Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Arianna Peluso April 9, 2021

TRKB Controls Value-Based Decision Making and Preserves Neuronal Structure in the Medial Orbital Frontal Cortex

By

Arianna Peluso

Dr. Shannon Gourley, PhD

Adviser

Neuroscience and Behavior Biology Program

Dr. Shannon Gourley, PhD

Adviser

Dr. David Weinshenker, PhD

Committee Member

Dr. Paul Lennard, PhD

Committee Member

Dr. Stephen Crist, PhD

Committee Member

2021

TRKB Controls Value-Based Decision Making and Preserves Neuronal Structure in the Medial Orbital Frontal Cortex

By

Arianna Peluso

Dr. Shannon Gourley

Adviser

An abstract of

a thesis submitted to the Faculty of Emory College of Arts and Sciences

of Emory University in partial fulfillment

of the requirements of the degree of

Bachelor of Science with Honors

Neuroscience and Behavior Biology Program

2021

Abstract

TRKB Controls Value-Based Decision Making and Preserves Neuronal Structure in the Medial Orbital Frontal Cortex

By Arianna Peluso

Abstract: Value-based decision making refers to executing actions based on the value of expected outcomes. The medial orbital frontal cortex (mOFC) is involved in value-based decision making, particularly when outcomes are not immediately available and must be envisioned. This process is important to our everyday life because we often make decisions without immediate access to outcomes. Here, we investigate how neurotrophin systems (specifically, Brain-Derived Neurotrophic Factor-TrkB) in the mOFC contribute to value-based decision making. We find that with TrkB activity, the mOFC is essential for the ability of mice to select actions based on the value of prospective outcomes, specifically when outcomes are not immediately observable. The receptor TrkB facilitates the formation and stabilization of dendritic spines, the primary sites of excitatory neuronal communication in the brain. We find that TrkB activity sustains the presence of mature dendritic spines – those most likely to be involved in learning and memory – on excitatory neurons in the mOFC. By investigating how neurotrophins in the mOFC contribute to value-based decision making and dendritic spine stabilization, we hope to identify novel therapeutic targets for neuropsychiatric illnesses in which decision making is disrupted or impaired.

TRKB Controls Value-Based Decision Making and Preserves Neuronal Structure in the Medial Orbital Frontal Cortex

By

Arianna Peluso

Dr. Shannon Gourley

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences

of Emory University in partial fulfillment

of the requirements of the degree of

Bachelor of Science with Honors

Neuroscience and Behavior Biology Program

2021

Acknowledgements

Author contributions: Ellen Woon and Shannon Gourley, designed research; Arianna Peluso and Ellen Woon performed research and analyzed data; Arianna Peluso wrote the thesis document; Ellen Woon and Shannon Gourley edited the thesis document.

This work was supported by the Emory University Integrated Cellular Imaging Core and Children's Healthcare of Atlanta, OD011132, NIH 5T32NS096050-23, and MH117103.

Table of Contents

Background

Value-based behaviors refer to executing behaviors based on the value of the expected outcomes. These behaviors are a critical component of value-based decision making. When the neurocircuitry that manages value-based decision making is disrupted, one may be unable to select actions based on value of potential outcomes and instead defer to habitual behavior, referring to engaging in behaviors regardless of outcome value. The neurocircuitry underlying value-based decision making is interrupted by many neuropsychiatric diseases, such as substance use disorder. For instance, habitual behavior is thought to contribute to the development and maintenance of substance addiction (Everitt & Robbins, 2016). Due to the looming public health concern of substance use and other neuropsychiatric disorders, the identification of molecular mechanisms and neurocircuitry which contribute to value-based decision making will provide insight into potential therapeutic agents. These agents may ultimately be able to target mechanisms of value-based decision making that are impaired in patients with neuropsychiatric disorders.

The orbital frontal cortex (OFC) is a region of the prefrontal cortex that receives input from the mediodorsal nucleus of the thalamus. It also receives information from visual, taste, olfactory, and somatosensory inputs (Rolls et al., 1994). There is sufficient evidence to show that the OFC is involved in updating behaviors when values of the outcomes are modified. Past studies show that damage to the OFC in monkeys affects their ability to assign and update the value of an expected outcome (Walton et al., 2010; Baxter et al., 2000). These findings have been replicated in studies with humans. For instance, Milner et al. (1982) showed that patients with frontal lobe damage had difficulty in changing direction in a stylus maze, after a sound indicated that the participant had left the correct path. Although the sound indicated the value of

the path, the participants were unable to change their direction, showing their inability to consider the value of their chosen path.

Connectivity studies show that the OFC in rats and primates contain similar organizations (Price, 2007). Additionally, quantifiable image processing shows that the anatomical organization of the OFC in monkeys and in humans is similar (Mackey & Petrides, 2009). Tensor imaging studies comparing the patterns of connectivity across species also support the similarity of OFC organization in monkeys and humans (Croxson et al., 2005). Thus, the OFC appears to be relatively conserved across species (Izquierdo, 2017; Wallis, 2011), increasing the translational significance of our study.

Value-based decision making can be tested by manipulating the value of different outcomes and then testing whether organisms make decisions based on the updated value of those outcomes. Our behavioral paradigm (represented in Figure 1) starts with an acquisition phase, when the mice learn to nose-poke on two different nose-poke apertures for two distinct, equally preferred food pellets (chocolate and grain). The mice then undergo a conditioned taste aversion procedure, when they are given unlimited access to one distinct pellet before it is paired with lithium chloride (LiCl). LiCl reduces the value of the pellet by causing temporary gastric malaise, and this pellet is now termed the “devalued” pellet. The next day, the mice are given unlimited access to the other distinct pellet. This pellet is paired with saline, which does not cause gastric malaise. This pellet is termed the “valued” pellet.

After all of the pairings are complete, the mice are then placed back in the operant conditioning chamber for a brief choice test, which tests whether the mice update their actions based on the new values of the food pellets. The choice test is conducted with two conditions: unobservable and observable. In the unobservable condition, nose-poking does not result in the

delivery of a food pellet. This contrasts the observable condition, when nose-poking does result in the delivery of a food pellet. Mice that are able to conduct value-based action will engage with the valued pellet's aperture over the devalued pellet's aperture in both conditions.

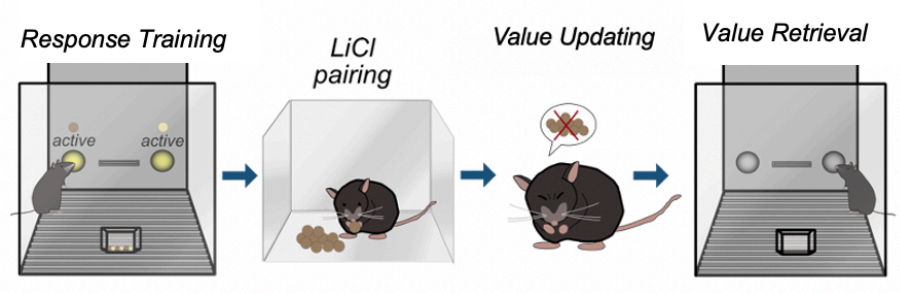

Figure 1. Behavioral paradigm which tests if mice update their actions based on outcome values. Mice are trained to nose poke for two different food pellets on two distinct apertures (left). The mice then undergo a conditioned taste aversion task, where one pellet is "devalued" by its pairing with LiCl and the other remains "valued" by its pairing with saline. The choice test (right) tests whether the mice update their behavior based on the modified value of the food pellets.

The medial orbital frontal cortex (mOFC) is essential for envisioning the values of likely outcomes to guide future decision making. Lesions to the mOFC result in the inability to compare values of different choices (Noonan et al., 2017). Patients with damage to the mOFC have difficulty using the value of available choices to guide their decision making (Walton et al., 2010). In 2015, Bradfield et al. further demonstrated that the mOFC is essential for value-based action, particularly when outcomes are not immediately delivered and must be envisioned. In a task similar to that described above, the authors in this study trained rats to lever-press for two distinct outcomes (grain pellet vs. sucrose). Then, the authors reduced the value of one of these outcomes. When rats were then placed back in the operant conditioning chambers, lesions to the mOFC rendered rats unable to select the action associated with the higher-valued outcome over the lower-valued outcome when outcomes were unobservable (not delivered). This deficit, however, did not persist when outcomes were observable (delivered). In other words, the mOFC helps organisms to envision outcomes to guide future action selection. This cognitive adaptation

of selecting actions when the value of likely outcomes must be envisioned is pertinent to everyday life, as we often do not have immediate access to the expected outcomes of our behaviors.

Though a functional mOFC is necessary for value-based action, *how* the mOFC coordinates this behavior remains unclear. Addictive drugs cause mOFC atrophy (Kuhn et al., 2010; Crombag et al., 2005; Barrientos et al., 2018) and impede mOFC function across species (Barrientos et al., 2018; Trantham et al., 2002; Adinoff et al., 2006; Goldstein et al., 2007; Tanabe et al., 2008; Bechara et al., 2001), emphasizing the need to fully understand the manner by which the mOFC coordinates value-based action.

One potential mechanism is Brain-Derived Neurotrophic Factor (BDNF). In 2016, Gourley et al. demonstrated that BDNF is essential for value-based action. Mice with a global knockdown of *Bdnf*, or with mOFC-selective *Bdnf* knockdown, were unable to engage in value-based action.

BDNF is essential for the development and maintenance of the brain, and is a predominant neurotrophin in the adult brain (Cattaneo et al., 2016). Its genomic structure is conserved throughout mammals (Sasi et al., 2017), and its effects are region specific, affected by temporal changes, receptor isoforms, intracellular signaling, and gene expression (Fenner, 2012). BDNF is known to enhance excitatory (glutamatergic) signaling and decrease inhibitory (GABAergic) signaling, supporting its role in long-term potentiation, a process that strengthens synaptic connections (Gerlai, 2018).

When BDNF binds to its high-affinity receptor TrkB, the receptor dimerizes and auto-phosphorylates the tyrosine residues located within the intracellular domains (Park & Poo, 2012). The dimerization initiates a downstream signaling cascade, resulting in the initiation of processes

necessary for neuronal survival, synaptic plasticity, and dendritic spine stabilization (Park and Poo, 2012). The three primary activated downstream signaling pathways through BDNF-TrkB activation are mitogen-activated protein kinase (MAPK), phospholipase C-γ (PLCγ), and phosphatidylinositol-3 kinase (P13K). These downstream signaling pathways are represented in Figure 2. Importantly, impaired BDNF-TrkB signaling is associated with many neuropsychiatric diseases, including those that cause deficits in value-based action (Autry and Monteggia, 2012).

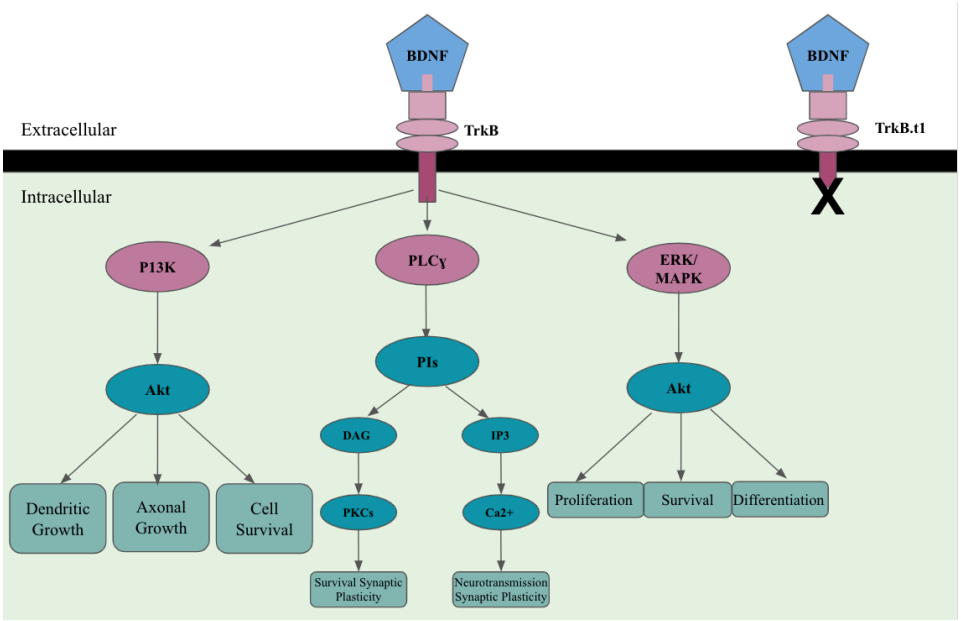

Figure 2. BDNF-TrkB signaling pathways. There are three major signaling pathways that are activated by BDNF-TrkB signaling: PLCγ, PI3K, and MAPK. Although not pictured here, all three pathways later converge on similar downstream mechanisms to influence gene transcription and long-term potentiation. TrkB.t1 is shown to demonstrate its interruption of the signaling pathways and the absence of an intracellular signaling domain, indicated by the "x". (modified from Figure 3 of Fenner, 2012)

Though the findings from Gourley et al. (2016) identify BDNF in the mOFC as essential for value-based action, OFC-selective *Bdnf* knockdown can deprive interconnected regions of the BDNF neurotrophin (Conner et al., 1997; Sobreviela et al., 1996). This is because BDNF is subject to both anterograde and retrograde transport. Thus, *where* BDNF binding is necessary for value-based action remains unclear. To begin to determine whether local BDNF-TrkB activity is

necessary for value-based action, my lab recently overexpressed the inactive isoform of TrkB, TrkB.t1, in the mOFC.

TrkB.t1 lacks the intracellular kinase-containing domain found on the active, full-length receptor (TrkB.FL), which eliminates the autophosphorylation of the tyrosine kinase domains that are responsible for initiating downstream signaling cascades activated by TrkB.FL. Typically, *Trkb.t1* is expressed throughout the central nervous system and is predominantly expressed in astrocytes, though it is also expressed on neurons (Saba et al., 2018). Under basal conditions, TrkB.t1 can act as part of a negative feedback loop: when there is overactivity of the neuron or extreme amounts of BDNF, TrkB.t1 decreases neuronal excitability to protect against the consequences of overactivity (Rose et al., 2003; Cao et al., 2020). Historically, TrkB.t1 is considered the inactive isoform of TrkB.FL, and indeed, overexpression of *Trkb.t1* interferes with TrkB.FL signaling (Pitts et al., 2018). By overexpressing *Trkb.t1* in the mOFC, my lab is able to determine whether BDNF-TrkB activity, specifically in the mOFC, facilitates value-based action, particularly when outcomes must be envisioned.

In addition to determining whether local TrkB activity in the mOFC is necessary for value-based action, I will determine how TrkB in the mOFC influences neuronal structure, particularly the structure of dendritic spines. Dendritic spines are critical sites of neuronal communication and serve as the primary sites of excitatory synaptic transmission (Uchizono, 1965). Dendritic spines are the products of dendritic arbor stabilization and are crucial for long-term circuit stability (Dailey and Smith, 1996; Wong et al., 2000; Wong and Wong, 2000; Wu et al., 1999). Dendritic spines are composed by filamentous (F) actin. F-actin builds the dendritic spine shape, organizes the postsynaptic signaling machinery, induces changes in dendritic spine structure, and maintains dendritic spine stability. The actin network fills the dendritic spine and

participates in modifying the structure during activity-driven remodeling of the dendritic spine (Tashiro and Yuste, 2003). Changes to the amount of F-actin in dendritic spines induce changes in spine size and synaptic efficacy (Okamoto et al., 2004; Matsuzaki et al., 2004). For example, long-term potentiation is induced by repeated firing of the synapse during high-frequency synaptic stimulation. This promotes actin polymerization, causing the dendritic spine to enlarge. Contrastingly, low-frequency stimulation can result in long-term depression, which causes a decrease of actin and dendritic spine shrinkage, weakening synaptic connections (Okamoto et al., 2004; Zhou et al., 2004).

Dendritic spines can be characterized by their morphology, which is tightly coupled to their function: mushroom, thin, and stubby spines (Fifkova and Delay, 1982; Matus et al., 1982; Fischer et al., 1998; Star et al., 2002; Hotulainen and Hoogenraad, 2010). Mushroom spines contain the largest excitatory synapses. These spines are known as "memory" spines because they develop during long-term potentiation (Bourne and Harris, 2007; Aguilar-Harnández et al., 2020). Thin spines contain smaller, immature excitatory synapses, but retain the capacity to grow, strengthen, and eventually develop into mushroom spines; thus, they are considered "learning" spines (Bourne and Harris, 2007). Stubby spines are prevalent in early postnatal development and are less seen in mature brains (Harris et al., 1992). Additionally, long-term depression, the process of weakening synaptic connections, can lead to the development of stubby spines (Aguilar-Hernández et al., 2020).

BDNF-TrkB activity facilitates the formation and stabilization of dendritic spines. TrkB activity increases trafficking of postsynaptic density protein 95 (PSD95), a marker of mature synapses (Kim & Sheng, 2004), to the dendritic spine head (Koleske et al. 2013). Kellner et al. (2014) observed that a loss of BDNF-TrkB activity decreases dendritic spine density. Further,

stimulation of TrkB via 7-8 dihydroxyflavone (7,8-DHF), a TrkB agonist, increases dendritic spinogenesis (Zimmermann et al., 2017). These studies directly implicate TrkB activity in dendritic spine stabilization.

In our project, we aim to determine how TrkB activity in the mOFC contributes to value-based action. To identify whether *local* TrkB activity within the mOFC is necessary for prospective value-based action, we interfered with TrkB.FL-activity via *Trkb.t1* overexpression in the mOFC. Then, using transgenic mice that express *Thy1*-Yellow Fluorescent Protein (YFP) in cortical layer V neurons, we examined how TrkB activity contributes to dendritic spine morphology and density in the mOFC.

Methods

I was added to IUCAC YER-4000010 for this project.

Mice and Intracranial Surgeries. For all experiments, subjects were adult male C57BL/6 mice. Dendritic spine imaging was accomplished by using mice that express YFP in cortical layer V neurons (Feng et al., 2000; H line) backcrossed onto a C57BL/6 background. Male mice were used throughout the experiment, as studies show that sex differences do not account for either BDNF or TrkB.FL protein counts in the mature frontal cortex (Barfield and Gourley, 2018)**.** The mOFC was bilaterally infused (AP=+2.75, DV=-2.35, ML=+/-0.15) (Gourley et al., 2016) with a control lenti-viral vector (LV-CMV-mCherry) or *Trkb.t1*-overexpressing virus (LV-CMV-Trkb.t1) with an HA tag. Lentiviruses contain a single stranded RNA genome. When infused, the virus integrates into the host genome, causing long-lasting gene expression (Ehrengruber et al., 2001). The spread of this particular viral vector type is notably contained relative to other viral vector types. This is significant because the mOFC region contains a

relatively small size (Figure 3). Further, lentiviruses can infect both neurons and glia but preferentially target neurons (Ehrengruber et al., 2001; Nathanson et al., 2009). The mice were undisturbed for 3 weeks to allow for surgical recovery and viral vector incubation.

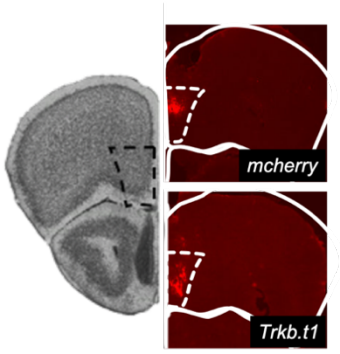

Figure 3. Representative images of viral vector expression in the mOFC. Left half of the figure is reprinted from the Mouse Brain Library.

Behavioral Testing. Mice were food restricted to 90% of their original body weight for all behavioral tasks. Mice were trained to nose poke for 2 unique food pellets (chocolate *vs.* grain) on 2 different nose poke apertures, to acquire the behavior of nose-poking for food reinforcers. Importantly, mice do not systematically prefer one flavor over the other (Kietzman et al., submitted). Mice were trained on a fixed ratio 1 (FR1) schedule of reinforcement; 30 pellets were available for responding on each of the 2 nose poke apertures, resulting in 60 pellets/session. Sessions ended at 70 min, or when mice acquired all 60 pellets. Mice required 7-8 daily sessions to acquire all pellets within the allotted time.

The conditioned taste aversion task can be used to reduce the value of one of the pellets used during training. Mice were placed in a clean cage and were given 2g of one of the pellets used during training. Sessions lasted 30 mins. Immediately following consumption of the pellet, mice were injected with 0.15M lithium chloride (LiCl, 4mL/100g, *i.p.*) (Sharp et al., 2017),

decreasing the value of the pellet by causing temporary gastric malaise. This procedure "devalues" the food pellet (termed "devalued"). Importantly, mice were also given 2g of the other pellet used during training the following day. They again fed for 30 mins, and were immediately injected with saline. The saline does not induce any gastric malaise, thus preserving its value (termed "valued"). This procedure was repeated for 10 days, with 5 LiCl pairings and 5 saline pairings.

After all the pairings were complete, mice were then placed back in the operant conditioning chamber for a brief choice test (10 min), which tested whether the mice modify their actions based on the updated value of the pellets. There are two conditions in the choice test: unobservable and observable. In the unobservable condition, nose-poking did not result in the delivery of food pellets. In contrast, pellets were delivered in the observable condition. Mice that preferentially engage on the aperture associated with the "valued" pellet are able to exhibit value-based action. Failure to differentiate between the apertures represents a failure in value-based action.

Lastly, the post-probe consumption test is conducted following the choice tests. 2g of each pellet were placed in the clean chamber, and the mice were allowed to feed for 30 mins. After 30 mins, the pellets remaining in the chamber were weighed. This procedure was used to confirm that the conditioned taste aversion task reduced consumption of the devalued pellet, while consumption of the valued pellet was maintained.

Euthanasia and immunohistochemistry. Mice were deeply anaesthetized using ketamine (120 mg/kg; *i.p.*) and dexdomitor (1 mg/kg; *i.p.*). The mice were then euthanized using intracardiac perfusion with ice-cold isotonic saline and 4% paraformaldehyde. The brains were collected and submerged in 4% paraformaldehyde for 24 hours. The brains were then transferred

to chilled 30% w/v sucrose. Brains were then sectioned into 50μm-thick sections on a microtome held at -20ºC for histological verification or dendritic spine analyses.

In control mice, mCherry was imaged to document transfected regions. For mice overexpressing *Trkb.*t1, transfected regions were visualized by immunostaining for the HA tag. Sections were blocked in a tris-buffered saline (TBS) solution containing 5% normal goat serum and 0.3% Triton X-100 (Sigma) for 1 hr. at room temperature. Sections were then incubated in a primary antibody solution containing the primary antibody for HA (1:500; Sigma), 1% normal goat serum and 0.3% Triton X-100 at room temperature overnight. Sections were then incubated in a secondary antibody solution containing 1% normal goat serum and 0.3% Triton X-100, with biotinylated secondary antibody (1:1000; Vector Laboratories) for 1 hr. at room temperature. Following secondary incubation, sections were incubated in streptavidin Dylight 594 (30g/ml; Vector Laboratories) for 30 mins before tissue was mounted.

Dendritic spine imaging. A confocal microscope was used to image the neurons that co-expressed the control mCherry or the experimental HA tag and YFP, with 0.1μm z-steps to resolve individual dendritic spines. Secondary and tertiary dendrites were analyzed from dendritic segments of at least 50μm from the soma and 15-30μm long. 3-9 independent dendritic segments per mouse were obtained.

Dendritic spine reconstruction. Using the FilamentTracer module of Imaris (Bitplane AG), dendritic spines were reconstructed in 3D. Dendritic spines were classified using established parameters (Radley et al., 2013). A single-blinded experimenter analyzed all images.

Statistics. Response rates for behavioral experiments were compared by ANOVA**.** Session, pellet, aperture selection, and group were factors in the analyses, with repeated measures as necessary. In the event of significant interactions, *post-hoc* comparisons were

conducted with Tukey's tests. The results are indicated graphically, with $p<0.05$ considered significant.

For dendritic spine analyses, comparisons were made by 2-tailed unpaired t-tests with $p<0.05$ considered significant. Each mouse, instead of each neuron, was treated as an independent sample to avoid power inflation. Each mouse contributed a single spine density per subtype, which reflected the average of all dendrites from that mouse. Spine lengths were analyzed using the Kolmogorov-Smirnov test with $p<0.01$ considered significant.
SPSS and GraphPad Prism were used throughout; *n* values for each group are reported in the figure captions.

Results

Trkb.t1 overexpression in the mOFC impairs value-based action, particularly when outcomes are unobservable.

Value-based action involves selecting actions based on the value of expected outcomes. The mOFC is critical for choosing behaviors that result in higher-valued outcomes, specifically when outcomes are unobservable. Bradfield et al. (2015) showed that rats with lesions to the mOFC were unable to engage in value-based action when outcomes were unobservable. Gourley et al. (2016) additionally showed that BDNF in the mOFC is essential for value-based action. However, OFC-selective knockdown can deprive interconnected regions of the mOFC of BDNF (Conner et al., 1997; Sobreviela et al., 1996). Thus, the exact area where BDNF-TrkB activity is necessary for value-based action is unknown. We began to determine whether *local* BDNF-TrkB activity is necessary for value-based action by overexpressing truncated TrkB isoform, TrkB.t1, in the mOFC.

To investigate whether TrkB activity in the mOFC is necessary for value-based action, we trained two groups of mice (controls and *Trkb.*t1-overexpressing mice) to acquire the behavior of nose-poking for food pellets (chocolate or grain) on two different nose poke apertures ("response training") (Figure 1 schematic; Figure 4a timeline). The rate of response acquisition did not differ between groups [main effect of session $F_{(6,108)}$=30.3, p<0.001, no interaction or main effect of group F<1] (Figure 4b). Responses on each aperture from the last 7 sessions were totaled to compare "to be valued" (dark colors) and "to be devalued" responses (light colors) (Figure 4b inset). There were no preferences for a specific response during acquisition training [control: $t_{(10)}$=-0.093, p=0.928, *Trkb.t1*: $t_{(8)}$=0.909=0.39]. In other words, mice did not prefer one pellet over the other.

The mice then underwent the conditioned taste aversion assay (Figure 1 schematic), where one pellet is "devalued" by pairing it with LiCl. Results indicated that across sessions, both groups decreased consumption of the devalued pellet [main effect of session $F_{(4,72)}$=3.742, p=0.008, main effect of pellet $F_{(1,18)}$=50.48, p<0.001, interaction of session*pellet $F_{(4,72)=}$1.42, p=0.001]. No differences between groups were detected [no main effect of group (F<1), no interaction of session*group (F<1), no interaction of pellet*group (F<1), no interaction of session*pellet*group (F<1)] (Figure 4c).

When all pairings were completed, mice were then placed back in the operant conditioning chamber to test whether they utilized updated value information to guide future decision making. The choice test was conducted under two different conditions, unobservable and observable. When outcomes were unobservable (meaning no pellets were delivered), control mice responded preferentially to the "valued" aperture *vs.* the "devalued" aperture; however, mice overexpressing *Trkb.t1* responded non-preferentially on both apertures [interaction of

aperture*group $F_{(1,18)}$=6.58, p=0.019, control: $t_{(10)}$=2.43, p=0.036, *Trkb.t1*: p>0.05, no main effect of aperture $F_{(1,18)}$=2.33, p=0.144] (Figure 4d).When the outcomes were observable (meaning responses now yielded a food pellet), both controls and *Trkb.t1*-overexpressing mice responded preferentially on the aperture associated with the valued pellet [main effect of aperture $F_{(1,9)}$=5.55, p=0.043, no interaction of aperture*group (F<1)] (Figure 4e).

To confirm that the conditioned taste aversion was successful (and thus, that the action selection patterns reported above were attributable to the ability of the mice to integrate value information into choice behavior), we conducted post-probe consumption tests. Both control and *Trkb.t1*-overexpressing mice consumed significantly more of the valued pellet than the devalued pellet [main effect of pellet $F_{(1,18)}$=117.06, p<0.001, no interaction of pellet*group (F<1), no main effect of group (F<1)] (Figure 4f), demonstrating that both groups appropriately updated pellet values. Thus, TrkB activity in the mOFC appears necessary for value-based action, particularly under circumstances in which outcomes must be envisioned.

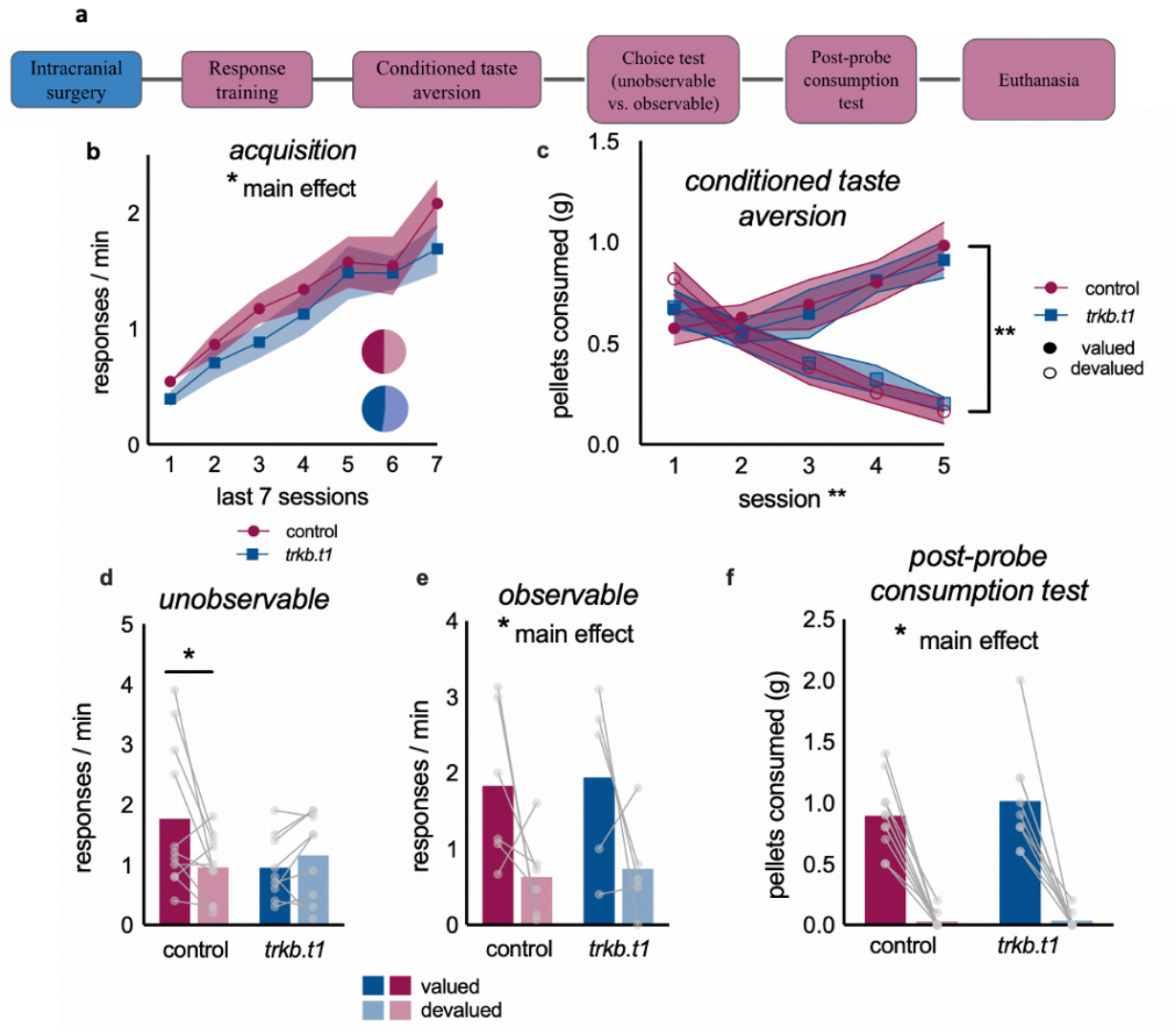

Figure 4. *Trkb.t1* overexpression in the mOFC impairs value-based action, specifically when outcomes are unobservable. (a) Experimental timeline. **(b)** Mice were trained to nose poke for 2 distinct food (last 7 sessions shown, and responses for both pellets are collapsed). We detected a main effect of session, but no differences between groups. **Inset:** Mice exhibit equivalent responding for the two distinct pellets (dark colors: "to be valued," light colors: "to be devalued." **(c)** Mice underwent conditioned taste aversion. No differences were found in the pellet consumption between the two groups, and the consumption of the devalued pellet decreased between sessions. **(d)** When outcomes were unobservable, the *Trkb.t1*-overexpressing mice responded non-preferentially for both pellets, showing an inability to engage in value-based action. Control mice demonstrated value-based action, with preferential responding for the valued pellet. **(e)** When outcomes were observable, both groups demonstrated an ability to engage in value-based action, with preferential responding to the valued pellet. **(f)** In the post-probe consumption test, both groups showed a preference for the valued pellet over the devalued pellet. n=5-11 mice/group. Group sizes in the observable condition are smaller because only one of two cohorts was tested in this condition. Bars and symbols represent means. Connected data points represent individual mice; * represents $p<0.05$, ** represents $p<0.01$.

Trkb.t1 overexpression reduces dendritic spine density and mushroom-shaped spines.

BDNF-TrkB activity initiates downstream signaling to facilitate dendritic spine formation and stabilization (Park and Poo, 2013). We investigated how interfering with TrkB activity via *Trkb.t1* overexpression influences dendritic spine morphology and density on mOFC neurons (Figure 5a). Mice were trained as previously to confirm the successful, or unsuccessful, engagement in value-based action. Following the choice test, mice were euthanized; brains were collected and immunostained; and sections were imaged for 3D reconstructions (Figure 5b). Quantifying dendritic spine density revealed that there is a significant decrease in the overall density of dendritic spines for *Trkb.t1*-overexpressing mice compared to controls [$t_{(10)}$= 3.13, p=0.011] (Figure 5c). Thus, TrkB signaling sustains robust dendritic spine densities on mOFC neurons.

We then classified dendritic spines into 3 dendritic spine types: stubby, mushroom, and thin spines (Figure 5d-f). There was a significant loss in mature, mushroom spines in the *Trkb.t1*-overexpressing mice when compared to the control mice [$t_{(10)}$=2.70, p=0.022] (Figure 5e). In contrast, there were no differences in the density of stubby spines and thin spines between the two groups [stubby: $t_{(10)}$=1.94, p=0.081, thin: $t_{(10)}$=0.969, p=0.355] (Figure 5d and 5f). This pattern suggests that interfering with TrkB activity via overexpression of *Trkb.t1* causes a loss of mature, mushroom spines.

Lastly, we analyzed dendritic spine lengths between the two groups. Results indicate that mice overexpressing *Trkb.t1* have longer spine lengths than control mice [D=0.104, p=0.00326] (Figure 5g). This increased spine length may be attributable to thin spines being aberrantly long in mice with *Trkb.t1*-overexpression. Inhibited TrkB activity may make it more challenging for

these aberrantly long thin spines to retract into mushroom-shaped spines or regress entirely when appropriate, which could also contribute to the overall lower density of mushroom spines.

Importantly, differences in dendritic spine density or spine-type were not biased by different dendrite lengths [$t_{(10)}$=-1.33, p=0.213] (Figure 5h).

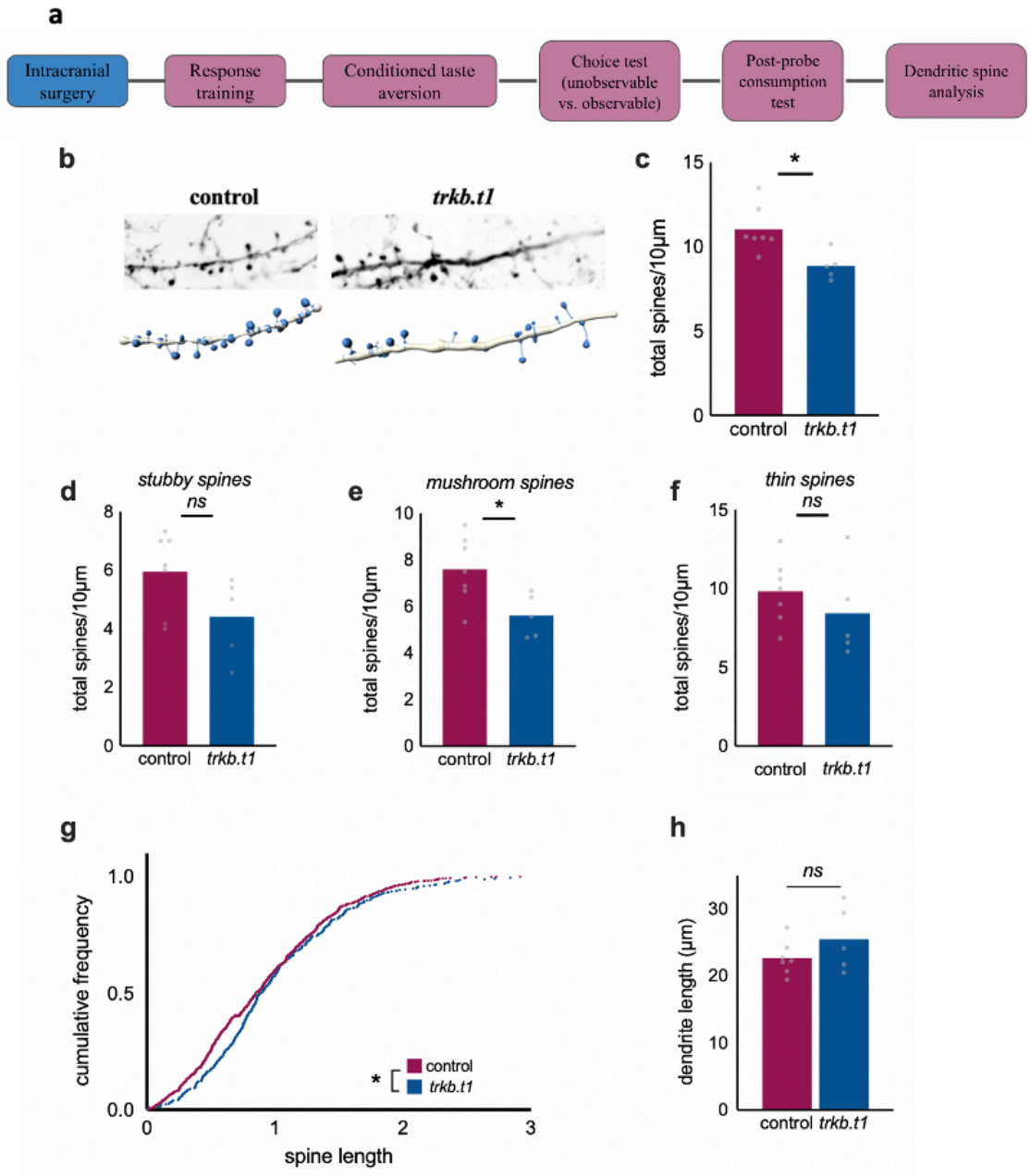

Figure 5. *Trkb.t1* overexpression decreased dendritic spine density and mushroom-shaped spines. (a) Experimental timeline. **(b)** Representative images of dendrites, reconstructed in 3D. **(c)** The number of total spines is decreased in the *Trkb.t1*-overexpressing mice. **(d)** Dendritic spines were classified by subtype. No significant difference in the density of stubby spines was detected between the two groups. **(e)** There was a significant decrease in the number of mushroom spines in the *Trkb.t1*-overexpressing mice. **(f)** The difference in thin spines was not significant. **(g)** The *Trkb.t1*-overexpressing mice had increased spine lengths, compared to the controls. **(h)** Dendrite lengths between the two groups were not significantly different. *n*=5-7 mice/group. Bars represent group means. Individual mice are represented by the grey points. Individual spines are represented in (g). *ns* signifies non-significant. * represents p<0.05.

Discussion

Value-based action, referring to choosing behaviors based on the values of the expected outcomes, is pertinent to everyday life. People use this decision-making strategy to make choices with little impact on one's life, such as where to purchase dinner, and large impacts, such as choices relating to one's career, health, etc. The OFC, particularly the mOFC, is conserved across rodent and primate species (Carlén, 2017; Wallis, 2011; Izquierdo, 2017) and is essential for this type of decision making.

Bradfield et al. (2015) showed that lesioning of the mOFC in rats caused an impairment in value-based action, particularly when outcomes are not immediately observable. The behavioral paradigm of this study contained a conditioned taste aversion procedure similar to ours: the rats were trained to lever-press for two distinct outcomes (grain pellet vs. sucrose). After one of the pellets was reduced in value, the rats were placed back into the operant conditioning chambers for a choice task. The lesioned rats were unable to select the action associated with the higher-valued outcome over the lower-valued outcomes when the outcomes were unobservable (pellets not delivered). Both groups were able to select actions appropriately when pellets were delivered. This study shows that the mOFC is involved in envisioning outcomes to guide behaviors.

Gourley et al. (2016) showed that BDNF, a predominant neurotrophin in the brain (Cattaneo et al., 2016), within the mOFC is necessary for mice to engage in value-based action. Though Gourley et al. (2016) identified BDNF in the mOFC as essential for value-based action, the exact region where BDNF-TrkB activity is needed is unknown. This is because BDNF can be transported in the anterograde and retrograde directions (Conner at al. 1997; Sobreviela et al. 1996). Thus, OFC-selective *Bdnf* knockdown can cause a loss of BDNF in the interconnected

regions of the OFC (see for examples, Pitts et al., 2018). To identify where BDNF is necessary for value-based action, we manipulated its receptor, TrkB, and overexpressed what is classically considered the inactive isoform of TrkB, TrkB.t1. Given that TrkB is a key mediator of neuronal plasticity (Park and Poo, 2013), we also characterized effects on dendritic spines, the primary sites of excitatory plasticity in the brain, in the mOFC.

In this study, we demonstrate that TrkB in the mOFC is necessary for value-based action, particularly when outcomes are unobservable. Mice with *Trkb.t1* overexpressed were unable to engage in value-based action, likely due to the inability to envision the values of expected outcomes. When outcomes were immediately observable, however, *Trkb.t1* overexpression did not impair value-based action. Our results replicate those of Bradfield et al.'s study in 2015, which used mOFC lesions to test value-based action. Notably, lesions identify *what* the brain region does, but not *how*. The results of our study help to clarify *how* the mOFC contributes to value-based action, *i.e.*, via local BDNF-TrkB-mediated signaling.

BDNF-TrkB activity facilitates the formation and stabilization of dendritic spines**.** Results indicated that impairing TrkB activity reduced the overall density of total spines. When further investigated, this reduction in overall dendritic spine density was likely due to a reduced density of mushroom spines, while the densities of other spine types (stubby and thin spines) did not differ. Additionally, results indicate that impairing TrkB activity increased the lengths of dendritic spines. This increased spine length may be attributable to thin spines being aberrantly long in mice with *Trkb.t1* overexpression. Though it may seem surprising that inhibiting TrkB activity increased spine length, this may be caused by aberrantly long thin spines failing to retract into mushroom-shaped spines.

Mature dendritic spines develop from immature, thin dendritic spines upon synaptic activity (Zagrebelsky et al., 2020; Yuste & Bonhoeffer, 2001; Harris et al., 1992; LeVay, 1973). Studies confirm that BDNF-TrkB activity induces changes in dendritic spine growth, maintaining "activity-dependent" maturation and stabilization of dendritic spines (Kellner et al., 2014; Ji et al., 2010; Zagrabelsky et al., 2020). Kellner et al. (2014) observed that BDNF deprivation resulted in a decrease in dendritic spine density and spine head width and an increase in spine length (as also observed here). These spine characteristics resemble those of immature spines, suggesting a decrease in mature spine phenotype and decreased synaptic communication (Kellner et al., 2014). Our results support those found by Kellner et al. (2014), adding to the literature that implicates BDNF-TrkB signaling in the development and maintenance of mature dendritic spines.

Our findings are the first, to the best of our knowledge, to investigate excitatory neurons in the mOFC, as opposed to the more commonly investigated hippocampus. Future studies should image dendritic spines *in vivo*. The advancement of *in vivo* two-photon microscopy has allowed researchers to analyze the same dendritic spines over time, such as during different developmental periods (Moyer and Zuo, 2018). Future studies may use this technology to analyze the effect of disrupted TrkB signaling on dendritic spines in the mOFC.

Does dendritic spine plasticity impact behavior?

Importantly, although our results suggest that TrkB activity preserves mushroom-shaped spines in the mOFC, we did not establish a direct, causal relationship between dendritic spine loss upon TrkB inhibition and impaired value-based decision making. One strategy to establish a causal relationship between dendritic spine morphology and value-based behavior is to

manipulate the regulatory factors of the actin cytoskeleton, which inform the shape of both neurons and dendritic spines. Our group found that cell adhesion and cytoskeleton regulatory factors are necessary for OFC function. Inhibition of these factors induces dendritic spine loss, occludes OFC-dependent learning, and inhibits the ability of mice to choose behaviors based on their likely consequences (Gourley et al., 2012a; Depoy et al., 2017; Depoy et al., 2019).

Other studies by the Gourley group (Pitts et al., 2020) showed that TrkB stimulation can rescue neurobehavioral deficits caused by cocaine exposure. In particular, cocaine exposure during adolescence causes habit-biased behaviors, greatly reducing one's ability to engage in value-based decision making. Additionally, cocaine exposure in adolescence causes reductions in dendritic spine density in the OFC that are apparent in adulthood (Gourley et al., 2012; Depoy et al., 2017). Administration of 7,8-DHF rescued goal-seeking behaviors following cocaine (Pitts et al., 2020), and it stimulated dendritic spinogenesis in the OFC (Zimmerman et al., 2017). Whether 7,8-DHF impacts reward-related decision making through the development and maintenance of mature dendritic spines is being investigated using some of the techniques that will be discussed next.

One strategy to assess the consequences of dendritic spine plasticity on behavior directly is through activated synapse targeting photoactivatable Rac1, or "As-PaRac1" (Hayashi-Takagi et al., 2015; Moda-Sava et al., 2019). This method labels recently potentiated dendritic spines and induces the shrinkage of these spines, obstructing dendritic spine structure at a highly localized level. This new tool can determine the functional consequences of dendritic spine plasticity when used in conjunction with behavioral assays.

In order to test whether value-based action is affected by dendritic spine plasticity, another approach would be to combine behavioral testing with manipulating actin dynamics. One

method of intervening in actin cytoskeleton plasticity is to administer Latrunculin A (Lat A), an inhibitor of F-actin polymerization. Future studies could administer Lat A while observing an organism's ability to engage in value-based action. If value-based decision making requires dendritic spinogenesis, then Lat A would be expected to disrupt it.

Another future direction is investigating whether there is an association between dendritic spine clustering and TrkB in the mOFC. Spine clustering is defined as the genesis of new spines within 5μm of another dendritic spine. Findings by Fu et al. (2012) suggest that dendritic spine clustering is induced by repetitive action during learning. This study trained mice to reach through a slit to grasp a food pellet and found that dendritic spines formed in clusters, with the new spine forming close to a stable, mature spine. It is believed that when spines are clustered, the synapses can be triggered more easily by activity. As TrkB facilitates dendritic spine clustering (Fu et al., 2012), one future direction may be to investigate whether TrkB stimulation (by 7,8-DHF or another TrkB agonist) induces dendritic spine clustering in the mOFC, and in conjunction with reward-related decision-making tasks.

In addition to BDNF-TrkB signaling, neurotoxins, social isolation, and stress are other factors that influence dendritic spine morphologies or densities. These factors are important to consider, due to their prevalence in everyday life. Tetanus toxin, one of the most potent neurotoxins known, has been shown to cause a loss of dendritic spine density (Heimer-McGinn et al., 2013). Heimer-McGinn et al. induced expression of tetanus toxin light chain on fluorescent labeled neurons in the adult mouse brain. The investigators found a 15% loss of dendritic spine density. However, the investigators could not identify which specific spine type was being lost. Further research should be conducted to deduce which types of spines are being affected by the tetanus neurotoxin through imaging microscopy.

The influence of social isolation on dendritic spine plasticity was explored in another study by the Gourley lab (Hinton et al., 2019). This study found that social isolation in adolescence decreased the ability of mice to update action-outcome associations. These authors also found that the disruption of action-outcome-based decision making, caused by social isolation, was unexpectedly associated with an excess of dendritic spines in the OFC. One potential explanation is that both an excess and depletion of dendritic spines can impair behavior due to an "inverted U-shape curve" function, in which case, both conditions imperil signal fidelity in the OFC. An important caveat, though, is that Hinton et al. (2019) did not classify spine subtypes. A future study may determine whether excess dendritic spines were of a mature or immature subtype and whether TrkB activity is affected by social isolation (Liu et al., 2014).

A final consideration is that chronic stress disrupts TrkB signaling, which could interfere with mature dendritic spine stability and induce long-lasting structural consequences that affect behavior. Barfield and Gourley (2019) showed that exposure to the primary stress hormone, corticosterone, in mice during early adolescence impaired decision making. Adolescence is a period that is particularly affected by chronic stress and susceptible to the development of neuropsychiatric disorders that are triggered by stress. The vulnerability to chronic stress during this period may be exhibited by changes in dendritic spines, as dendritic spines experience dynamic changes throughout adolescence into adulthood (Bai et al., 2015).

Studies have reported that chronic stress decreases PSD-95 in the OFC, which are proteins that facilitate spine function, stabilization, and maturation (Ehrlich et al., 2007; Ma et al., 2003). Additionally, Barfield and Gourley (2018) showed that exposure to excess corticosterone in adolescent mice caused a loss of dendritic spines in the lateral orbital prefrontal cortex. Further, p-ERK2 (a signaling process associated with TrkB) levels increase in

the frontal and prefrontal cortex in rats following acute and chronic stress (Galeotti and Ghelardini, 2012; Meller et al., 2003; Shen et al., 2004). Further research should be conducted to show whether an interruption of BDNF-TrkB signaling in the mOFC, caused by exposure to stress, would affect the maturation of dendritic spines and the capacity to choose behaviors based on the value of outcomes.

Our current study has a wide range of implications for everyday decision making, substance use disorder, and neuropsychiatric diseases. The results developed in this study add to the growing literature that connects BDNF-TrkB signaling to value-based decision making; our results showed that mice with inhibited TrkB signaling were unable to envision likely outcomes and choose actions that resulted in outcomes of higher value. Additionally, we observed that BDNF-TrkB activity is essential for dendritic spine maturation and maintenance, as *Trkb.t1*-overexpressing mice exhibited a decrease in mature-shaped mushroom spines. Although further research should be conducted to determine if there is a direct association between dendritic spines and value-based decision making, our results confirm the association of BDNF-TrkB with these two processes. Our research thus renders us closer to targeting mechanisms that disrupt value-based decision making in neuropsychiatric disorders.

Conclusion

Value-based decision making is a critical adaptation necessary for everyday life. It allows us to prospectively evaluate which outcome may benefit us most, thereby informing our decision making. This type of decision making is disrupted in many neuropsychiatric diseases, such as substance use disorder. In individuals whose value-based decision making is impaired, they may be unable to envision the value of differing outcomes, such as using an addictive drug or seeking treatment. These individuals are susceptible to perpetuating an action that can ultimately harm them. Further, dendritic spines are the primary sites of synaptic communication and are heavily implicated in learning behaviors. Our study tested how TrkB activity in the mOFC influences value-based decision making and dendritic spine maturation. We find that TrkB activity is required to envision values of likely outcomes to guide value-based action. Additionally, our results show that TrkB facilitates the maintenance of mature, mushroom-shaped dendritic spines. Thus, therapeutic agents targeted at modulating neurotrophin systems, specifically BDNF-TrkB, may be effective treatments for those suffering from neuropsychiatric disease in which value-decision making is impaired.

References

Adinoff, B., Williams, M. J., Best, S. E., Harris, T. S., Chandler, P., & Devous, M. D. (2006). Sex differences in medial and lateral orbitofrontal cortex hypoperfusion in cocaine-dependent men and women. Gender Medicine, 3(3), 206-222. doi:10.1016/s1550-8579(06)80209-3

Aguilar-Hernández, L., Gómez-Villalobos, M. D., & Flores, G. (2020). Cerebrolysin ameliorates prefrontal cortex and HIPPOCAMPUS NEURAL atrophy of Spontaneous Hypertensive rats WITH HYPERGLYCEMIA. Synapse, 74(9). doi:10.1002/syn.22156

Autry AE, Monteggia LM. (2012) Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 64(2):238-58. doi: 10.1124/pr.111.005108. PubMed PMID: 22407616; PMCID: PMC3310485.

Bai YY, Ruan, CS, Yang CR, Li JY, Kang ZL, Zhou, L, Liu D, Zeng YQ, Wang TH, Tian CF, Liao H, Bobrovskaya L, & Zhou XF. (2016). ProBDNF Signaling Regulates Depression-Like Behaviors in Rodents under Chronic Stress. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 41(12), 2882–2892. https://doi.org/10.1038/npp.2016.100

Barfield ET, & Gourley SL. (2018). Prefrontal cortical trkB, glucocorticoids, and their interactions in stress and developmental contexts. Neuroscience and biobehavioral reviews, 95, 535–558. https://doi.org/10.1016/j.neubiorev.2018.10.015

Barrientos, C., Knowland, D., Wu, M. M., Lilascharoen, V., Huang, K. W., Malenka, R. C., & Lim, B. K. (2018). Cocaine-Induced structural plasticity in Input regions to distinct cell types in nucleus accumbens. Biological Psychiatry, 84(12), 893-904. doi:10.1016/j.biopsych.2018.04.019

Baxter MG, Parker A, Lindner CC, Izquierdo AD, & Murray EA. (2000). Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. The Journal of neuroscience : the official journal of the Society for Neuroscience, 20(11), 4311–4319. https://doi.org/10.1523/JNEUROSCI.20-11-04311.2000

Bechara, A., Dolan, S., Denburg, N., Hindes, A., Anderson, S. W., & Nathan, P. E. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. Neuropsychologia, 39(4), 376-389. doi:10.1016/s0028-3932(00)00136-6

Bednarek E, Caroni P. (2011) β-Adducin is required for stable assembly of new synapses and improved memory upon environmental enrichment. Neuron. 69(6):1132-46. doi: 10.1016/j.neuron.2011.02.034. PMID: 21435558.

Bradfield LA, Dezfouli A, Van Holstein M, Chieng B, & Balleine BW. (2015) Medial Orbitofrontal Cortex Mediates Outcome Retrieval in Partially Observable Task Situations. Neuron, 88(6), 1268–1280. doi: 10.1016/j.neuron.2015.10.044 PubMEd PMID: 26627312

Bourne J, Harris KM. (2007) Do thin spines learn to be mushroom spines that remember? Curr Opin Neurobiol. 17:381–386. doi:10.1016/j.conb.2007.04.009 PubMed PMID: 17498943

Cao T, Matyas JJ, Renn CL, Faden AI, Dorsey SG, & Wu J. (2020). Function and Mechanisms of Truncated BDNF Receptor TrkB.T1 in Neuropathic Pain. Cells, 9(5), 1194. https://doi.org/10.3390/cells9051194

Carlén M. (2017). What constitutes the prefrontal cortex?. Science (New York, N.Y.), 358(6362), 478–482. https://doi.org/10.1126/science.aan8868

Cattaneo A, Cattane N, Begni V, Pariante CM, & Riva MA. (2016). The human Bdnf gene: Peripheral gene expression and protein levels as biomarkers for psychiatric disorders. Translational Psychiatry, 6(11). doi:10.1038/tp.2016.214

Crombag, H. S. (2004). Opposite effects of amphetamine Self-administration experience on dendritic spines in the medial and ORBITAL prefrontal cortex. Cerebral Cortex, 15(3), 341-348. doi:10.1093/cercor/bhh136

Conner JM, Lauterborn JC, Yan Q, Gall CM, Varon S. (1997) Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport. J Neurosci. 17(7):2295-313. doi: 10.1523/JNEUROSCI.17-07-02295.1997. PMID: 9065491

Croxson PL, Johansen-Berg H, Behrens TE, Robson MD, Pinsk MA, Gross CG, Richter W, Richter MC, Kastner S, & Rushworth MF. (2005). Quantitative investigation of connections of the prefrontal cortex in the human and macaque using probabilistic diffusion tractography. The Journal of neuroscience : the official journal of the Society for Neuroscience, 25(39), 8854–8866. https://doi.org/10.1523/JNEUROSCI.1311-05.2005

Dailey ME, Smith SJ. (1996) The dynamics of dendritic structure in developing hippocampal slices. J Neurosci. 16:2983–2994. doi: 10.1523/JNEUROSCI.16-09-02983.1996 PubMed PMID: 8622128

DePoy LM, Zimmermann KS, Marvar PJ, & Gourley SL. (2017). Induction and Blockade of Adolescent Cocaine-Induced Habits. Biological psychiatry, 81(7), 595–605. https://doi.org/10.1016/j.biopsych.2016.09.023

DePoy, L. M., Shapiro, L. P., Kietzman, H. W., Roman, K. M., & Gourley, S. L. (2019). β1-Integrins in the Developing Orbitofrontal Cortex Are Necessary for Expectancy Updating in Mice. The Journal of neuroscience : the official journal of the Society for Neuroscience, 39(34), 6644–6655. https://doi.org/10.1523/JNEUROSCI.3072-18.2019

Ehrengruber, M. U., & Lundstrom, K. (2000). Alphavirus-mediated gene transfer into neurons. Current Protocols in Neuroscience, 13(1). doi:10.1002/0471142301.ns0422s13

Ehrlich I, Klein M, Rumpel S, & Malinow R. (2007). PSD-95 is required for activity-driven synapse stabilization. Proceedings of the National Academy of Sciences of the United States of America, 104(10), 4176–4181. https://doi.org/10.1073/pnas.0609307104

Everitt BJ, Robbins TW. (2016) Drug Addiction: Updating actions to habits to compulsions ten years on. Annu Rev Psychol. 67:23-50. Epub 2015/08/09. doi: 10.1146/annurev-psych-122414-033457. PubMed PMID: 2625354

Feng G, Mellor RH, Bernstein M, Keller-Peck C, Nguyen QT, Wallace M, Nerbonne JM, Lichtman JW, & Sanes JR. (2000). Imaging neuronal subsets in transgenic mice expressing multiple spectral variants of GFP. Neuron, 28(1), 41–51. https://doi.org/10.1016/s0896-6273(00)00084-2

Fenner BM. (2012). Truncated TrkB: beyond a dominant negative receptor. Cytokine & growth factor reviews, 23(1-2), 15–24. https://doi.org/10.1016/j.cytogfr.2012.01.002

Fiala JC, Feinberg M, Popov V, Harris KM. (1998) Synaptogenesis via dendritic filopodia in developing hippocampal area CA1. J Neurosci. 18(21):8900-11. doi: 10.1523/JNEUROSCI.18-21-08900.1998. PMID: 9786995; PMCID: PMC6793554.

Fiala JC, Allwardt B, and Harris KM. (2002). Dendritic spines do not split during hippocampal LTP or maturation. Nat. Neurosci. 5: 297–298. doi: 10.1038/nn830 PubMed PMID: 11896399

Fifkova E, Delay RJ. (1982) Cytoplasmic actin in neuronal processes as a possible mediator of synaptic plasticity. J Cell Biol. 95:345–350. doi: 10.1083/jcb.95.1.345. PubMed PMID: 6890558

Fischer M, Kaech S, Knutti D, Matus A. (1998) Rapid actin-based plasticity in dendritic spines. Neuron. 20:847–854. doi: 10.1016/S0896-6273(00)80467-5 PubMed PMID: 9620690

Fu M, Yu X, Lu J, & Zuo Y. (2012). Repetitive motor learning induces coordinated formation of clustered dendritic spines in vivo. Nature, 483(7387), 92–95. https://doi.org/10.1038/nature10844

Galeotti N & Ghelardini C. (2011). Regionally selective activation and differential regulation of erk, JNK AND P38 MAP kinase signaling pathway by protein kinase C in mood modulation. The International Journal of Neuropsychopharmacology, 15(06), 781-793. doi:10.1017/s1461145711000897

Gerlai RT. (2018). Molecular-genetic and statistical techniques for behavioral and neural research. London: Academic Press, an imprint of Elsevier.

Goldstein R, Tomasi D, Rajaram S, Cottone L, Zhang L, Maloney T, Volkow N. (2007). Role of the anterior cingulate and medial orbitofrontal cortex in processing drug cues in cocaine addiction. *Neuroscience, 144*(4), 1153-1159. doi:10.1016/j.neuroscience.2006.11.024

Gourley SL., Olevska A, Warren MS, Taylor JR, & Koleske AJ. (2012). Arg kinase regulates prefrontal dendritic spine refinement and cocaine-induced plasticity. The Journal of neuroscience : the official journal of the Society for Neuroscience, 32(7), 2314–2323. https://doi.org/10.1523/JNEUROSCI.2730-11.2012

Gourley SL, Zimmermann KS, Allen AG, & Taylor JR. (2016). The Medial Orbitofrontal Cortex Regulates Sensitivity to Outcome Value. The Journal of neuroscience : the official journal of the Society for Neuroscience, 36(16), 4600–4613. https://doi.org/10.1523/JNEUROSCI.4253-15.2016

Harris KM, Jensen FE, Tsao B. (1992) Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. J Neurosci. 12:2685–2705. doi: 10.1523/JNEUROSCI.12-07-02685.1992 PubMed PMID: 1613552

Harris KM. Structure, development, and plasticity of dendritic spines. (1999) Curr Opin Neurobiol.(3):343-8. doi: 10.1016/s0959-4388(99)80050-6. PMID: 10395574.

Hayashi-Takagi A, Yagishita S, Nakamura M, Shirai F, Wu YI, Loshbaugh AL, Kuhlman B, Hahn KM, & Kasai H. (2015). Labelling and optical erasure of synaptic memory traces in the motor cortex. Nature, 525(7569), 333–338. https://doi.org/10.1038/nature15257

Heimer-McGinn V, Murphy AC, Kim JC, Dymecki SM, & Young PW. (2013). Decreased dendritic spine density as a consequence of tetanus toxin light chain expression in single neurons in vivo. Neuroscience letters, 555, 36–41. https://doi.org/10.1016/j.neulet.2013.09.007

Hinton EA, Li DC, Allen AG, & Gourley SL (2019). Social Isolation in Adolescence Disrupts Cortical Development and Goal-Dependent Decision-Making in Adulthood, Despite Social Reintegration. eNeuro, 6(5), ENEURO.0318-19.2019. https://doi.org/10.1523/ENEURO.0318-19.2019

Hotulainen P, Hoogenraad CC. (2010) Actin in dendritic spines: connecting dynamics to function. J Cell Biol. 189:619–629. doi: 10.1083/jcb.201003008 PubMed PMID: 20457765

Hu B, Nikolakopoulou AM, Cohen-Cory S. (2005) BDNF stabilizes synapses and maintains the structural complexity of optic axons in vivo. Development. 132(19):4285-98. doi: 10.1242/dev.02017. PMID: 16141221.

Izquierdo A. (2017). Functional Heterogeneity within Rat Orbitofrontal Cortex in Reward Learning and Decision Making. The Journal of neuroscience : the official journal of the

Society for Neuroscience, 37(44), 10529–10540. https://doi.org/10.1523/JNEUROSCI.1678-17.2017

Ji Y, Lu Y, Yang F, Shen W, Tang TT, Feng L, Duan S, & Lu B. (2010). Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. Nature neuroscience, 13(3), 302–309. https://doi.org/10.1038/nn.2505

Jung Y, Mulholland PJ, Wiseman SL, Chandler LJ, Picciotto MR. (2013) Constitutive knockout of the membrane cytoskeleton protein beta adducin decreases mushroom spine density in the nucleus accumbens but does not prevent spine remodeling in response to cocaine. Eur J Neurosci. 37(1):1-9. doi: 10.1111/ejn.12037. PMID: 23106536

Kellner Y, Gödecke N, Dierkes T, Thieme N, Zagrebelsky M, & Korte M. (2014). The BDNF effects on dendritic spines of mature hippocampal neurons depend on neuronal activity. Frontiers in synaptic neuroscience, 6, 5. https://doi.org/10.3389/fnsyn.2014.00005

Kim E, Sheng M. (2004) PDZ domain proteins of synapses. Nat Rev Neurosci. (10):771-81. doi: 10.1038/nrn1517. PMID: 15378037.

Koleske AJ. (2013) Molecular mechanisms of dendrite stability. Nat Rev Neurosci. 2013(8):536-50. doi: 10.1038/nrn3486. PMID: 23839597

Kühn, S., Schubert, F., & Gallinat, J. (2010). Reduced Thickness of Medial Orbitofrontal Cortex in Smokers. Biological Psychiatry, 68(11), 1061-1065.

LeVay S. (1973) Synaptic patterns in the visual cortex of the cat and monkey. Electron microscopy of Golgi preparations. J Comp Neurol. 150:53–85. doi: 10.1002/cne.901500104. PubMed PMID: 4124647

Liu J, You Q, Wei M, Wang Q, Luo Z, Lin S, Gao T. (2014). Social isolation during adolescence strengthens retention of fear memories and facilitates induction of late-phase long-term potentiation. *Molecular Neurobiology, 52*(3), 1421-1429. doi:10.1007/s12035-014-8917-0

Ma XM, Huang J, Wang Y, Eipper BA, & Mains RE. (2003). Kalirin, a multifunctional Rho guanine nucleotide exchange factor, is necessary for maintenance of hippocampal pyramidal neuron dendrites and dendritic spines. The Journal of neuroscience : the official journal of the Society for Neuroscience, 23(33), 10593–10603. https://doi.org/10.1523/JNEUROSCI.23-33-10593.2003

Mackey S, & Petrides M. (2009). Architectonic mapping of the medial region of the human orbitofrontal cortex by density profiles. Neuroscience, 159(3), 1089–1107. https://doi.org/10.1016/j.neuroscience

Marshak S, Nikolakopoulou AM, Dirks R, Martens GJ, Cohen-Cory S. (2007) Cell-autonomous TrkB signaling in presynaptic retinal ganglion cells mediates axon arbor growth and synapse maturation during the establishment of retinotectal synaptic connectivity. J

Neurosci. 27:2444–2456. doi: 10.1523/JNEUROSCI.4434-06.2007. PubMed PMID: 17344382

Matsuzaki M, Honkura N, Ellis-Davies GC, Kasai H. (2004) Structural basis of long-term potentiation in single dendritic spines. Nature. 429(6993):761-6. doi: 10.1038/nature02617. PMID: 15190253; PMCID: PMC4158816.

Mattila P & Lappalainen P. (2008) Filopodia: molecular architecture and cellular functions. Nat Rev Mol Cell Biol 9, 446–454. doi: 10.1038/nrm2406 PMID: 18464790

Matus A, Ackermann M, Pehling G, Byers HR, Fujiwara K. (1982) High actin concentrations in brain dendritic spines and postsynaptic densities. Proc Natl Acad Sci USA. 79:7590–7594. doi: 10.1073/pnas.79.23.7590 PubMed: 6760199

Meller E, Shen C, Nikolao TA, Jensen C, Tsimberg Y, Chen J, & Gruen RJ. (2003). Region-specific effects of acute and repeated restraint stress on the phosphorylation of mitogen-activated protein kinases. *Brain Research, 979*(1-2), 57-64. doi:10.1016/s0006-8993(03)02866-x

Milner BA. (1982). Some cognitive effects of frontal-lobe lesions in man. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 298(1089), 211-226. doi:10.1098/rstb.1982.0083

Moda-Sava RN, Murdock MH, Parekh PK, Fetcho RN, Huang BS, Huynh TN, Witztum, J, Shaver DC, Rosenthal DL, Alway EJ, Lopez K, Meng Y, Nellissen L, Grosenick L, Milner TA, Deisseroth K, Bito H, Kasai H, & Liston C. (2019). Sustained rescue of prefrontal circuit dysfunction by antidepressant-induced spine formation. Science (New York, N.Y.), 364(6436), eaat8078. https://doi.org/10.1126/science.aat8078

Moyer, C. E., & Zuo, Y. (2018). Cortical dendritic spine development and plasticity: insights from in vivo imaging. Current opinion in neurobiology, 53, 76–82. https://doi.org/10.1016/j.conb.2018.06.002

Nathanson, J., Yanagawa, Y., Obata, K., & Callaway, E. (2009). Preferential labeling of inhibitory and excitatory cortical neurons by endogenous tropism of adeno-associated virus and lentivirus vectors. Neuroscience, 161(2), 441-450. doi:10.1016/j.neuroscience.2009.03.032

Noonan MP, Chau BK, Rushworth MF, Fellows LK. (2017). Contrasting effects of medial and Lateral orbitofrontal Cortex lesions on credit assignment and decision-making in humans. The Journal of Neuroscience, 37(29), 7023-7035. doi:10.1523/jneurosci.0692-17.2017

Okamoto K, Nagai T, Miyawaki A, Hayashi Y. (2004) Rapid and persistent modulation of actin dynamics regulates postsynaptic reorganization underlying bidirectional plasticity. Nat Neurosci. (10):1104-12. doi: 10.1038/nn1311. PMID: 15361876.

Park H, Poo MM. (2013) Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci. 14(1):7-23. Epub 2012/12/21. doi: 10.1038/nrn3379. PubMed PMID: 23254191.

Peters A, Kaiserman-Abramof IR. (1970) The small pyramidal neuron of the rat cerebral cortex. The perikaryon, dendrites and spines. Am J Anat. (4):321-55. doi: 10.1002/aja.1001270402. PMID: 4985058.

Pitts EG, Li DC, Gourley SL. (2018) Bidirectional coordination of actions and habits by TrkB in mice. Sci Rep. 8(1):4495. doi: 10.1038/s41598-018-22560-x. PubMed PMID: 29540698

Pitts EG, Barfield ET, Woon EP, & Gourley SL. (2020). Action-Outcome Expectancies Require Orbitofrontal Neurotrophin Systems in Naïve and Cocaine-Exposed Mice. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics, 17(1), 165–177. https://doi.org/10.1007/s13311-019-00752-1

Price JL. (2007). Definition of the orbital cortex in relation to specific connections with limbic and visceral structures and other cortical regions. Annals of the New York Academy of Sciences, 1121, 54–71. https://doi.org/10.1196/annals.1401.008

Radley JJ, Anderson RM, Hamilton BA, Alcock JA, & Romig-Martin SA. (2013). Chronic stress-induced alterations of dendritic spine subtypes predict functional decrements in an hypothalamo-pituitary-adrenal-inhibitory prefrontal circuit. The Journal of neuroscience : the official journal of the Society for Neuroscience, 33(36), 14379–14391. https://doi.org/10.1523/JNEUROSCI.0287-13.2013

Rolls ET, Hornak J, Wade D, & McGrath J. (1994). Emotion-related learning in patients with social and emotional changes associated with frontal lobe damage. Journal of Neurology, Neurosurgery & Psychiatry, 57(12), 1518-1524. doi:10.1136/jnnp.57.12.1518

Rose CR, Blum R, Pichler B, Lepier A, Kafitz KW, & Konnerth A. (2003). Truncated TrkB-T1 mediates neurotrophin-evoked calcium signalling in glia cells. Nature, 426(6962), 74–78. https://doi.org/10.1038/nature01983

Saba, J., Turati, J., Ramírez, D., Carniglia, L., Durand, D., Lasaga, M., & Caruso, C. (2018). Astrocyte truncated tropomyosin receptor Kinase B mediates brain-derived neurotrophic FACTOR ANTI-APOPTOTIC effect leading to NEUROPROTECTION. Journal of Neurochemistry, 146(6), 686-702. doi:10.1111/jnc.14476

Sharp WG, Allen AG, Stubbs KH, Criado KK, Sanders R, McCracken CE, Parsons RG, Scahill L, & Gourley SL. (2017). Successful pharmacotherapy for the treatment of severe feeding aversion with mechanistic insights from cross-species neuronal remodeling. Translational psychiatry, 7(6), e1157. https://doi.org/10.1038/tp.2017.126

Sasi M, Vignoli, B, Canossa M, & Blum R. (2017). Neurobiology of local and intercellular BDNF signaling. *Pflügers Archiv - European Journal of Physiology, 469*(5-6), 611-611. doi:10.1007/s00424-017-1971-5

Shen CP, Tsimberg Y, Salvadore C, Meller E. (2004) Activation of Erk and JNK MAPK pathways by acute swim stress in rat brain regions. BMC Neurosci 5, 36. https://doi.org/10.1186/1471-2202-5-36

Star EN, Kwiatkowski DJ, Murthy VN. (2002) Rapid turnover of actin in dendritic spines and its regulation by activity. Nature Neurosci. 5:239–246. doi: 10.1038/nn811 PubMed PMID: 11850630

Sobreviela T, Pagcatipunan M, Kroin JS, Mufson EJ. Retrograde transport of brain-derived neurotrophic factor (BDNF) following infusion in neo- and limbic cortex in rat: relationship to BDNF mRNA expressing neurons. (1996) J Comp Neurol. 375(3):417-44. doi: 10.1002/(SICI)1096-9861(19961118)375:3<417::AID-CNE6>3.0.CO;2-5. PMID: 8915840.

Tashiro A, Yuste R. (2003) Structure and molecular organization of dendritic spines. Histol Histopathol. (2):617-34. doi: 10.14670/HH-18.617. PMID: 12647812.

Tanabe, J., Tregellas, J. R., Dalwani, M., Thompson, L., Owens, E., Crowley, T., & Banich, M. (2009). Medial orbitofrontal CORTEX gray matter is reduced in abstinent Substance-Dependent Individuals. Biological Psychiatry, 65(2), 160-164. doi:10.1016/j.biopsych.2008.07.030

Trantham, H., Szumlinski, K., McFarland, K., Kalivas, P., & Lavin, A. (2002). Repeated cocaine administration alters the electrophysiological properties of prefrontal cortical neurons. *Neuroscience, 113*(4), 749-753. doi:10.1016/s0306-4522(02)00246-4

Uchizono K. (1965) Characteristics of excitatory and inhibitory synapses in the central nervous system of the cat. Nature. 207:642–643. Doi: 10.1038/207642a0. PubMed PMID: 5883646

Wallis JD. (2011). Cross-species studies of orbitofrontal cortex and value-based decision-making. *Nature neuroscience*, *15*(1), 13–19. https://doi.org/10.1038/nn.2956

Walton ME, Behrens TE, Buckley MJ, Rudebeck PH, & Rushworth MF. (2010). Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning. Neuron, 65(6), 927–939. https://doi.org/10.1016/j.neuron.2010.02.027

Wong WT, Faulkner-Jones BE, Sanes JR, Wong RO. (2000) Rapid dendritic remodeling in the developing retina: dependence on neurotransmission and reciprocal regulation by Rac and Rho. J Neurosci. 20:5024–5036. doi: 10.1523/JNEUROSCI.20-13-05024.2000 PubMed PMID: 10864960

Wong WT, Wong RO. (2000) Rapid dendritic movements during synapse formation and rearrangement. Curr Opin Neurobiol. 10:118–124. doi: 10.1016/s0959-4388(99)00059-8. PubMed PMID: 10679440

Wu GY, Zou DJ, Rajan I, Cline H. (1999) Dendritic dynamics in vivo change during neuronal maturation. J Neurosci. 19:4472–4483. doi: 10.1523/JNEUROSCI.19-11-04472.1999 PubMed PMID: 10341248

Yasuda R, Harvey CD, Zhong H, Sobczyk A, van Aelst L, Svoboda K. Supersensitive Ras activation in dendrites and spines revealed by two-photon fluorescence lifetime imaging. (2006) Nat Neurosci. 2:283-291. doi: 10.1038/nn1635. PMID: 16429133.

Zagrebelsky M, Tacke C, & Korte M. (2020). BDNF signaling during the lifetime of dendritic spines. Cell and tissue research, 382(1), 185–199. https://doi.org/10.1007/s00441-020-03226-5

Zhou Q, Homma KJ, Poo MM. (2004) Shrinkage of dendritic spines associated with long-term depression of hippocampal synapses. Neuron. 44(5):749-57. doi: 10.1016/j.neuron.2004.11.011. PMID: 15572107.