psychopharmacology 124: 231-240.

Drew MR, Simpson EH, Kellendonk C, Herzberg WG, Lipatova O, Fairhurst S, Kandel E, Malapani C, Balsam PD. 2007. Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. Journal of Neuroscience 27: 7731-7739.

Eshel N, Tian J, Bukwich M, Uchida N. 2016. Dopamine neurons share common response function for reward prediction error. Nature neuroscience 19: 479-486.

Filla I, Bailey MR, Schipani E, Winiger V, Mezias C, Balsam PD, Simpson EH. 2018. Striatal dopamine D2 receptors regulate effort but not value-based decision making and alter the dopaminergic encoding of cost. Neuropsychopharmacology 43: 2180-2189.

Floresco SB, Ghods-Sharifi S. 2007. Amygdala-prefrontal cortical circuitry regulates effort-based decision making. Cerebral cortex 17: 251-260.

Floresco SB, Onge JRS, Ghods-Sharifi S, Winstanley CA. 2008. Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. Cognitive, Affective, & Behavioral Neuroscience 8: 375-389.

Gardner MP, Schoenbaum G, Gershman SJ. 2018. Rethinking dopamine as generalized prediction error. Proceedings of the Royal Society B 285: 20181645.

Green, M. F., Horan, W. P., Barch, D. M., & Gold, J. M. 2015. Effort-based decision making: a novel approach for assessing motivation in schizophrenia. Schizophrenia bulletin, 41(5), 1035-1044.

Hauber W, Sommer S. 2009. Prefrontostriatal circuitry regulates effort-related decision making. Cerebral Cortex 19: 2240-2247.

Mata J, Dallacker M, Hertwig R. 2017. Social nature of eating could explain missing link between food insecurity and childhood obesity. Behavioral and Brain Sciences: 40.

Mingote S, Weber SM, Ishiwari K, Correa M, Salamone JD. 2005. Ratio and time requirements on operant schedules: effort-related effects of nucleus accumbens dopamine depletions. European Journal of Neuroscience 21: 1749-1757.

Nowend KL, Arizzi M, Carlson BB, Salamone JD. 2001. D1 or D2 antagonism in nucleus accumbens core or dorsomedial shell suppresses lever pressing for food but leads to compensatory increases in chow consumption. Pharmacology Biochemistry and Behavior 69: 373-382.

Pessiglione M, Seymour B, Flandin G, Dolan RJ, Frith CD. 2006. Dopamine-dependent prediction errors underpin reward-seeking behaviour in humans. Nature 442: 1042-1045.

Robles CF, Johnson AW. 2017. Disruptions in effort-based decision-making and consummatory behavior following antagonism of the dopamine D2 receptor. Behavioural brain research 320: 431-439.

Salamone JD, Steinpreis RE, McCullough LD, Smith P, Grebel D, Mahan K. 1991. Haloperidol and nucleus accumbens dopamine depletion suppress lever pressing for food but increase free food consumption in a novel food choice procedure. Psychopharmacology 104: 515-521.

Salamone JD, Correa M, Farrar A, Mingote SM. 2007. Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology 191: 461-482.

Schultz W. 1997. Dopamine neurons and their role in reward mechanisms. Current opinion in neurobiology 7: 191-197.

Sharpe MJ, Batchelor HM, Mueller LE, Chang CY, Maes EJ, Niv Y, Schoenbaum G. 2019. Dopamine transients delivered in learning contexts do not act as model-free prediction errors. bioRxiv: 574541.

Simpson EH, Winiger V, Biezonski DK, Haq I, Kandel ER, Kellendonk C. 2014. Selective overexpression of dopamine D3 receptors in the striatum disrupts motivation but not cognition. Biological psychiatry 76: 823-831.

Steinberg EE, Boivin JR, Saunders BT, Witten IB, Deisseroth K, Janak PH. 2014. Positive reinforcement mediated by midbrain dopamine neurons requires D1 and D2 receptor activation in the nucleus accumbens. PloS one 9: e94771.

Treadway MT, Bossaller NA, Shelton RC, Zald DH. 2012. Effort-based decision-making in major depressive disorder: a translational model of motivational anhedonia. Journal of abnormal psychology 121: 553.

Treadway MT, Peterman JS, Zald DH, Park S. 2015. Impaired effort allocation in patients with schizophrenia. Schizophrenia research 161: 382-385.

Trifilieff P, Feng B, Urizar E, Winiger V, Ward RD, Taylor KM, Martinez D, Moore H, Balsam PD, Simpson EH et al. 2013. Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. Molecular psychiatry 18: 1025-1033.

Waelti P, Dickinson A, Schultz W. 2001. Dopamine responses comply with basic assumptions of formal learning theory. Nature 412: 43-48.

Walton ME, Groves J, Jennings KA, Croxson PL, Sharp T, Rushworth MF, Bannerman DM. 2009. Comparing the role of the anterior cingulate cortex and 6-hydroxydopamine nucleus accumbens lesions on operant effort-based decision making. European Journal of Neuroscience 29: 1678-1691.

Wardle MC, Treadway MT, Mayo LM, Zald DH, de Wit H. 2011. Amping up effort: effects of d-amphetamine on human effort-based decision-making. Journal of Neuroscience 31: 16597-16602.

Winstanley CA, Theobald DE, Cardinal RN, Robbins TW. 2004. Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. Journal of Neuroscience 24: 4718-4722.

Figure Legends

Figure 1. Representative photomicrograph showing immunohistochemical **(a)** verification of Cre-dependent ChR2 (green) in tyrosine-hydroxylase positive cells (red) in VTA. In addition, **(b)** dense viral transfection was noted in terminals in nucleus accumbens. ACBs = nucleus accumbens shell; ACBc = nucleus accumbens core; aca = anterior commissure.

Figure 2. Optogenetic stimulation of VTA dopamine cells disrupts effort-based decision-making. **(a)** During stimulated trials, ChR2 mice displayed reduced preference for the high choice lever, whereas **(b)** eYFP controls gradually decreased performance on the high choice lever during both stimulated and non-stimulated test sessions. **(c,d)** Optogenetic stimulation had no effect on omissions during choice trials, with comparable omissions during both **(c)** stimulated and **(d)** non-stimulated test sessions. # indicates significant effect of optogenetic stimulation at each trial block (p's≤0.02). * indicates significant difference in high choice responding between FR1 and FR40 testing blocks during stimulated ($p<0.0001$) and non-stimulated ($p<0.01$) test sessions.

Figure 3. Following reversal of the lever contingencies such that the low effort lever always produced the preferred high value outcome, ChR2 mice continually maintained their preference for the low effort lever irrespective of laser stimulation or trial block.