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A Transient Dopamine Signal Represents Avoidance Value and Causally Influences the Demand to Avoid

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A transient dopamine signal represents avoidance value and causally influences the demand to avoid

dopamine modifies value of negative reinforcement

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Abstract

While an extensive literature supports the notion that mesocorticolimbic dopamine plays a role in negative reinforcement, recent evidence suggests that dopamine exclusively encodes the value of positive reinforcement. In the present study, we employed a behavioral economics approach to investigate whether dopamine plays a role in the valuation of negative reinforcement. Using rats as subjects, we first applied fast-scan cyclic voltammetry to determine that dopamine concentration decreases with the number of lever presses required to avoid electrical footshock (i.e. the economic price of avoidance). Analysis of the rate of decay of avoidance demand curves, which depict an inverse relationship between avoidance and increasing price, allows for inference of the worth an animal places on avoidance outcomes. Rapidly decaying demand curves indicate increased price sensitivity, or low worth placed on avoidance outcomes, while slow rates of decay indicate reduced price sensitivity, or greater worth placed on avoidance outcomes. We therefore used optogenetics to assess how inducing dopamine release causally modifies the demand to avoid electrical footshock in an economic setting. Increasing release at an avoidance predictive cue made animals more sensitive to price, consistent with a negative reward prediction error (i.e., the animal perceives they received a worse outcome than expected). Increasing release at avoidance made animals less sensitive to price, consistent with a positive reward prediction error (i.e., the animal perceives they received a better outcome than expected). These data demonstrate that transient dopamine release events represent the value of avoidance outcomes and can predictably modify the demand to avoid.

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Significance Statement

Dopamine is thought to play a crucial role in reward learning and directing actions towards beneficial outcomes. While the avoidance of harmful stimuli is similarly pertinent to an organism's survival, the role of dopamine in avoidance remains controversial. Using in vivo electrochemistry, we observed that dopamine concentration decreased when the effort (lever presses) required to avoid electrical footshock increased. We also found that increasing dopamine at an avoidance predictive cue decreased avoidance, consistent with a negative prediction error. In contrast, increasing release at successful avoidance increased avoidance, consistent with a positive prediction error. These data demonstrate that transient dopamine release events represent the value of avoidance outcomes and capably modify avoidance.

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Introduction

Dopamine release events within the mesocorticolimbic pathway actively guide behavior in positive reinforcement, where behavior is strengthened by the occurrence of a rewarding event (Schultz et al., 1997; Glimcher, 2011). A growing literature shows that a transient dopamine signal represents reward value and causally modifies the valuation of reward (Schelp et al., 2017; Schultz et al., 2017). It was first demonstrated that bursts of dopamine neural activity occur when animals are presented with an unexpected reward or a reward predictive cue but are suppressed when reward is withheld. These observations led to the view that dopamine neurons represent a reward prediction signaled by the cue (Schultz et al., 1997). It was then shown that the extent of the dopamine response corresponds to predicted reward magnitude (Enomoto et al., 2011; Lak et al., 2014; Schelp et al., 2017; Stauffer et al., 2014; Tobler et al., 2005), suggesting that dopamine actually represents a value prediction signaled by the cue (Schelp et al., 2017; Schultz et al., 2017).

An extensive body of literature suggests that dopamine also plays a role in avoiding harm (i.e. negative reinforcement). Dopamine terminal lesions (McCullough et al., 1993) and dopamine receptor antagonists (Arnt, 1982; Wadenberg et al., 1990) impair avoidance of electrical footshock while genetic restoration of dopamine within the striatum of otherwise dopamine-deficient mice is necessary to maintain avoidance (Darvas et al., 2011). Microdialysis studies have demonstrated that striatal dopamine concentration is increased during the avoidance of footshock and in post-surgical pain relief (McCullough et al., 1993; Navratilova and Porreca, 2014). More recently, studies utilizing fast-scan cyclic voltammetry (FSCV) showed that transient increases in

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105 dopamine concentration occur in response to cues signaling the opportunity to avoid
106 electrical footshock (Gentry et al., 2016; Oleson et al., 2012). While these studies
107 indicate a role for dopamine in avoidance, it remains controversial whether dopamine
108 plays a role in the valuation of avoidance. Indeed, a notable electrophysiology study
109 concluded that dopamine neurons predominately encode the value of positive
110 reinforcement (Fiorillo, 2013). Similarly, a recent FSCV study found that transient
111 dopamine release events encode ultrasonic vocalizations associated with a positive, but
112 not a negative affective state—indicating that the behavior response to negatively
113 reinforcing stimuli is regulated by distinct brain areas (Willuhn et al., 2014).
114 To investigate the role of dopamine in the valuation of avoidance, we utilized a
115 behavioral economics task in which rats were presented with an avoidance predictive
116 cue and provided the opportunity to avoid the onset of electrical footshock by
117 responding on a lever. We then increased the price of avoidance by increasing the
118 number of lever presses required to avoid footshock at fixed intervals over the course of
119 a session. To measure changes in price sensitivity we generated demand curves to
120 model avoidance as a function of price. The rate at which these demand curves decay
121 depicts price sensitivity and allows for inference of the worth an animal places on an
122 outcome.
123 If, as in reward seeking (Schultz et al., 2015; Schultz et al., 2017), dopamine represents
124 the value to avoid aversive outcomes, than dopamine concentration at avoidance-
125 predictive cues should decrease as the price to avoid increases. Furthermore, optically
126 increasing release at cue presentation should increase price sensitivity. In theory,
127 artificially augmenting release at cue presentation would lead the animal to expect a

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128 better outcome. When the animal's expectation is negatively violated by the recurrence
129 of the same amplitude of footshock, the animal becomes more sensitive to price
130 because the worth of avoidance is diminished. By contrast, optically increasing release
131 at successful avoidance should increase the demand to avoid. In this case, we infer that
132 the worth of avoidance is increased because the animal perceives that they received a
133 better bargain than predicted.
134 As hypothesized, dopamine scaled inversely with cost; however, both dopamine and
135 avoidance were concurrently attenuated at session onset. Augmenting dopamine
136 release at an avoidance predictive cue rendered animals more sensitive to avoidance
137 costs. We attribute this finding to a negative reward prediction error, whereby the animal
138 perceives they received a worse value than anticipated when they receive the same
139 amplitude of electrical footshock. Increasing release at successful avoidance made
140 animal less sensitive to avoidance costs. We attribute this finding to a positive reward
141 prediction error, whereby the animal perceives they received a better value than
142 anticipated despite still receiving footshock in other trials. From these data we conclude
143 that dopamine release events represent the value of avoidance and casually modify the
144 demand to avoid.

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Materials and Methods

Subjects and surgeries:

Male Long-Evans rats provided by Charles River Laboratories as well as Transgenic rats (LE-Tg(TH-Cre)3.1Deis) expressing Cre-recombinase under the tyrosine hydroxylase (TH) promotor (TH::Cre+/-) supplied by Rat Resource and Research Center were used as subjects. Rats were singly housed and maintained on a 12:12 light dark cycle with the dark cycle beginning at 10am. All experiments were conducted during the dark (active) cycle with food, water, and crinkle paper enrichment provided ad lib. Surgeries were conducted at 300g-350g using Kopf stereotaxic equipment with rats anesthetized at 5% isoflurane and maintained at 2% ±1%. For FSCV, rats were implanted with a microdialysis guide cannula (BASi) targeted at the nucleus accumbens core [+1.3AP, +1.4ML] of the right hemisphere and a contralateral Ag/AgCl reference electrode. For optogenetic surgeries, rats were unilaterally transfected with 4μL of a Cre-dependent virus (rAAV2/EF1a-DIO-hChR2(H134R)-EYFP; UNC Vector Core) targeted at the ventral tegmental area (VTA). 1 μL viral aliquots were infused at 4 areas surrounding the VTA [-5.2AP and -6.0AP, -0.5ML, -7.4AP and -8.4AP] at a rate of 50nL/min. A fiber optic cannula (ThorLabs: 200μM core) was then implanted unilaterally, directed at the VTA [-5.6AP, +0.5ML, -7.9DV]. These methods were utilized for both the transgenic rats as well as wild-type (WT) counterparts, which served as controls in optogenetic experiments. Post-surgery, rats were given >3 days to recover during which time each received a daily 3mL intraperitoneal injection of a 1% carprofen solution to reduce inflammation and post-operative pain. All animal procedures were performed in accordance with the CU Denver animal care committee's regulations.

Behavioral Tasks

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Rats were maintained on a daily training schedule (7d/wk) within operant boxes (Med-Associates) outfitted with footshock grid floors. Rats were first trained to escape electrical footshock with each daily escape-only session lasting 15min. At the onset of each session both an active and inactive lever were extended, a cue light placed above the active lever was illuminated and 0.5mA electrical current (i.e., footshock) was applied to the grid floor of the operant chamber. A response on the active lever terminated the ongoing foot shock and allowed the rat to escape into a 30s-safety period accompanied by a tone, while a response on the inactive lever had no effect. This safety signal played for the entirety of the safety period, the end of which coincided with the onset of the next escape trial. In this initial training task, escape behavior was shaped by a researcher who could simulate a lever response using a wireless keyboard, thereby reinforcing a series of behaviors that would lead to the acquisition of operant escape. The experimenter first shaped the animal to the quadrant of the operant box containing the extended lever. They then reinforced rearing behavior in front of the lever before finally requiring the rat to respond on the lever to self-terminate electrical footshock. This training persisted until the animal demonstrated an association between lever response and shock termination through consistent and researcher-independent escape (>20 sequential escape responses) (**Fig. 2A**).

Following the acquisition of escape behavior animals were moved into a daily 1-hr avoidance task in which reinforcement was maintained under a fixed ratio1 (FR1) schedule of reinforcement. This task added the potential for an avoidance outcome. Each session was initiated by the extension of both an active an inactive lever and the illumination of a cue light placed directly above the active lever. In each trial, rats were

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given 1s from cue presentation to respond on the active lever to successfully avoid footshock. If rats failed to respond, recurrent footshock (0.5s, 0.5mA every 1s) was applied until a lever response was made to escape further shock. Responses made on the inactive lever had no effect on behavioral outcome. Rats remained on this task until they consistently performed (≥3 sessions) ≥50% avoidance (**Fig. 2A**).

After successful acquisition of 50% avoidance under an FR1 schedule, rats moved into a behavioral economics-based shock avoidance task. Here the unit-price (response requirement/mA shock avoided) increased throughout each session by increasing the response requirement to both avoid or escape footshock. Within this task, the unit-price epoch duration increased in length to allow 20 avoidance opportunities and provide sufficient time to meet increasing response requirements as well as to account for safety period duration following avoidance or escape responses. (**Fig. 2B,C column 4**). Failure to meet the response requirement on the active lever within the allotted time (**Fig. 1A-C column 3**) resulted in the onset of a 0.5s, 0.5mA footshock and reset any lever responses made prior to footshock onset to zero. Footshock recurred until the response requirement was fully met or 15 sequential footshock were received and the session terminated. Similarly, 20 sequential escape responses resulted in session termination. Rats were first trained to perform multiple lever presses to avoid footshock across 6 unit-prices (**Fig. 2B**). Once animals demonstrated independent multiple lever press responding, they were moved into an extended, 16 unit-price economic task until they acquired stable response output across daily sessions (**Fig. 2C**). Acquisition was defined by rats reaching a final unit price (within a range of three price points) for three consecutive days, without showing any ascending or descending trend.

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Unit-price was additionally manipulated through changes in the mA of shock received. Within this task, the response requirement was held constant at an FR2 and rats were given 2s to respond prior to the onset of a 0.5s footshock across six unit-prices (**Fig. 1A**) (upper). Within this task animals were presented with descending unit-prices (ascending mA shock) to prevent avoidances in higher unit-prices due to elevated mA shock amplitude in lower unit-prices. Safety period duration was additionally modulated to address opportunity cost confounds in the response requirement task and unit-price epoch duration was similarly modulated to allow for 20 avoidance opportunities at each unit-price. Utilizing this task, response output failed to scale in response to changing unit-price (**Fig. 1A**) (lower).

We further attempted to address order effect confounds within the ascending response requirement task by randomizing the presentation of five unit-prices within a single session (**Fig. 1C** (upper)). Randomization of unit-price resulted in inconsistent behavioral output and preemptive extinction of avoidance (lower).

Within all tasks, the cue was defined as the presentation of a lever and the illumination of a light above the lever which was also accompanied by white noise. Both an escape or avoidance response resulted in the retraction of the lever, the dimming of the light, termination of the white noise, as well as the beginning of the 30s safety tone and illumination of a secondary house light for the duration of the safety period.

Fast-Scan Cyclic Voltammetry

FSCV recordings (n=7) were performed during the economics-based task. For these recordings, glass carbon-fiber electrodes were lowered into the nucleus accumbens utilizing micromanipulators (UC-Illinois; Schmidt) and locked into place at a depth where

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transient dopamine release events were apparent. Electrodes were first cycled at 60Hz *in vivo* for 30-40mins prior to being reduced to 10Hz for data collection. An initial waveform (-0.4V to 1.3 V, tarheelCV filtered with cutoff frequency of 2kHz for a scan rate of 400 V/s) was applied which allowed for the detection of dopamine via fast-scan cycle voltammograms taken every 100 ms. To extract the dopamine component, principle component regression (PCR) was applied to the raw voltammetric data as previously described (Heien et al., 2004). Specifically, DA and pH were resolved from the FSCV recordings using recording-specific training sets (n=7/anylate) to produce pH background-subtracted (10 consecutive scans) dopamine concentration files. To increase the validity of calibration factors for dopamine assessment, we applied a recently developed computational model (Roberts et al., 2013) designed to calculate calibration factors for individual electrodes by applying known constants to background current values from each *in vivo* recording. By replicating Roberts et al. 2013, using 10 electrodes we obtained a set of empirical values using multiple linear regression analysis. Our lab-specific coefficients are: $\alpha=4.71e^{-5}$, $\beta=17.185$, $\gamma=8.324$, $\delta=-0.656$. Using these coefficients, we can calculate calibration factors for individual electrodes used *in vivo* by simply entering the observed total background current and the switching potential used for each individual recording. For additional information see the supplemental information of (Schelp et al., 2017).

We used different approaches to analyze dopamine concentration at the avoidance predictive cue versus throughout the safety period. The concentration of dopamine at the presentation of the cue preceding avoidance was defined as the peak concentration ±0.5s surrounding the event. Avoidance predictive cue-associated dopamine

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concentrations from individual trials were aggregated, split according to avoidance or escape outcome, and averaged across all animals per unit-price for analysis (**Fig. 4A,C & Fig. 6A,C**). Voltammograms which contained excessive electrical noise were excluded from this analysis.

As previously described (Oleson et al., 2012), dopamine concentration begins to increase prior to cue presentation in tasks where the animal can anticipate the timing of its presentation. Because varying the duration of the safety period is known to alter avoidance (Sidman, 1953), and theoretically would alter the valuation of avoidance, we opted to use a fixed, 30s safety period for the behavioral economics task. As such, it should be noted that the dopamine concentration at the warning signal likely results from both anticipation of the warning signal its actual presentation.

The pattern of dopamine release during the safety period was distinct as this period is not transient; rather, each safety period lasted 30s in duration. Thus, to more accurately assess dopamine transient activity during the safety period, rather than analyzing mean dopamine concentration at the onset of the 30s-safety signal we performed an analysis of the amplitude of individual transient events throughout this period. A custom Matlab program previously described by Schelp et al. (2018) was utilized to fit a polynomic line to individual 30s safety period dopamine concentration traces. For each individual safety period trace a polynomic line shift down 1/3 a standard deviation was fit to the dopamine concentration trace as baseline. This standard deviation was then multiplied by 3 and added to the baseline polynomic line to generate a first fit threshold line. Any transients which reached above this threshold line were considered true transients and these concentrations were averaged across each unit-price for both avoidances and

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escapes (**Fig. 5C-E**). Notably, this program differed from Schelp et al. (2018) through the calculation of standard deviation. Within this program, a region of 2-3 seconds without dopamine was declared as a baseline to determine the standard deviation of the background signal. Voltammograms which contained excessive electrical noise were excluded from this analysis.

Fitting demand curves to avoidance

Demand curves depict the relationship between the consumption of a commodity (in this case avoidance) and changing unit-price. Because demand decreases or decays with increasing price, the desirability of a given commodity may be inferred based on the relative rate of decay of a demand curve. More rapidly decaying demand curves indicate lower demand, or heightened sensitivity to increasing price, while more gradually decaying demand curves indicated greater demand for a given commodity, or reduced sensitivity to increasing price. In order to generate demand curves depicting avoidance as a function of increasing price, the avoidance responding observed within the task was fit to an exponentially decaying model though a custom Matlab script utilizing non-linear least squares. Within this analysis, total avoidance per unit-price epoch was graphed on the y axis against unit-price on the x axis.

$$Q = Q_{min} + (Q_{max} - Q_{min})e^{-\alpha C}$$

Analysis revealed that exclusion of unit-price 2 resp/mA, at which attenuated avoidance responding was observed, generated lower X_v^2 values and, therefore, was not included in these fits. Within this equation the variable α, which describes the rate of decay of the avoidance demand curve, was utilized to contrast changes in price sensitivity across optogenetic manipulations (**Fig. 7**).

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306 **Optogenetic manipulation**

307 In order to allow sufficient expression of the channelrhodopsin-2 (ChR2) protein, both
308 the transgenic TH-Cre group (n=10) as well as the WT control group (n=10) were given
309 30 days following surgery prior to optogenetic stimulation. Both groups received
310 intracranial blue light (473nm) delivered at 10 pulses, 20Hz, 0.5s duration from a laser
311 (opto-engine) controlled by a custom Arduino system (Ng-Evans). Laser output was
312 determined through the Stanford brain transmission calculator to produce a 1mm cone
313 of light of 1mW/mm2 within the brain tissue with a 15mW output from the ferrule
314 cannula tip and was designed to encompass exclusively the VTA dopamine cell bodies.
315 Stimulation was unilaterally applied to the right hemisphere in all animals. These
316 stimulation parameters have previously been utilized within the lab and have
317 demonstrated successful augmentation of dopamine within the NAcc (Schelp et al.,
318 2017). Within the economic based avoidance task, rats first established a baseline rate
319 of avoidance across three sessions. Following baseline performance, in a
320 counterbalanced design, rats received optogenetic stimulation for three sessions either
321 at the presentation of the avoidance predictive cue, or when the animal fully met the
322 response requirement and successfully avoided footshock (referred to as 'cue' and
323 'avoidance' stimulation respectively). Following cue or avoidance stimulation, rats
324 reestablished baseline behavior across three sessions, after which the animals received
325 stimulation under the complementary paradigm (cue or avoidance) across three
326 sessions (**Fig. 8A**).

327 **Locomotor Assessment**

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To assess for locomotor changes as a result of optogenetic stimulation, transgenic TH-Cre rats (n=8) were placed in locomotor chambers and their movement was tracked using Med-Associates Activity Software. Rats first acclimated to the chambers over the course of 1hr before immediately moving into either a 1hr 'stimulation' paradigm wherein they received optogenetic stimulation every 30s, paralleling the frequency of stimulation animals receive within the economic based avoidance task, or a 'baseline' paradigm wherein rats attached to a mock fiber optic patch cable received no stimulation. These conditions were counterbalanced against each other and total distance moved every 5min was analyzed for both conditions (**Fig. 11D**).

Histology

Upon completion of optogenetic experiments, animals were anesthetized with a ketamine xylazine mixture and transcardially perfused with 0.01M PBS followed by a 4% paraformaldehyde solution. The brains were harvested and rested in a 4% paraformaldehyde solution for 24hrs before soaking in a 30% sucrose solution for 48hrs after which the neural tissue was then frozen and kept at -80^{o}C. The frozen neural tissue was sectioned in coronial in 50μM slices and floated in a 0.01 M PBS/15% Normal Donkey serum solution for 24hrs at 4^{o}C. Tissues was washed with a 0.1% Tween 20/0.01 M PBS for 5min followed by three 5min washes of 0.01 M PBS. Tissue was then floated in a 0.1% solution of Immunostar Tyrosine Hydroxylase primary antibody and 0.01M PBS for 48hrs at 4^{o}C after which they were washed with Tween 20 and PBS as described above. Following washes, tissue was floated in a 0.1% Alexafluor647 donkey ant-mouse IgG secondary antibody and 0.01M PBS at 4^{o}C for 12hrs after which the tissue was washed once more with Tween 20 and PBS. Once

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351 washed, tissue was mounted on slides with Vector Vectasheild hardset mounting media
352 with DAPI and imaged to identify expression of ChR2, tyrosine hydroxylase, and DAPI
353 in both the NAcc and VTA (**Fig. 11A,B**).
354 Upon completion of voltammetry experiments, animals were sacrificed utilizing CO2.
355 With a micromanipulator, a stainless-steel electrode was lowered to the depth of the
356 recording site and a current was applied to electrically lesion the region. Following
357 lesioning, the brains were extracted, frozen, and stored at -80^{o}C. Tissue was sliced at
358 50μM and dry mounted on slides. The mounted tissue was submerged in 95%
359 ethanol/DI water for 15min followed by 1min submersions in 70% ethanol, 50% ethanol,
360 and two washes in DI water. Tissue was then soaked in Crysal Violet for 1min after
361 which it was bathed in two 1min washes in DI water followed by 15s washes in 50%,
362 70%, 95%, and 100% ethanol. This was followed by a ≥8 min wash in Histo-Clear. Once
363 complete, slides were mounted with Paramount and imaged to confirm lesion placement
364 (**Fig. 11E**).
365 **Statistics**
366 All statistics were performed using SigmaPlot11. First, Shapiro-Wilk was used to assess
367 for normality and Brown-Forsythe was used to assess for equal variance. If these tests
368 passed, ANOVA were used; if these tests failed, equivalent non-parametric statistics
369 were used.

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Results

Relationship between alpha and price elasticity of demand

Demand curves are a common tool used by economists to measure price sensitivity. Demand curves depict the relationship between consumption of a commodity (in this case the avoidance of harm) and price. Demand curves usually show a negative gradient (i.e., the law of demand), where consumption decreases with increasing price. The rate at which the negative slope decays can be used to make inferences regarding the value individuals place on the commodity being consumed. When demand curves decay at a faster rate they are said to be more elastic. Price elasticity of demand is defined as the change in the quantity of the commodity being consumed in response to an increase in price. In the present study, the number of successful avoidance responses at each price represents the quantity of the demanded commodity. We measure the elasticity of demand by computing the variable α, which represents the cost at which the elasticity of demand is exactly -1, meaning consumption drops by one percent in response to a one percent increase in price. A higher α value indicates the demand for the good is more elastic, suggesting the value of the commodity is diminished. In contrast, a lower α value indicates the demand for the good is relatively inelastic, suggesting the value of the commodity is enhanced.

A behavioral economics task was used to investigate the role of dopamine in the valuation of avoidance

To assess the role of mesocorticolimbic dopamine release events in the valuation of avoidance, we utilized a behavioral economic-based shock avoidance task. Our approach both builds upon previous within session designs that characterized the

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394 demand for sucrose and cocaine (Schelp et al., 2017; Oleson and Roberts, 2009) as
395 well as expands upon a recent between sessions approach that was the first to apply
396 behavioral economics to negative reinforcement (Fragale et al., 2017). As a within
397 session design is more conducive to neural monitoring, we began by exploring the
398 effects of increasing the unit-price (response requirement/mA shock avoided) of
399 avoidance within individual sessions. We first sought to increase unit-price by
400 manipulating the mA shock avoided (1.0-0.13mA) across fixed epochs. To accomplish
401 this, a range of six unit-prices were presented in descending order (**Fig. 1A**) and
402 avoidance was analyzed as a function of unit-price. Avoidance failed to consistently
403 scale with price when price was manipulated by changing shock amplitude across within
404 session epochs. Next, we sought to manipulate unit-price by increasing the response
405 requirement to avoid across within session epochs. As would be predicted by the law of
406 demand, this task engendered a stable pattern of behavior hallmarked by a negative
407 price elasticity—meaning that the demand to avoid decreased with increasing price
408 (**Fig. 1B**). However, we also observed an initial attenuation of behavior at session onset
409 which is distinct from previous reports investigating the demand to obtain sucrose
410 (Schelp et al., 2017) or cocaine (Oleson and Roberts, 2009). To address order effects,
411 we attempted to randomize the order in which the response requirements were
412 presented (**Fig 1C**) (upper). As was previously reported for sucrose demand (Schelp et
413 al., 2017), animals showed aberrant response patterns with randomization of unit-price
414 (lower). Thus, we proceeded with a task in which the unit-price was manipulated by
415 increasing response requirement and presented prices in a consistent ascending order.

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Finally, to ensure that all animals reached a price at which they failed to sustain demand, the total number of unit-prices was increased from six to 16.

Within the selected ascending response requirement task, a compound cue signaled the opportunity to respond on a lever in order avoid an unchanging electrical footshock (0.5s, 0.5mA). Failure to meet the response requirement within a set timeframe resulted in recurrent footshock until the full response requirement was met. This outcome is defined as escape. Successfully meeting the response requirement within a set timeframe resulted in a safety period signaled by a tone without the occurrence of footshock. This outcome is defined as avoidance. Following either avoidance or escape, a tone accompanied by house light illumination signaled a 30s safety period (**Fig. 2A**). The end of each safety period coincided with the onset of the next trial, starting with presentation of the avoidance predictive cue. Upon acquisition of avoidance under a fixed ratio 1 (FR1) reinforcement schedule, animals were introduced to unit-price manipulation through an increase in response requirement across discrete, time-based epochs in each daily session. Rats were initially trained to perform multiple lever press responding across six unit-prices (**Fig 2B**). Unit-price epoch duration was modulated to allow for a maximum of 20 avoidance opportunities within each unit-price (column 4) and additional time was provided to rats to meet increasing response requirement (column 3). Upon acquisition of multiple lever press responding rats were placed in an economic based task with 16 ascending unit-prices (**Fig 2C**). Once animals were successfully trained to escape, then avoid at ≥50% avoidance on an FR1 schedule, animals took an average of 13.9±3.1 sessions to acquire multiple lever press responding and 19.5±2.9 sessions within the economic task to establish stable baseline

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behavior (**Fig 2D**). Upon establishment of baseline behavior, animals entered optogenetic and FSCV experimentation.

While data were primarily analyzed in terms of successful avoidance outcomes at each unit-price, note that the additional delay required to meet increasing response requirements adds a conceptually important opportunity cost (**cf. columns 2 and 3, Fig. 2B,C**). Thus, total cost to the animal results from both effort and opportunity costs.

Unique avoidance demand curves were generated to measure price sensitivity

This task allowed us to generate within session demand curves by plotting total avoidance responses per epoch against unit-price. Unique demand curves emerged over the course of acquisition of the economic task, with an attenuation of avoidance at 2 resp/mA becoming apparent in experienced animals (**Fig. 3A**)**.** The ratio of total avoidance at 4 resp/mA vs. 2 resp/mA significantly increased over the course of training (one-way RM ANOVA: $F_{(2,44)}=9.87$, $p<0.001$; Tukey Post-Hoc: start of training vs. end of training $p<0.001$) (**Fig. 3B)**. Similar economic-based food-seeking tasks do not demonstrate this attenuation at session onset (Schelp et al., 2017) (**Fig. 3C**), suggesting that additional inhibitory neural circuitry might be recruited during economic assessments of avoidance (Jhou et al., 2009). The ratio of avoidance 4 resp/mA vs. attenuated avoidance at 2 resp/mA in both a transgenic TH-Cre and WT group develops and stabilizes over the course of training (**Fig. 3D**). While a measure of demand, α, similarly stabilizes over the course of training in both the TH-Cre and WT group (**Fig. 3E**), suggesting this attenuation might be a learned suppression that develops across repeated sessions, each of which terminates when the animal receives 15-20 consecutive footshocks.

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Dopamine release events correspond to value associative cues in avoidance

To evaluate the role of NAcc dopamine during the valuation of avoidance, we employed FSCV to measure transient changes in dopamine concentration time-locked (±0.5s) to associative cues in the behavioral economics-based task. We exclusively analyzed dopamine concentration within the second through fourth unit-price because 100% of animals maintained responding in this range and a concurrent attenuation in behavior and dopamine concentration was observed at the first price point. We independently address these attenuated responses later in the manuscript (**Fig. 10**). The nA changes in current were converted to nM concentration by utilizing principle component regression and lab-specific computational factors (see methods). Dopamine concentration files were arranged around one of two events: 1) avoidance predictive cue, 2) safety associated cue. Dopamine at the avoidance predictive cue was quantified and analyzed as a group mean because the mean data were representative of individual trials (cf. **Fig. 4A inset** vs **Fig. 4B,D**). Dopamine during the safety period was quantified and analyzed at the level of the individual transient because mean data were not representative of individual trial (cf. **Fig. 5C** vs **Fig. 5A,B**).

Dopamine at the avoidance predictive cue

In successful avoidance trials we found that dopamine concentration at the avoidance predictive cue scaled inversely to unit-price (one way RM ANOVA: $F_{(2,20)}$=29.507, $p<0.001$; Tukey Post-Hoc: Unit-price 4 vs. 6 $p<0.001$, 4 vs 10 $p<0.001$) **(Fig. 4A)**. A representative avoidance trial (**Fig. 4A inset**) demonstrates individual trial similarity to mean dopamine concentration plots for all animals. Average color plots demonstrate these avoidance cue trends at each unit-price (bottom) with corresponding

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485 concentration traces (top) **(Fig. 4B)**. Contrary to our predictions (Oleson et al., 2012), in
486 escape trials we found that dopamine concentration also showed an inverse relationship
487 to unit-price (one way RM ANOVA: $F_{(2,20)}$=3.916, p=0.049; Tukey Post-Hoc: Unit-price 4
488 vs. 10 p=0.040) **(Fig. 4C).** Within escape trials, dopamine concentration did not,
489 however, demonstrate sensitivity to number of shocks subsequently received (one way
490 RM ANOVA: $F_{(3,23)}$=2.14; p=0.14) **(Fig. 4C inset**). As would be predicted (Oleson et al.,
491 2012), an increase in dopamine concentration was observed prior to cue presentation.
492 Given that the safety period duration preceding cue presentation is consistent (30s) is it
493 possible that this preemptive increase in dopamine reflects anticipation of the cue. As
494 such, it should be noted that the dopamine concentration at the warning signal likely
495 results from both anticipation of the warning signal its actual presentation. However,
496 varying the duration of the safety period is known to alter avoidance (Sidman, 1953),
497 and theoretically would alter the valuation of avoidance, thus it is necessary to use a
498 fixed, 30s safety period for the behavioral economics task. Together these data
499 demonstrate that, as in reward seeking, dopamine concentration at an avoidance
500 predictive cue generally scales with price.

501 **Dopamine at the safety associated cue**

502 We further analyzed the amplitude of dopamine transient events during the 30s safety
503 period following both avoidance and escape outcomes. Unlike dopamine concentration
504 transients time-locked to cue presentation, averaged safety period color plots (bottom)
505 and corresponding concentration traces (top) following avoidance (**Fig. 5A**) and escape
506 (**Fig. 5B**) are not representative of individual safety period trials (**Fig. 5C**). Therefore, to
507 analyze safety period transients, dopamine concentration traces from individual trials

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were fit with a baseline polynomial line (**Fig 5D**) (red) and a standard deviation was added to the baseline fit to generate a cutoff (blue). The baseline was set to zero and concentrations above the first fit line were analyzed as transients (**Fig 5E**). Analysis of individual safety period trials revealed an inverse relationship between average transient amplitude and unit-price following successful avoidance (one way RM ANOVA: $F_{(3,27)}$=3.50, p=0.037; Tukey Post-Hoc: Unit-price 2 vs. 10 p=0.029) **(Fig. 6A)**. This trend is demonstrated with representative color plots (bottom) and corresponding concentration traces (top) **(Fig. 6B)**. Conversely, concentration directly following escape demonstrated no relationship to unit-price (one way RM ANOVA: $F_{(3,27)}$=0.902; p=0.460) **(Fig. 6C,D)**. These data suggest that dopamine only represents the valuation of avoidance during the safety period following successful avoidance.

Modeling the elasticity of demand in avoidance

To measure changes in price sensitivity, total avoidance versus unit-price was fit with an exponentially decaying model:

$$Q = Q_{min} + (Q_{max} - Q_{min})e^{-\alpha C}$$

with the variable α describing the rate of decay, Q_{max} depicting avoidance at zero price, Q_{min} depicting minimum avoidance, and *C* representing unit-price. Notably, α is inversely related to avoidance performance, with lower α values signifying higher avoidance while larger α values reflect lower avoidance **(Fig. 7A)**. As previously noted **(Fig. 3A)**, an initial attenuation in demand occurs at 2 resp/mA. Similarly to the loading phase of cocaine self-administration (Oleson et al., 2011; Bentzley et al., 2014), this initial attenuation may be influenced by additional variables other than price. Thus, we investigated whether removing the first data point resulted in better-fitted demand

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profiles. To account for differences in the degrees of freedom between fitting 16 unit-prices and 15 unit-prices, the reduced chi squared (X_v^2) was calculated with both the inclusion and exclusion of the lowest unit-price. It was revealed that the exclusion of the lowest unit-price yielded significantly lower X_v^2 (included: 0.178±0.0059; excluded: 0.104 ±0.0053) (Mann-Whitney Rank Sum Test: $U_{(n1=n2=272)}$=20155.0, p<0.001) **(Fig. 7B)** as well as increased R^2 values (included: 0.85±0.005; excluded: 0.91±0.005) **(Fig. 7C).** Given that removal of the first data point results in significantly lower X_v^2 we opted to analyze demand profiles generated in the avoidance task after removing the first data point—as is done when analyzing demand profiles of cocaine self-administration (Oleson et al., 2011). This approach, combined with optogenetics, allowed us to investigate how dopamine neurons causally modify economic demand in avoidance.

Dopamine release events causally modify demand for avoidance

Optogenetics can be used to assess the causal relationship between patterns of neural activity and behavior (Steinberg et al., 2013). Thus, we next sought to investigate how optically increasing dopamine release through selective optogenetic activation (10 pulses, 20Hz, 0.5s duration) of ChR2 expressing dopamine neurons of the VTA alters demand for avoidance. While future studies are necessary to parse the role of dopamine release at its various terminal projections, we opted to begin by augmenting mesoscorticolimbic dopamine release at its origin. Initially, animals from both experimental TH-Cre and wild type (WT) control groups were trained to respond on a lever to terminate and escape footshock. Upon acquisition of escape behavior animals were moved into an FR1 avoidance task until >50% avoidance was reached and maintained. Subsequent to the establishment of >50% avoidance behavior, animals

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were trained to respond multiple times on the lever to avoid footshock across six unit-prices**.** Upon acquisition of multiple lever presses, animals were trained on the economic based shock avoidance task until a baseline rate of avoidance was established across three sessions. After establishing stable baseline behavior, we provided unilateral optogenetic stimulation of the VTA cell bodies of the right hemisphere at either the presentation of the avoidance predictive cue or upon successful avoidance. All animals were tested under both stimulation conditions. We investigated the effects of optical stimulation at the first event for three sessions followed by reestablishment of baseline behavior and then stimulation under the complimentary event. The order of stimulation (avoidance predictive cue vs successful avoidance) was counterbalanced within both the TH-Cre group and the WT group **(Fig. 8A).** The number of successful avoidances out of 20 avoidance opportunities within each unit-price was fit with an exponentially decaying model (**Fig 7A**) and optogenetic-induced changes in demand were assessed by comparing α values to baseline values. Representative cumulative response records, response-price curves and corresponding demand curves from a single TH-Cre animal depict the resulting patterns of behavior across all stimulation conditions **(Fig. 8B-E).**

Analysis of the TH-Cre group revealed that stimulation at the avoidance predictive cue significantly increased α (decreased demand to avoid) while stimulation at successful avoidance significantly decreased α (increased demand to avoid). Cue associated baseline and avoidance associated baseline were not significantly different (Friedman RM ANOVA on ranks: $X^2_{(3)}=17.88$, $p<0.001$; Tukey Post-Hoc: Baseline-1 vs. Cue $p=0.017$, Baseline-2 vs. Avoid $p=0.017$, Cue vs. Avoid $p=0.003$, Baseline-1 vs.

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Baseline-2 p=0.072) **(Fig. 9A)**. Conversely, the WT group demonstrated no change in α value with optogenetic stimulation (one way RM ANOVA: $X^2_{(3)}$=1.08, p=0.78) (**Fig. 9B)**. Averaged avoidance within the TH-Cre group depicts changes in demand with cue (**Fig. 9C**) and avoidance (**Fig 9D**) stimulation. Neither stimulation condition produced a significant change in avoidance or escape response latency when compared to baseline (one-way RM ANOVA: Cre avoidance: $F_{(3,39)}$=0.824, p=0.49; Cre escape $F_{(3,39)}$=2.87, p=0.055) (Friedman RM ANOVA on ranks: WT avoidance: $X^2_{(3)}$=1.2, p=0.75; WT escape: $X^2_{(3)}$=2.0, p=0.56). As in reward seeking (Schelp et al., 2017), both Q_{min} and Q_{max} demonstrated no significant change in response to optogenetic manipulations, suggesting that α is more highly associated with changes in avoidance price sensitivity. These data demonstrate that optical stimulation of dopamine neurons influences the demand to avoid electrical footshock, supporting the notion that dopamine causally modifies the valuation of avoidance.

Dopamine is concurrently attenuated with avoidance at session onset

We additionally sought to analyze the attenuation of avoidance at 2 resp/mA (**Fig. 10A**). Dopamine time-locked to cues (±0.5s) preceding avoidance at 2 resp/mA is significantly lower than cue evoked dopamine at 4 resp/mA. (**Fig. 10B**) Averaged color plots (bottom) and dopamine concentration traces (top) depict these trends. Conversely, dopamine time-locked (±0.5s) to cues preceding escape at 2 resp/mA was greater than at 4 resp/mA. (**Fig. 10C**) Averaged color plots (bottom) and dopamine concentration traces (top) demonstrate this trend. Optical stimulation of dopamine neurons similarly influences the attenuation in demand observed as session onset. In the TH-Cre group, optical stimulation at successful avoidance, but not at the avoidance predictive cue,

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reduced attenuation relative to baseline performance (one way RM ANOVA: $F(3,39)=3.76$, $p=0.022$; Tukey Post-Hoc: Baseline-1 vs Cue $p=0.28$, Baseline-2 vs. Avoid $p=0.042$). Conversely, the WT group demonstrated no change in attenuated avoidance in response to optical stimulation (one way RM ANOVA: $F(3,39)=1.00$, $p=0.41$) (**Fig. 10D**). Our electrochemistry data demonstrate that dopamine at the avoidance predictive cue is concurrently attenuated with behavior at session onset in this task. We believe this attenuation at session onset is a learned suppression that develops following repeated training sessions in which sessions terminate with repeated electrical footshock. Our optogenetics data reveal that dopamine release is sufficient to rectify attenuation of avoidance demand at session onset. Together, these observations support the view that heightened dopamine release can overcome the effects of negative affect on behavior (Chaudhury et al., 2013; Tye et al., 2013).

Additional experimental controls and considerations

Upon completion of optogenetic experiments, neural tissue was harvested and expression of ChR2 in the VTA and NAcc was confirmed in all TH-Cre animals (**Fig 11A,B)**. Optical ferrule cannulae, which were assessed before and after surgical implantation, demonstrated no significant decrease in retention (Paired Two-tailed t-test: $t_{(7)}=1.59$, $p=0.15$) (**Fig 11C**)**.** In order to address potential locomotor confounds produced by optical stimulation, recurrent optogenetic stimulation of VTA dopamine neurons was conducted within the TH-Cre group during an open field test. This augmentation failed to increase horizontal activity relative to baseline (Paired Two-tailed t-test: $t_{(7)}=-0.363$, $p=0.727$)**,** suggesting changes in overall activity played a minimal role in our demand profiles. Upon completion of the voltammetric experiments, animals were

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622 sacrificed and electric lesions at the depth of the recording site confirmed electrode
623 placement within the NAcc (**Fig 11E**).

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Discussion

We investigated the role of mesocorticolimbic dopamine release events in the valuation of avoidance using a combination of behavioral economics, modeling, electrochemistry and optogenetics. After an initial attenuation of dopamine release and behavior at session onset, accumbal dopamine concentration scaled inversely with price during the active avoidance of signaled footshock. Dopamine at the warning signal decreased with price irrespective of the outcome (avoidance vs. escape); however, dopamine only decreased with price at the safety associated cue following successful avoidance. We interpret these distinct outcome-specific responses to suggest that dopamine concentration prior to the behavioral action was predictive of value; whereas, dopamine concentration following action was reflective of the outcomes value.

We next sought to assess the causal role of dopamine in the valuation of avoidance within an economic framework. A central principle of economics is that as the price of a commodity increases, its demand decreases. This principle is commonly demonstrated using demand curves, in which consumption of a commodity is plotted against price. According to the law of demand, consumption typically decreases with increasing price. The resulting negative gradient represents the elasticity of demand, or how sensitive consumption of the commodity is to increasing price. In the present study, consumption is defined as the number of successful avoidance responses at each price (responses/mA). If avoidance became more sensitive to price, the resulting demand curve would decay at a faster rate; if avoidance became less sensitive to price, the resulting demand curve would decay at a slower rate. We can then make inferences regarding the value of avoidance by measuring the rate of decay. We compute the rate

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at which demand curves decay by solving for the variable α, which represents the cost at which the elasticity of demand is exactly -1. At this point, consumption drops by one percent in response to a one percent increase in price. A higher α value indicates the demand for the good is more elastic, suggesting the value of the commodity is diminished. In contrast, a lower α value indicates the demand for the good is relatively inelastic, suggesting the value of the commodity is enhanced. In accordance with our previous work assessing the role of dopamine in the valuation of a sugar reward (Schelp et al., 2017), we predicted that increasing dopamine release at an avoidance predictive cue would decrease α; whereas, increasing release at successful avoidance would increase α.

Optically stimulating dopamine cell bodies in the VTA at an avoidance predictive cue rendered animals more sensitive to price (i.e., α increased), consistent with a negative reward prediction error. We infer that heightened release at cue presentation signaled a beneficial outcome, a prediction that was then violated by the occurrence of footshock outcomes of the same amplitude. Optically increasing release at successful avoidance made animals less sensitive to price (i.e., α decreased), consistent with a positive reward prediction error. We infer that heightened release at successful avoidance signaled the outcome was better than predicted, indicating a good value worth seeking. Our data build upon the notion that transient dopamine release events can represent subjective value (Schelp et al., 2017; Schultz et al., 2017), and further clarify that these value signals not only represent the value of pursuing reward, but also the value of avoiding harm.

The mesocorticolimbic pathway originates from dopamine neurons in the VTA and

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projects to motivational circuitry throughout the brain, most prominently the NAcc of the basal ganglia. The NAcc is thought to integrate transient dopamine signals with an array of converging neural input to generate goal directed actions via the basal ganglia (Floresco, 2015; Graybiel and Grafton, 2015; Haber, 2014; Jin et al., 2014). Both the pursuit of reward (positive reinforcement) and the active avoidance of harm (negative reinforcement) require an increase in action to ultimately promote behavioral fitness and survival. While it is likely that individual dopamine neurons heterogeneously represent rewarding and aversive stimuli (Lammel et al., 2014; Pignatelli and Bonci, 2015), our data suggest that the summation of dopamine neural output is increased in the NAcc during active avoidance. Overall, we believe that these transient release events act on the basal ganglia to strengthen action sequences directed toward optimal outcomes. However, it is important to note that optical stimulation of the VTA dopamine neuron cell bodies leads to increases in concentration in various neural substrates implicated in avoidance (Darvas et al., 2011; Pignatelli and Bonci, 2015). Future studies are needed to investigate the specific role that dopamine plays in the valuation of negative reinforcement in various regions including the frontal cortex, amygdala, and striatum.

It is important to note that there are distinct forms of avoidance and dopamine may play unique roles in each. For example, if transient dopamine release events are significant for action generation, then dopamine release should be suppressed in passive avoidance—a situation where animals must inhibit action in order to avoid a negative outcome (Ögren and Stiedl, 2010). Unsignaled 'Sidman' avoidance is another interesting consideration. In Sidman avoidance animals show active avoidance despite the absence of an exteroceptive warning signal (Sidman, 1953). It is possible that

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693 during unsignaled avoidance the lever itself, in addition to proprioceptive associations
694 that develop during the lever response, function as conditioned stimuli. Another
695 possibility is that dopamine may guide unsignaled avoidance through representation of
696 interoceptive timing cues (Oleson et al., 2014; van Jijn et al., 2014). Indeed, as
697 previously noted (**Fig 4B,D**), we observed an increase in dopamine concentration
698 preceding the cue, implying an anticipation of cue presentation as animals perform
699 within this task. While additional studies are required to fully understand how dopamine
700 is related to the various forms of avoidance, it is important to note that distinct patterns
701 of dopamine release likely distinguish them.
702 In contrast to active avoidance, transient dopamine release is suppressed by
703 unavoidable aversive events and their associative stimuli. Using FSCV, the Roitman
704 group has demonstrated that a variety of aversive stimuli transiently decrease accumbal
705 dopamine concentration (Ebner et al., 2010; Fortin et al., 2016; McCutcheon et al.,
706 2012; Roitman et al., 2008). Similarly, stimuli associated with inescapable electrical
707 footshock suppress accumbal dopamine concentration within the NAc (Badrinarayan et
708 al., 2012; Oleson et al., 2012). While these dopamine release events likely represent
709 the summation of VTA dopamine neural activity, there is growing evidence to support
710 heterogeneous representation of rewarding and aversive stimuli at the level of the single
711 unit (Lammel et al., 2012; Pignatelli and Bonci, 2015). While the majority of
712 electrophysiology studies report that dopamine neurons are inhibited by aversive stimuli
713 (Mileykovskiy and Morales, 2011; Tan et al., 2012), numerous reports of excitations
714 exist as well (Brischoux et al., 2009; Matsumoto and Hikosaka, 2009). While some of
715 these reports were likely confounded by the inclusion of non-dopamine neurons

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716 (Ungless and Grace, 2012), it is likely that many factors influence whether individual
717 neurons are excited or inhibited by an aversive event, including the subpopulation of
718 neurons surrounding the recording site (Lammel et al., 2012) and the behavioral context
719 in which aversion is introduced (Matsumoto et al., 2016).
720 Dopamine responses in active avoidance further depend on the behavioral history of the
721 subject and the behavioral context in which avoidance is assessed. Notably, our
722 observations here are discrepant from a previous FSCV characterization of dopamine in
723 signaled active avoidance (Oleson et al., 2012) which observed distinct responses at
724 the avoidance predictive cue during the escape outcome. In the present study,
725 dopamine scaled with price at avoidance predictive cues—even in escape. Conversely,
726 Oleson et al. (2012) reported that dopamine was inhibited at the avoidance predictive
727 cues preceding escape. We believe that this discrepancy is due to a combination of
728 behavioral history and behavioral context. While Oleson et al. (2012) observed a
729 suppression in dopamine concentration and behavior when the avoidance predictive
730 cue resulted in escape rather than avoidance, animals were simply trained to avoid
731 footshock under an FR1 schedule in up to 50% of trials and sessions terminated after a
732 fixed amount of time. In the current task, animals were extensively trained to avoid in
733 several different paradigms (see methods) prior to being tested in the much more
734 strenuous behavioral economics task. Furthermore, in the current task, sessions only
735 terminate after 15 shocks or 20 escapes occur in a consecutive order. Thus, we believe
736 that this initial attenuation of both behavior and dopamine release is induced by session
737 onset, which comes to predict a negative session end. This powerful negative prediction
738 develops and stabilizes over the course of economic avoidance training (**Fig. 3D,E**)

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739 supporting the notion that it is a learned association and distinct from changes in
740 performance with optical stimulation. Once the animals had established stable baseline
741 performance in the behavioral economics task, the negative prediction represented by
742 session onset was sufficient to attenuate dopamine release and active avoidance.
743 Optically augmenting dopamine release at avoidance rectified this behavioral
744 attenuation, further supporting recent evidence that heightened dopamine release can
745 overcome the effects of negative affect on behavior (Chaudhury et al., 2013; Tye et al.,
746 2013).

747 Dopamine responses at the avoidance predictive cue depend on the behavioral
748 outcome (avoidance vs. escape). We observed distinct responses to avoidance
749 associative cues depending on whether animals avoided or escaped footshock. During
750 successful avoidance, cue-evoked dopamine concentration scaled with changing price,
751 resulting from both an increase in effort and opportunity costs, and corresponded to the
752 avoidance outcome. During escape outcomes, cue-evoked dopamine concentration
753 scaled with price irrespective of behavior. We interpret the distinct responses at the
754 avoidance predictive cue to suggest that dopamine is continually representing
755 avoidance value but is being simultaneously attenuated with behavior at session onset.
756 We surmise this concurrent attenuation at session onset results from afferent inhibitory
757 input, possibly from the lateral habenula via the rostromedial tegmentum (Jhou et al.,
758 2009).

759 Dopamine responses during the safety period also depend on the behavioral outcome.
760 We observed dopamine concentration scaled with price during the signaled safety
761 period when animals successfully avoided footshock, but not following escape. The

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762 distinct dopamine responses observed during the safety period may be relevant in the
763 context of avoidance learning. Successfully avoiding an aversive event was the optimal
764 outcome under our experimental conditions. Thus, dopamine might exclusively convey
765 value during the safety period after optimal outcomes—thereby promoting their
766 recurrence. While entering safety after escape is a beneficial outcome as well,
767 dopamine may fail to represent the value of this less optimal outcome. Rather, we
768 reason that dopamine generally represents the safety signal proceeding escape as a
769 positive relief from pain (Navratilova and Porreca, 2014).
770 Avoidance outcomes produce unique behavioral and neurochemical responses when
771 compared to rewarding outcomes (Schelp et al., 2017), suggesting that avoidance is
772 more than simply reward redux. While the avoidance of an aversive event is rewarding,
773 the avoidance outcome still carries aversive qualities. When animals run toward a goal
774 box containing a footshock-associated rewarding outcome they show an approach-
775 avoidance conflict, wherein retreat from the goal box accompanies the pursuit of reward
776 (Geist and Ettenberg, 1997). In addition, unique demand profiles and dopamine
777 responses are observed during the valuation of avoidance and reward (Roitman et al.,
778 2008). The aversive attributes of footshock avoidance likely recruit additional neural
779 circuits that interact with the mesocortiolimbic pathway during negative reinforcement.
780 Thus, while mesocorticolimbic dopamine release events similarly represent the value of
781 both rewarding and avoidance outcomes, we surmise that the overall neural circuitry
782 underlying positive and negative reinforcement is distinct.
783 In conclusion, we demonstrate that accumbal transient dopamine release events scale
784 proportionally to the value of avoidance outcomes and capably modify the valuation of

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785 active avoidance. Our data refute the notion that mesocorticolimbic dopamine is
786 exclusively involved in positive reinforcement (Fiorillo 2013) and, rather, indicate that
787 dopamine represents the value of all advantageous outcomes, including the avoidance
788 of harm.

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922 **Figure Captions**

923 **Figure 1.** Unit-price randomization and manipulation of unit-price through changing mA
924 shock avoided prevents establishment of baseline avoidance performance during a
925 within session design in which unit-price epoch duration was modulated to allow 20
926 avoidance opportunities at each unit-price (**A**) The first 6 unit-prices, achieved through
927 decreases in the mA of shock avoided (upper), were presented in descending order to
928 animals and avoidance behavior was assessed. Representative avoidance with mA
929 shock manipulation demonstrates variability and failure of avoidance to scale with
930 increasing price (lower). (**B**) Ascending unit-price through increase in response
931 requirement (upper) demonstrates a unique, but stable avoidance demand curve with
932 increasing unit-price (lower). (**C**) The first 5 unit-prices, achieved through increasing
933 response requirement (upper) were randomly presented to animals and avoidance
934 behavior was assessed, representative avoidance behavior demonstrates variability
935 between sessions and failure to establish a baseline of avoidance (lower).

936 **Figure 2.** Shock avoidance paradigm and behavioral economics methodology. (**A**) An
937 avoidance predictive cue, characterized by the presentation of a lever and the
938 illuminator of a cue light, is presented to signal the opportunity for an animal to respond
939 on the lever to avoid. If a response is made within a set interval (**Fig 2B,C column 3**)
940 the animal does not receive footshock. This is considered an avoidance (blue). If the
941 animal fails to meet the response requirement with a set interval, the animal receives
942 recurrent footshock until the full response requirement is met and the animal escapes
943 further shock (red). (**B**) To increase the cost of avoidance, unit-price (column 1: defined
944 as response requirement/mA shock) increases through an increase in response

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945 requirement (column 2). Animals were initially presented 6 unit-prices to train multiple
946 lever press responding with additional time provided to meet higher response
947 requirements prior to shock onset (column 3). The epoch length increases in duration to
948 allow for 20 avoidance opportunities at every unit-price (column 4). (**C**) Upon acquisition
949 of multiple lever press responding, animals were moved to the economic based task
950 wherein 16 ascending unit-prices were presented. Here seconds to respond prior to
951 shock onset (column 3) was restricted relative to the 6 unit-price training task. (**D**)
952 Timeline depicting animals' progression through active operant avoidance training. 38
953 animals were first trained to respond to escape unavoidable footshock. The 37 which
954 animals fully acquired escape behavior moved into a FR1 avoidance task until rats
955 consistently (≥3 sessions) performed avoidance at ≥50% avoidance performance. Upon
956 acquisition of avoidance behavior, animals were trained to meet increasing response
957 requirements across 6 unit-price epochs. Of the 37 animals introduced to this task, 26
958 demonstrated avoidance at ≥3 unit-prices, taking an average of 13.9±3.1 sessions to
959 acquire, and proceeded to the next task. Animals were moved into an economic
960 footshock avoidance task with 16 ascending unit-prices. Here seconds (column 3) to
961 respond prior to shock onset was restricted relative to the training task. Upon
962 establishing a stable, baseline rate of avoidance (average 19.5±2.9 sessions), animals
963 were placed either in the optogenetics or FSCV group.

964 **Figure 3.** Attenuation of avoidance at 2 resp/mA develops during acquisition of the
965 economic shock avoidance task. (**A**) As animals acquired this economic shock
966 avoidance task (**cf. Fig 1C**) an attenuation of avoidance at the lowest unit-price (2
967 resp/mA) developed relative to responding at the second unit-price (4 resp/mA). (**B**) The

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968 ratio between avoidance at 4 resp/mA and avoidance at 2 resp/mA significantly
969 increased as animals established baseline rates of performance. (**C**) Demand curves
970 depicting economic food seeking does not demonstrate attenuation of consumption at
971 session onset. (**D**) The ratio of avoidance at 4:2 resp/mA increases and stabilizes over
972 the course of training in both the TH-Cre and WT group. (**E**) α, a measure of avoidance
973 demand, similarly stabilizes over the course of training. Error bars are mean ±SEM.

974 **Figure 4.** Accumbal dopamine concentration scales as a function of unit-price. **(A)** The
975 concentration of dopamine at cues preceding avoidance at unit-prices 4,6, and 10
976 resp/mA scale inversely to unit-price. Representative avoidance cue trial (inset) **(B)**
977 Average color plots (bottom) and dopamine concentration traces (top) depicting
978 avoidance cues demonstrate this trend. **(C)** Conversely, the concentration of dopamine
979 at cues preceding escape decreased with increasing unit-price, while the concentration
980 at cues preceding 1,2,3, and 4 shocks received across all unit-prices demonstrated no
981 sensitivity to unit-price (inset) **(D)** Average color plots (bottom) and dopamine
982 concentration traces (top) depict dopamine concentration at cues preceding escape with
983 increasing unit-price. Error bars are mean ±SEM.

984 **Figure 5.** Dopamine during the 30s safety period following avoidance and escape. (**A**)
985 Averaged color plots (bottom) and dopamine concentration traces (top) limit viable
986 analysis of dopamine transients following successful avoidance and escape (**B,C**).
987 Individual color plot (bottom) and dopamine concentration trace (top) may be fit (**D**) with
988 a baseline polynomic line (red). Addition of a standard deviation to this baseline fit

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989 determines the fit (blue). (**E**) The baseline fit is normalized to zero and concentration
990 above this first fit line are considered transients.

991 **Figure 6.** Dopamine during the 30s safety period following avoidance, but not escape,
992 scales as a function of unit-price. **(A)** The average concentration of accumbal dopamine
993 transients following successful avoidance decreases with increasing unit price, while the
994 concentration of dopamine following escape did not change as a function of unit-price
995 **(C).** Representative color plots (bottom) and dopamine concentration traces (top) depict
996 these trends at the first 4 unit-prices following avoidance **(B)** and escape **(D)**. Error bars
997 are mean ±SEM.

998 **Figure 7.** Modeling demand for avoidance. **(A)** An exponentially decaying model fit
999 successful avoidances out of 20 potential cue/response parings as a function of unit-
1000 price. Here the variable α is inversely related to the rate of decay or elasticity of
1001 avoidance demand, allowing for a direct assessment of motivation to avoid. **(B)** The
1002 attenuation of avoidance at session onset (**cf. Fig 3A**) necessitated the exclusion of the
1003 lowest unit-price from economic modeling. The reduced chi squares (X_v^2) generated
1004 from the exclusion vs inclusion of the lowest unit-price rationalized this exclusion. **(C)**
1005 The fits garnered from this equation revealed relatively high R^2 values (0.92±0.005).
1006 Error bars are mean ±SEM.

1007 **Figure 8.** Representative behavior from a single TH-Cre animal. **(A)** Cue and
1008 optogenetic augmentation of VTA dopamine cell bodies was counterbalanced across
1009 both the TH-Cre and WT groups. Following the establishment of baseline across three
1010 behavioral sessions, both groups initially received optical stimulation at either the

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1011 presentation of the cue (left) or upon successful avoidance (right) for three sessions.
1012 Upon completion of the first stimulation paradigm, animals reestablished behavior over
1013 three sessions followed by stimulation at the complementary paradigm for three
1014 sessions. **(B,C)** Successful avoidances out of 20 potential avoidance opportunities from
1015 a representative TH-Cre animal were fit with the exponentially decaying model during
1016 cue, avoidance and baseline conditions. **(D)** Avoidance lever responses as a function of
1017 unit price. **(E)** Cumulative avoidance record.

1018 **Figure 9.** Optogenetic augmentation of VTA dopamine at the cue upon avoidance alters
1019 the valuation of avoidance. **(A)** α values of the TH-Cre group increase with cue
1020 stimulation but decrease with stimulation upon successful avoidance. **(B)** Cue and
1021 avoidance stimulation did not change avoidance performance within the WT control
1022 group. **(C,D)** Averaged behavioral trends within the TH-Cre group depict avoidance
1023 demand (out of 20 potential avoidance opportunities at each unit-price) with cue and
1024 avoid stim relative to baseline. Error bars are mean ± SEM.

1025 **Figure 10.** Dopamine scales with avoidance attenuation at session onset and optical
1026 augmentation of dopamine attenuation at 2 resp/mA (**A)** Avoidance at the 2 resp/mA is
1027 attenuated relative to avoidance at 4 resp/mA. (**B**) Dopamine concentrations at 2
1028 resp/mA avoidance cues are significantly less than dopamine at 4 resp/mA avoidance
1029 cues. Averaged color plots (bottom) with corresponding dopamine concentration traces
1030 (top) depict these trends. (**C**) Dopamine concentrations at 2 resp/mA escape cues are
1031 significantly less than dopamine at 4 resp/mA escape cues. Averaged color plots
1032 (bottom) with corresponding dopamine concentration traces (top) depict these trends.

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(**D**) Avoidance stimulation, but not cue stimulation, altered avoidance at 2 resp/mA within the TH-Cre group (left), but not within the WT group (right). Error bars are mean ± SEM.

Figure 11. Histology, locomotor assessment and optical ferrule retention. At the end of optogenetic experimentation, rats were deeply anesthetized with a 50:50 ketamine:xylazine solution and transcardially perfused. Neural tissue was harvested, sliced coronally into 50 µm slices and stained for tyrosine hydroxylase (TH) and DAPI. Expression of ChR2 in VTA (**A**) and NAcc (**B**) was verified in all TH-Cre animals. **(C)** Light retention of the optical ferrule cannula was assessed both prior to and upon completion of the experiment. A t test revealed no significant difference in ferrule retention. **(D)** To assess for potential locomotor confounds associated with optogenetic stimulation of VTA dopamine neuron cell bodies, rats were first placed in open field chambers and allowed to acclimate for 1hr before moving into either a 1hr stimulation paradigm wherein they received optical stimulation every 30s, mirroring the max frequency of stimulation animals may receive in the economic task. Baseline and stimulation conditions were counterbalanced across 8 animals and did not significantly alter locomotion. **(E)** Upon completion of the voltammetric experiments rats were euthanized with CO2 and, utilizing micromanipulators identical to those used for the voltammetry recordings, stainless steel electrodes were lowered to the same depth as the working electrode during the voltammetry recording. A current to both the stainless-steel electrode and the reference electrode was applied for ~40s to lesion the neural tissue at the recording site. Tissue was coronally sectioned into 50 µm slices and

placement of the electrodes within the NAcc was assessed. Lesion locations are indicated in red.

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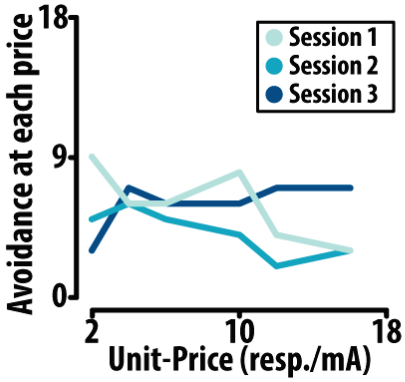
Figure 1

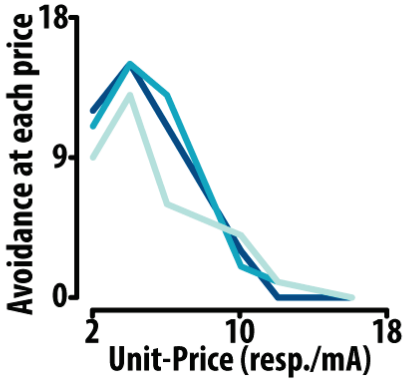
A Shock mA Manipulation

Unit-Price Resp/mA	Shock (mA)	Safety Period (sec)	Bin length (min)
2	1.0	1.82	0.64
4	0.5	3.33	1.78
6	0.33	4	3
10	0.2	5	5.83
12	0.17	6.67	8.67
16	0.13	10	16



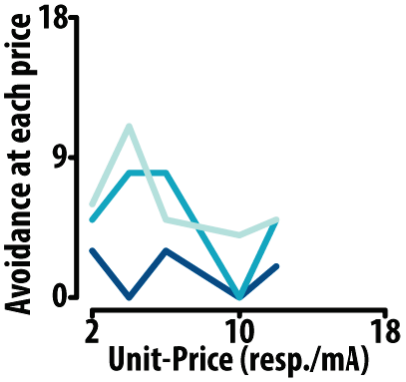
B Ascending Response Req

Unit-Price Resp/mA	Response Req.	Seconds to Respond	Bin length (min; cum)
2	1	1	10.33
4	2	2	21
6	3	3	32
10	5	4	43.33
12	6	5	55
16	8	6	67



C Randomized Unit-Price

Unit-Price Resp/mA	Response Req.	Seconds to Respond	Bin length (min)
2	1	1	10.33
4	2	2	10.67
6	3	3	11
10	5	4	11.33
12	6	5	11.67



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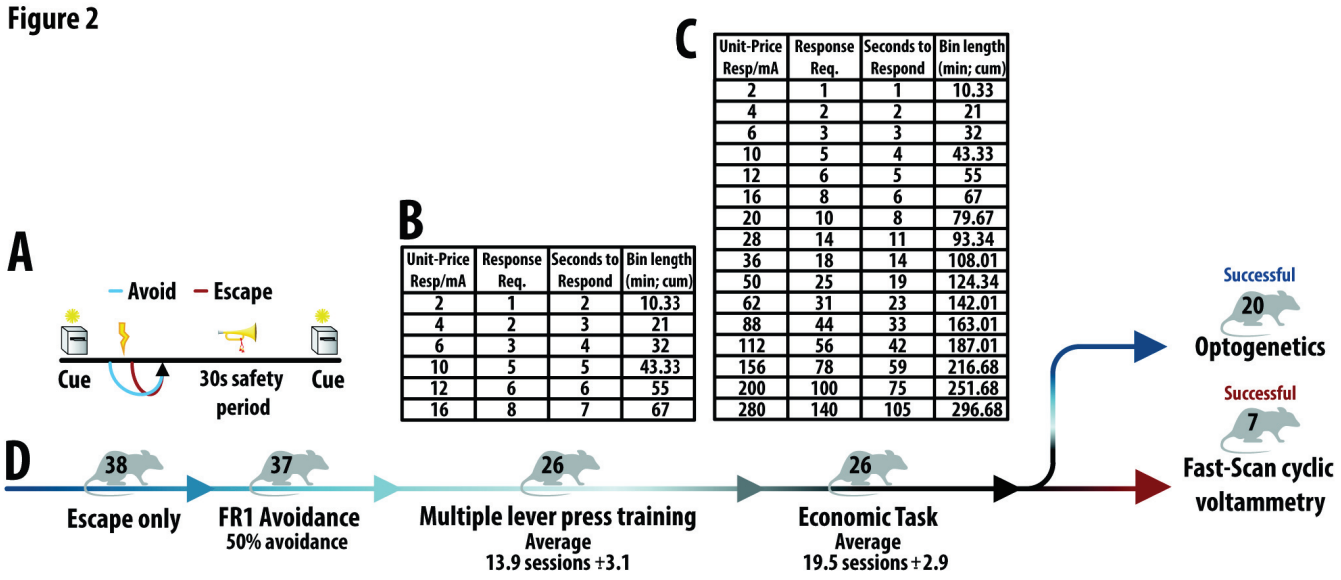

Unit-Price Resp/mA	Response Req.	Seconds to Respond	Bin length (min; cum)
2	1	2	10.33
4	2	3	21
6	3	4	32
10	5	5	43.33
12	6	6	55
16	8	7	67

Unit-Price Resp/mA	Response Req.	Seconds to Respond	Bin length (min; cum)
2	1	1	10.33
4	2	2	21
6	3	3	32
10	5	4	43.33
12	6	5	55
16	8	6	67
20	10	8	79.67
28	14	11	93.34
36	18	14	108.01
50	25	19	124.34
62	31	23	142.01
88	44	33	163.01
112	56	42	187.01
156	78	59	216.68
200	100	75	251.68
280	140	105	296.68

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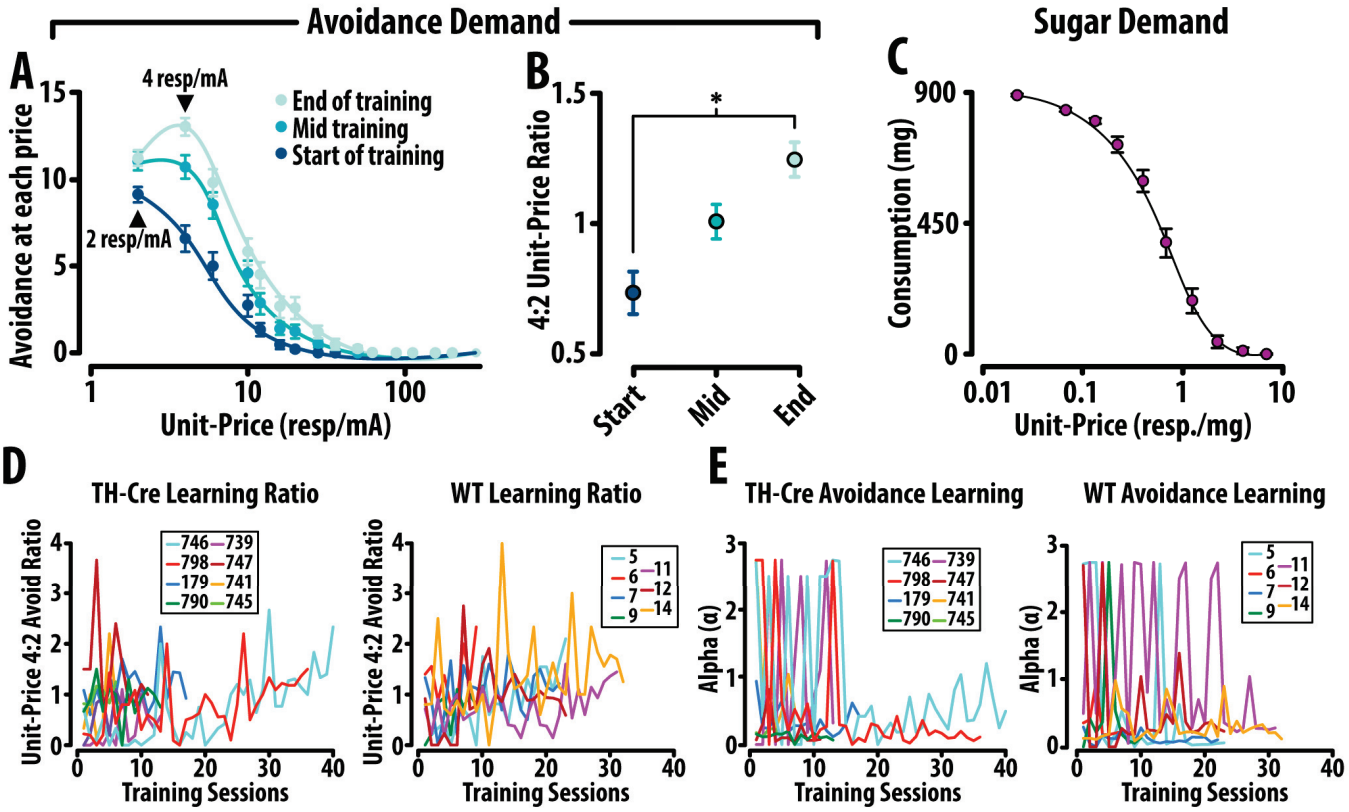
Figure 3



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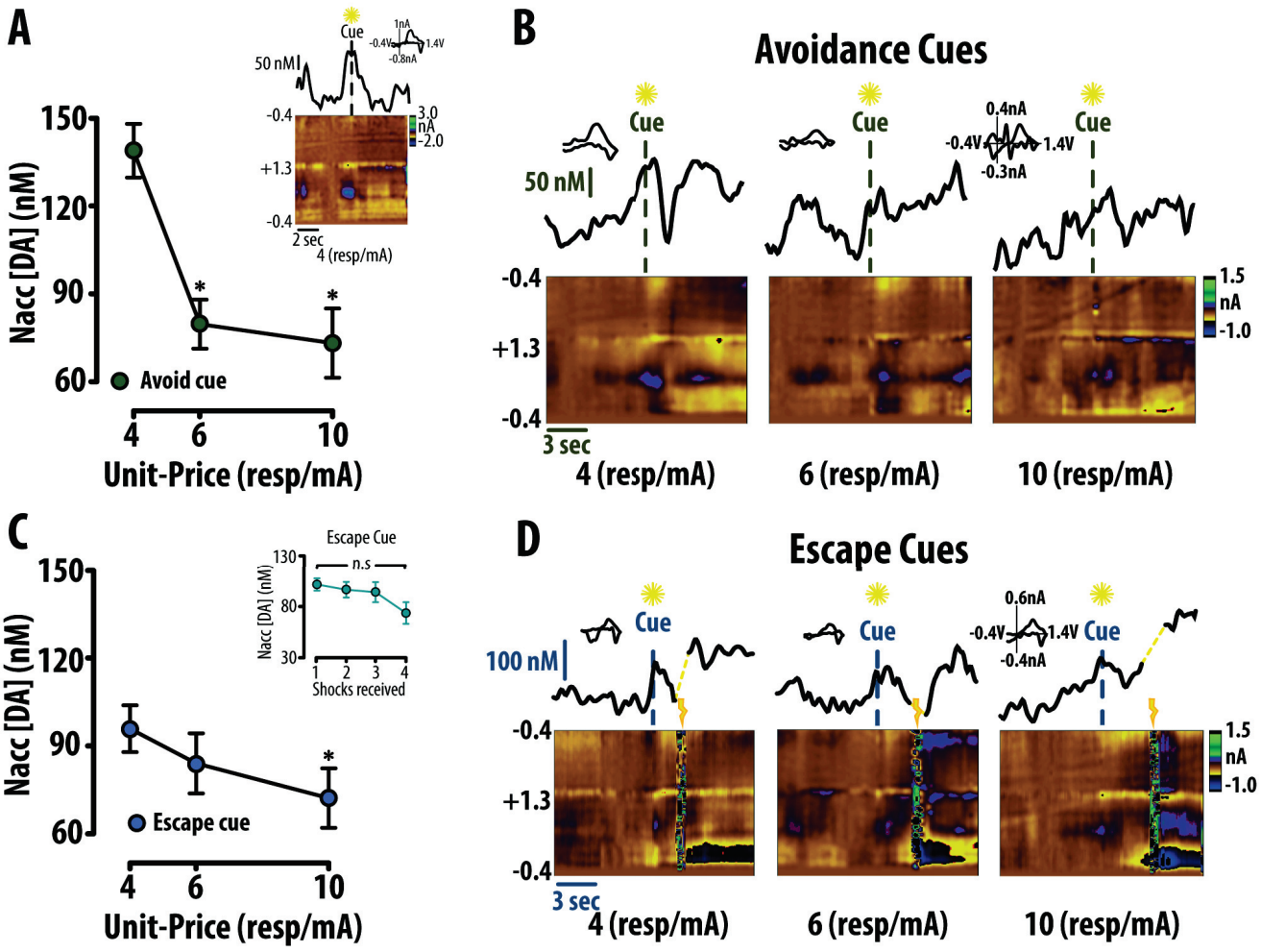

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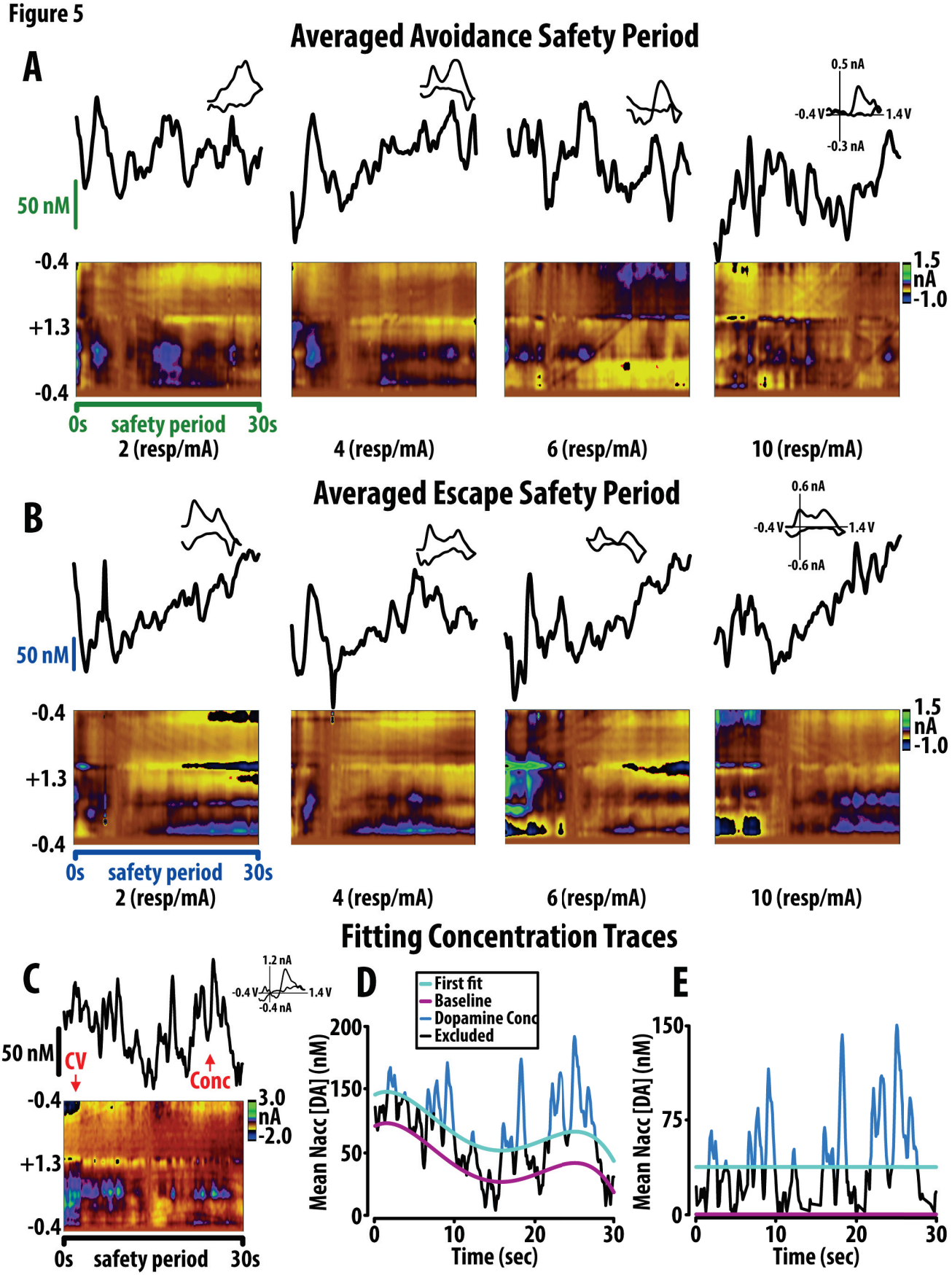

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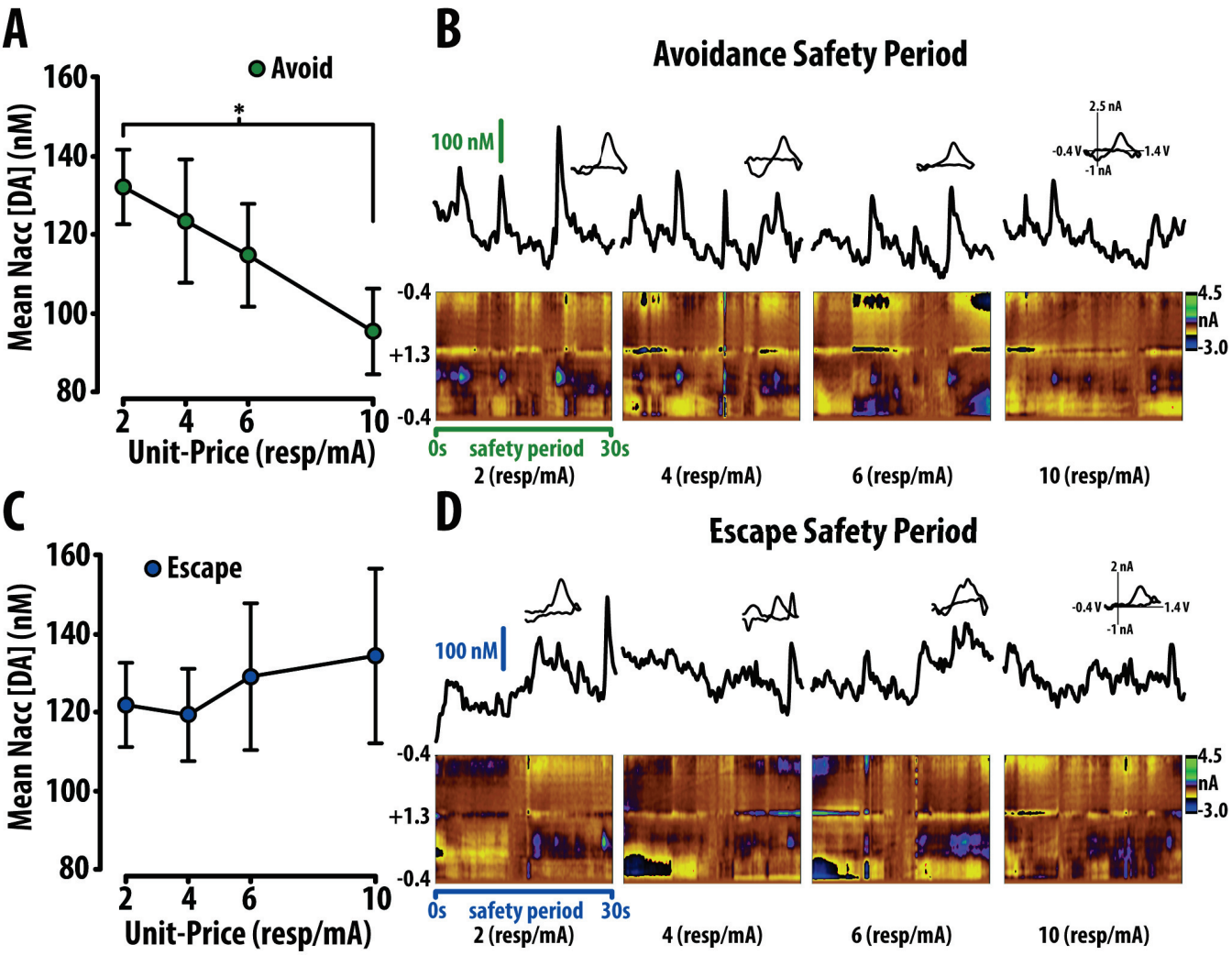

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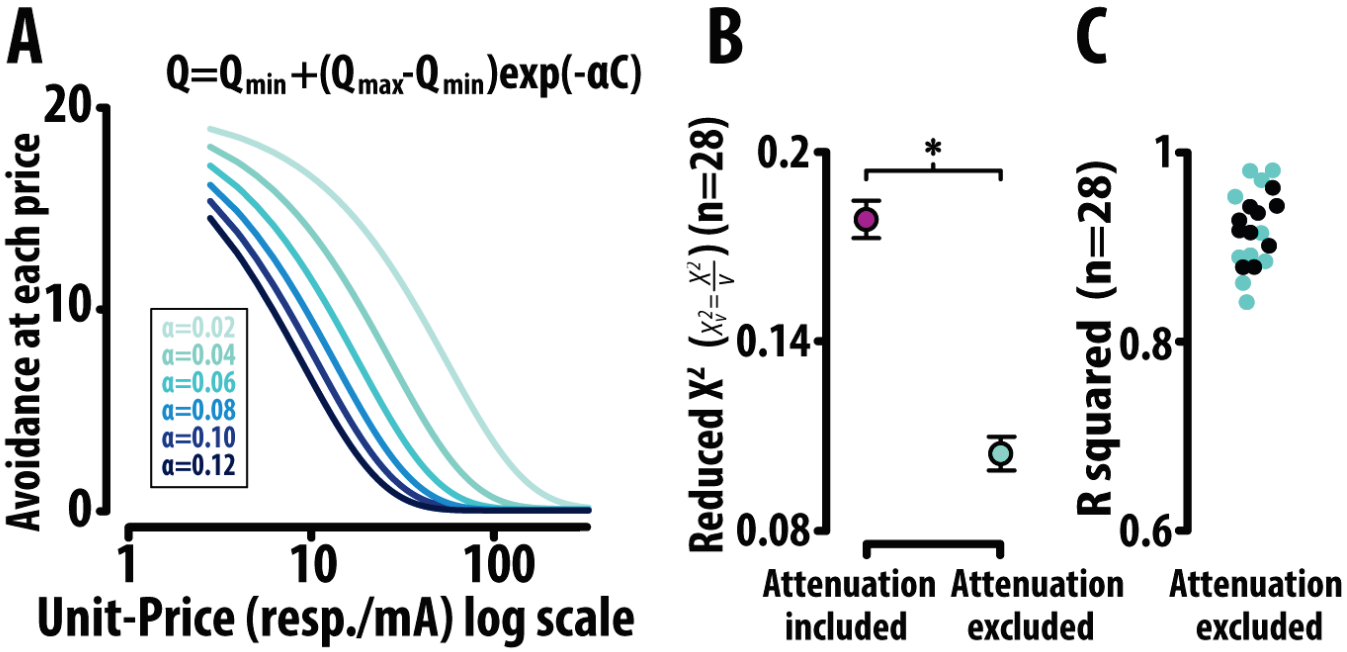

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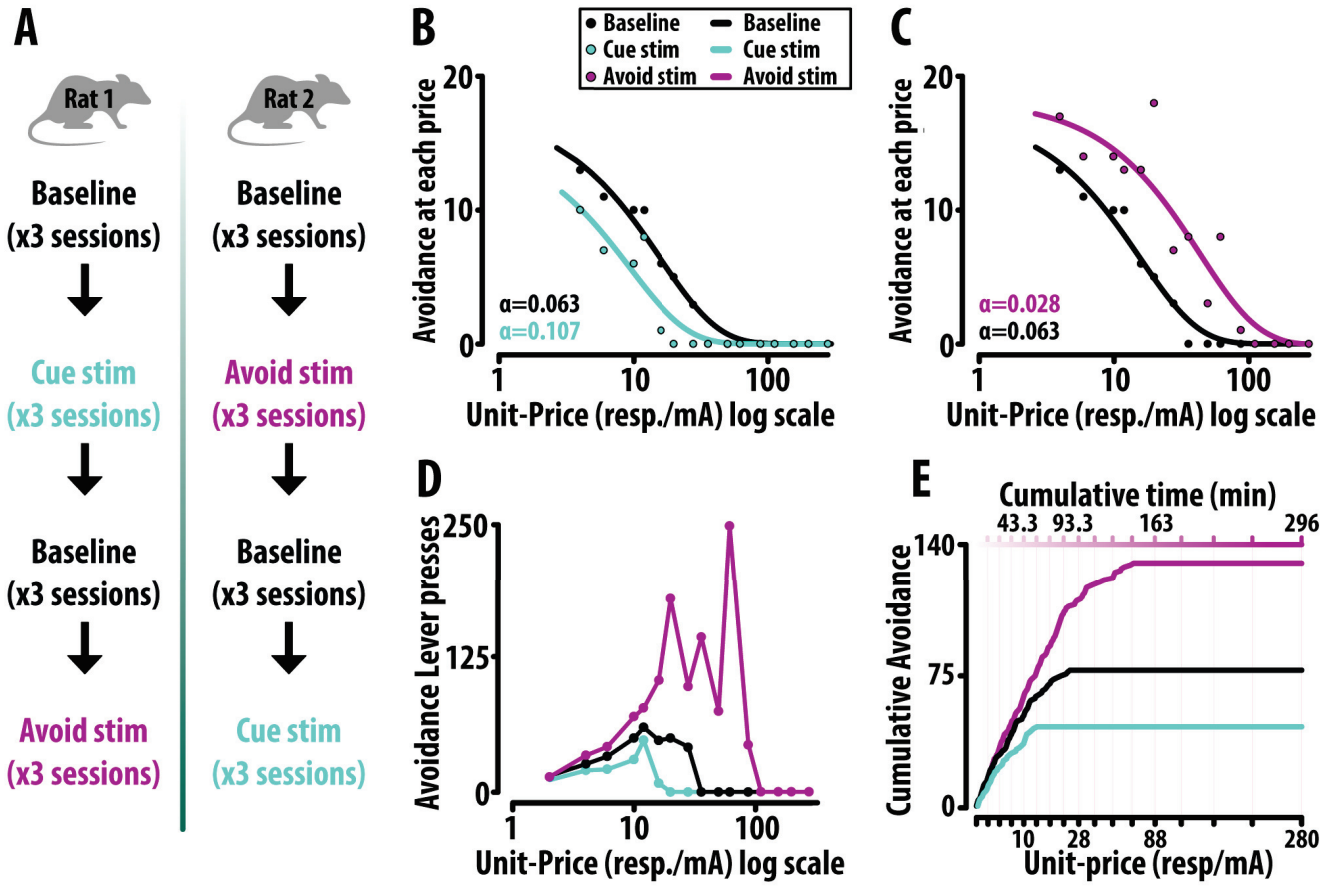
Figure 8



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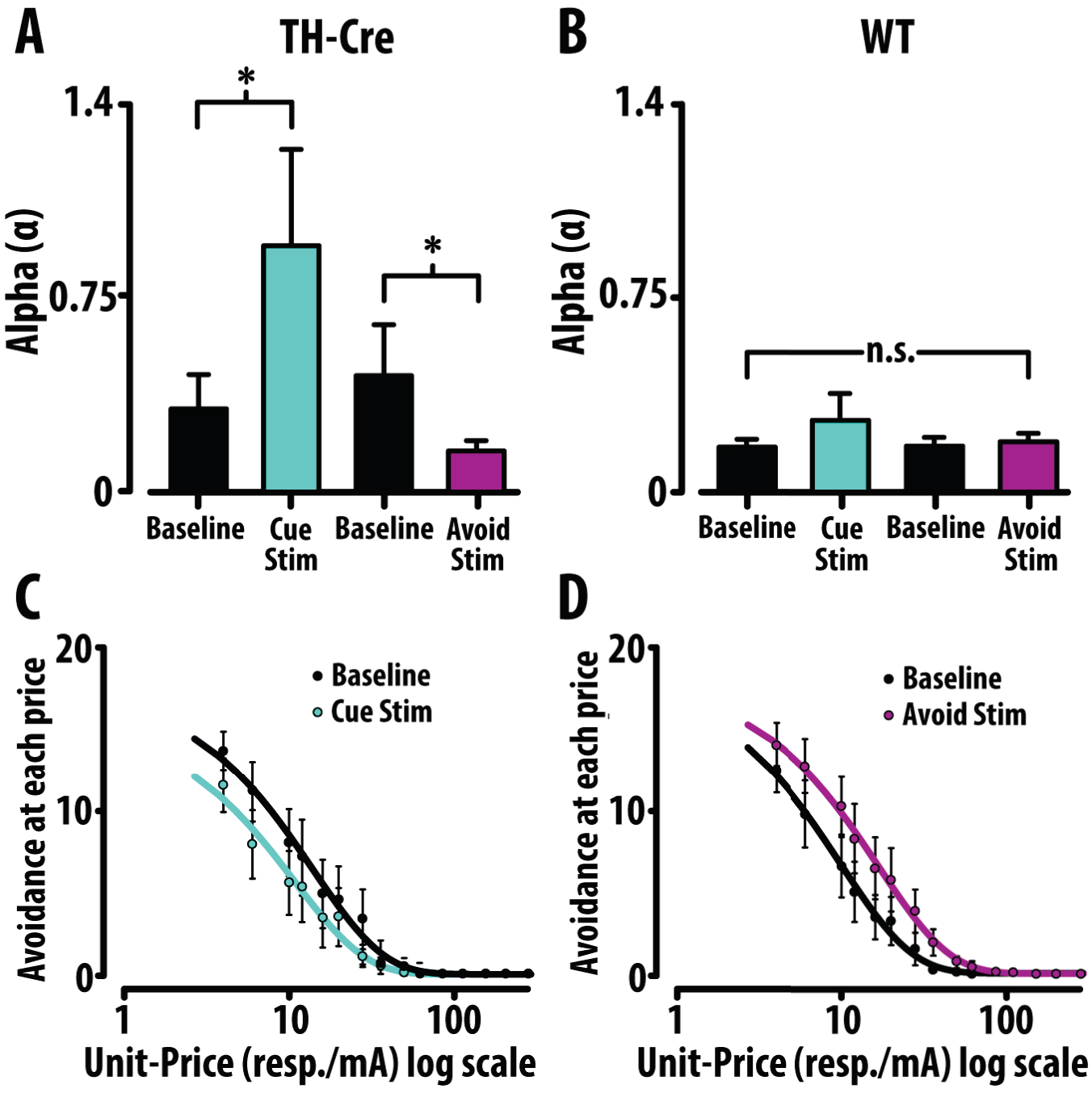

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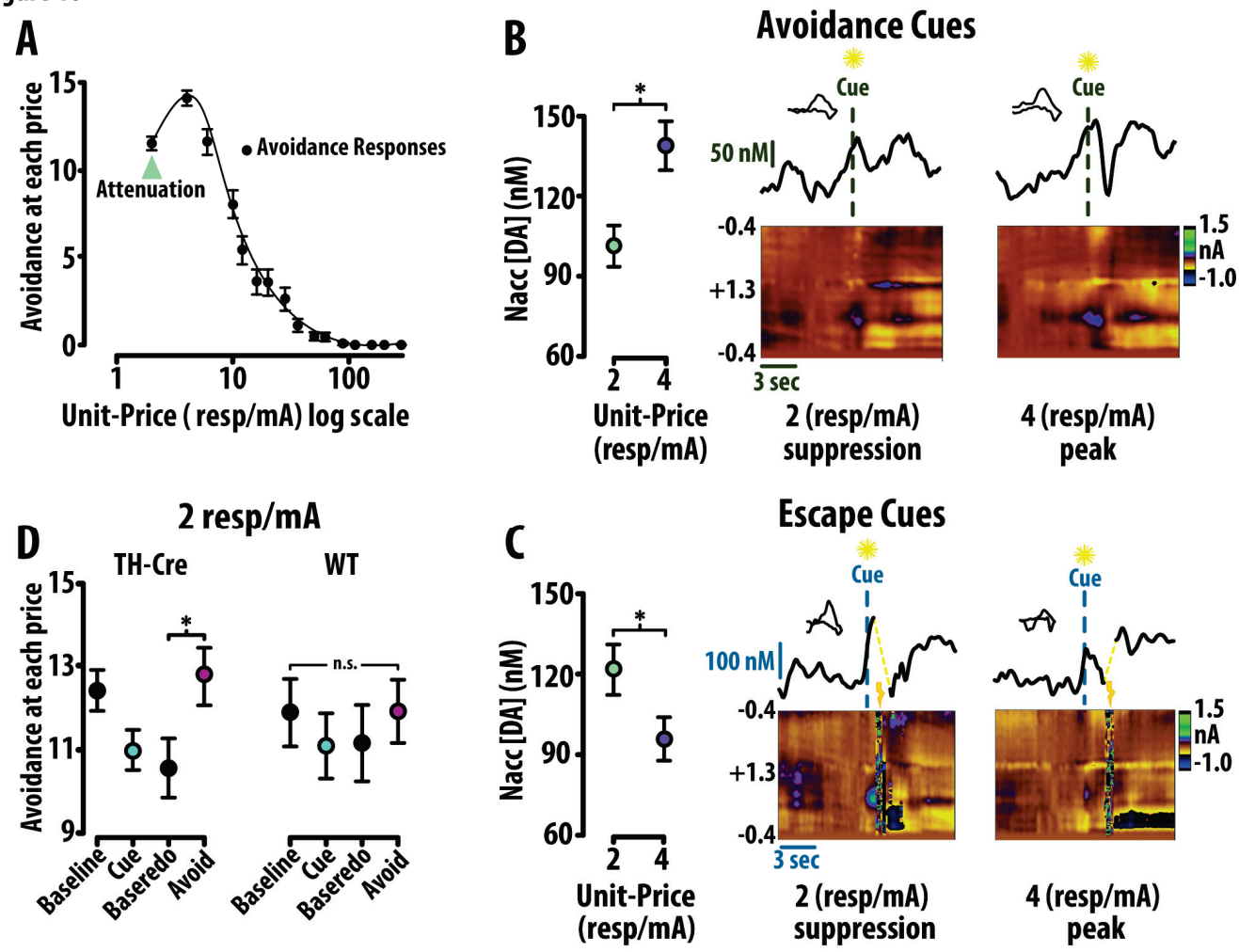
Figure 10



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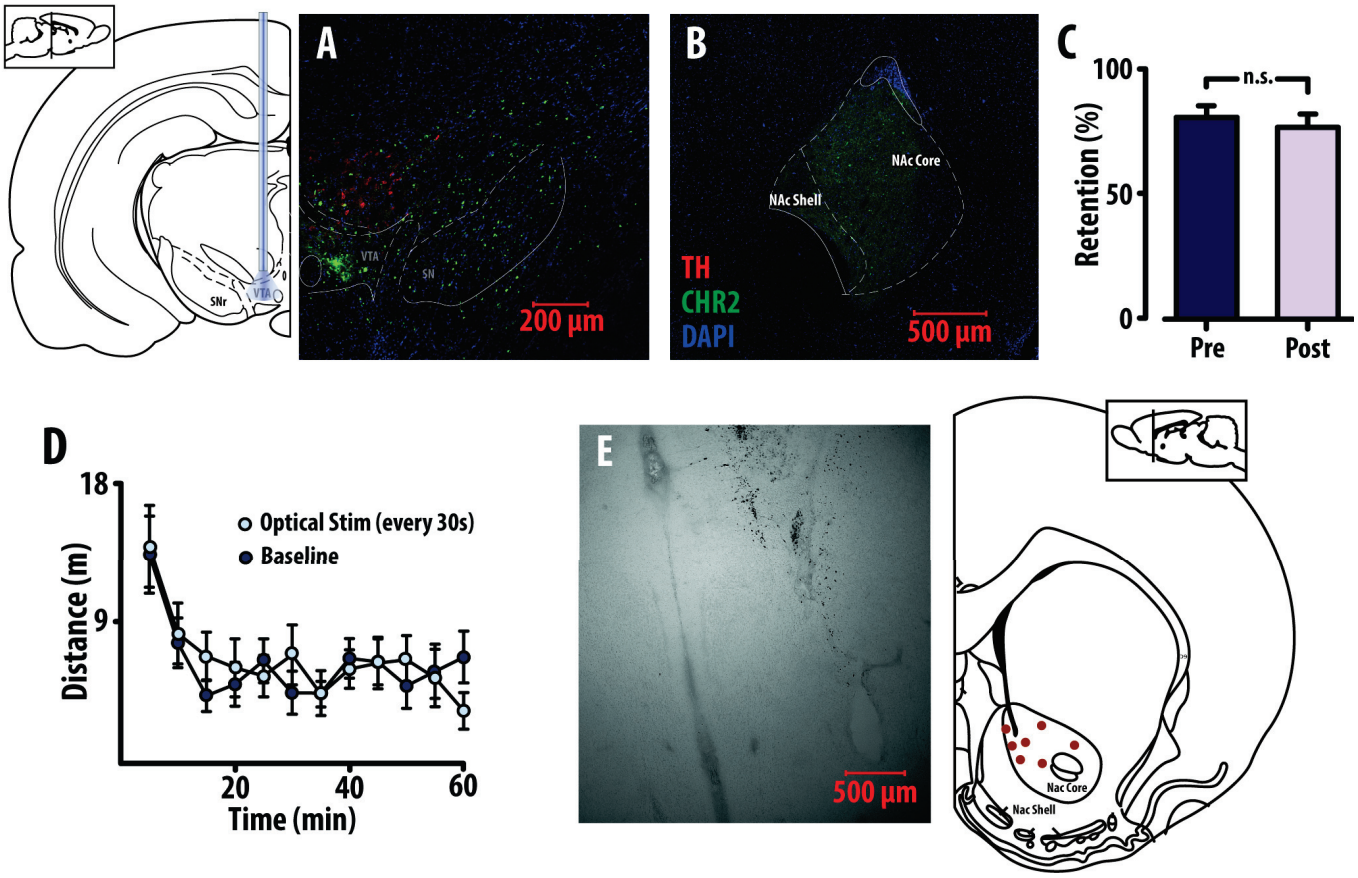
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