

## Acute vagus nerve stimulation enhances reversal learning in rats

Lindsay K-P. Altidor<sup>a</sup>, Matthew M. Bruner<sup>a</sup>, Josue F. Deslauriers<sup>a</sup>, Tyler S. Garman<sup>a</sup>, Saúl Ramirez<sup>a</sup>, Elliott W. Dirr<sup>c</sup>, Kaitlynn P. Olczak<sup>c</sup>, Andrew P. Maurer<sup>a,c,d,f</sup>, Damon G. Lamb<sup>a,b,c,d,e</sup>, Kevin J. Otto<sup>a,c,d</sup>, Sara N. Burke<sup>a,d</sup>, Argyle V. Bumanglag<sup>a,d</sup>, Barry Setlow<sup>b,d</sup>, Jennifer L. Bizon<sup>a,d,\*</sup>

<sup>a</sup> Department of Neuroscience, University of Florida, Gainesville, FL, USA

<sup>b</sup> Department of Psychiatry, University of Florida, Gainesville, FL, USA

<sup>c</sup> J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, USA

<sup>d</sup> Evelyn F. & William L. McKnight Brain Institute, University of Florida, USA

<sup>e</sup> Brain Rehabilitation Research Center, Malcom Randall VAMC, Gainesville, FL, USA

<sup>f</sup> Engineering School of Sustainable Infrastructure and Environment, University of Florida, Gainesville, FL, USA

### ARTICLE INFO

#### Keywords:

Vagus nerve stimulation  
Reversal learning  
Cognitive flexibility

### ABSTRACT

Cognitive flexibility is a prefrontal cortex-dependent neurocognitive process that enables behavioral adaptation in response to changes in environmental contingencies. Electrical vagus nerve stimulation (VNS) enhances several forms of learning and neuroplasticity, but its effects on cognitive flexibility have not been evaluated. In the current study, a within-subjects design was used to assess the effects of VNS on performance in a novel visual discrimination reversal learning task conducted in touchscreen operant chambers. The task design enabled simultaneous assessment of acute VNS both on reversal learning and on recall of a well-learned discrimination problem. Acute VNS delivered in conjunction with stimuli presentation during reversal learning reliably enhanced learning of new reward contingencies. Enhancement was not observed, however, if VNS was delivered during the session but was not coincident with presentation of to-be-learned stimuli. In addition, whereas VNS delivered at 30 Hz enhanced performance, the same enhancement was not observed using 10 or 50 Hz. Together, these data show that acute VNS facilitates reversal learning and indicate that the timing and frequency of the VNS are critical for these enhancing effects. In separate rats, administration of the norepinephrine reuptake inhibitor atomoxetine also enhanced reversal learning in the same task, consistent with a noradrenergic mechanism through which VNS enhances cognitive flexibility.

### 1. Introduction

Cognitive flexibility refers to the ability to modify behavior in response to a change in environmental contingencies. Given our rapidly changing environment, this neurocognitive process is essential for effectively navigating everyday life. Behavioral rigidity and perseveration occur in a host of neuropsychiatric conditions (including schizophrenia, obsessive compulsive disorder, attention-deficit/hyperactivity disorder, autism, and substance use disorders) as well as during the normal aging process (Beas, Setlow, & Bizon, 2013; Bizon, Foster, Alexander, & Glisky, 2012; Stuchlik & Sumiyoshi, 2014). As such, therapeutic strategies to enhance cognitive flexibility could have far-reaching benefits (Groman et al., 2013; Izquierdo & Jentsch, 2012).

Cognitive flexibility can be parsed into several distinct forms (e.g., set shifting and reversal learning), but all share a critical dependence on the prefrontal cortex (PFC) and can be modulated by monoaminergic and cholinergic afferents (Birrell & Brown, 2000; Bissonette et al., 2008; Borodovitsyna, Flamini, & Chandler, 2017; Dias, Robbins, & Roberts, 1996; Kim, Johnson, Cilles, & Gold, 2011; McAlonan & Brown, 2003; Tait, Chase, & Brown, 2014). Pharmacological manipulations targeting these neurochemical systems can enhance cognitive flexibility in both healthy and cognitively-compromised subjects (Chamberlain & Robbins, 2013; Floresco & Jentsch, 2011; Prado, Janickova, Al-Onaizi, & Prado, 2017; Sadacca, Wikenheiser, & Schoenbaum, 2017; Samanez-Larkin et al., 2013); the efficacy of these drugs is accompanied by off-target effects on behavior, however, which can counter-indicate their

\* Corresponding author at: Department of Neuroscience, University of Florida, Gainesville, FL 32608, USA.

E-mail address: [bizonj@ufl.edu](mailto:bizonj@ufl.edu) (J.L. Bizon).

<https://doi.org/10.1016/j.nlm.2021.107498>

Received 30 April 2021; Received in revised form 1 July 2021; Accepted 24 July 2021

Available online 29 July 2021

1074-7427/© 2021 Elsevier Inc. All rights reserved.

utility for intervention.

Electrical vagus nerve stimulation (VNS) has been approved for 30 years to treat intractable epilepsy and depression (Aaronson & Conway, 2018; Dibué-Adjei, Brigo, Yamamoto, Vonck, & Trinka, 2019; Dibué-Adjei, Kamp, & Vonck, 2019b; McDonald, 2016; Reuter, McClure, Liebler, & Pozo-Rosich, 2019; Smucny, Visani, & Tregellas, 2015; van Hoorn et al., 2019). Some individuals in these VNS treatment groups report cognitive benefits, particularly after long-term use (Aaronson & Conway, 2018; Clark, Naritoku, Smith, Browning, & Jensen, 1999; Clark et al., 1998; Desbeaumes Jodoin, Richer, Miron, Fournier-Gosselin, & Lespérance, 2018; Ghacibeh, Shenker, Shenal, Uthman, & Heilman, 2006; Helmstaedter, Hoppe, & Elger, 2001) (Jacobs, Riphagen, Razat, Wiese, & Sack, 2015). Moreover, a year-long trial of chronic VNS in Alzheimer's disease patients reported improved cognitive outcomes (Merrill et al., 2006; Sjögren et al., 2002). Research in animal models further supports the efficacy of VNS for facilitating cognition. Acute VNS enhances performance in novel object recognition, water maze, and extinction learning tasks in rodents (Noble, Meruva, et al., 2019; Sun et al., 2017). Moreover, VNS facilitates learning to extinguish fear-related responses to a cue previously predictive of electrical shock (i. e., extinction of fear conditioning), which depends critically upon the medial PFC (Morgan & LeDoux, 1995; Morgan, Romanski, & LeDoux, 1993; Noble, Meruva, et al., 2019; Peters, Kalivas, & Quirk, 2009; Peña, Engineer, & McIntyre, 2013; Quirk, Garcia, & González-Lima, 2006).

Vagus nerve afferents project to the nucleus of the solitary tract (NTS) which in turn directly innervates the locus coeruleus (LC). The LC provides noradrenergic innervation to much of the brain, including the PFC (Aston-Jones & Waterhouse, 2016; Chandler & Waterhouse, 2012; Chandler, Gao, & Waterhouse, 2014; Chandler, Lamperski, & Waterhouse, 2013; Poe et al., 2020; Waterhouse & Navarra, 2019; Waterhouse, Devilbiss, Fleischer, Sessler, & Simpson, 1998; Waterhouse, Lin, Burne, & Woodward, 1983). VNS enhancement of cognitive function and neuroplasticity involves signaling through modulatory neurotransmitters, including norepinephrine. Indeed, VNS robustly drives LC neurons and stimulates norepinephrine release in forebrain, including hippocampus and neocortical regions (Hassert, Miyashita, & Williams, 2004; Hulsey et al., 2017; Naritoku, Terry, & Helfert, 1995; Roosevelt, Smith, Clough, Jensen, & Browning, 2006). Moreover, intact LC neurons are critical for VNS-induced cortical plasticity (Hulsey, Sadmaan, Abe, Hays, & Kilgard, 2018; Shen, Fuchino, Miyamoto, Nomura, & Matsuki, 2012). Noradrenergic neurons in LC innervate PFC and hippocampus, and norepinephrine signaling in these structures influences many forms of cognition, including flexibility (Arnsten, 2011; Cain, Wasserman, Waterhouse, & McGaughy, 2011; Cope, Vazey, Floresco, & Aston Jones, 2019; Glennon et al., 2019; Hvoslef-Eide et al., 2015; Janitzky et al., 2015; Rorabaugh et al., 2017; Sara, 2009; Seu & Jentsch, 2009; Seu, Lang, Rivera, & Jentsch, 2009).

In the current study, the utility of VNS for enhancing cognitive flexibility was assessed in rats using a novel visual discrimination reversal learning task conducted in touchscreen operant chambers. The task was designed to 1) evaluate the effects of VNS using a within-subjects experimental design; and 2) enable concomitant evaluation of performance on both reversal learning and recall of a well-learned discrimination problem. In Experiment 1, this new task was validated using acute administration of the GABA(B) receptor agonist baclofen, which robustly enhances cognitive flexibility (Beas, McQuail, Banuelos, Setlow, & Bizon, 2017; Beas, Setlow, & Bizon, 2016). In Experiment 2, the effects of VNS paired with presentation of the reversed problem were evaluated. Additional experiments tested effects of varying VNS timing and/or stimulation parameters. Given that VNS can modulate norepinephrine release in the forebrain, Experiment 3 determined whether pharmacologically enhancing norepinephrine availability with

atomoxetine mimics the effects of VNS on reversal learning.

## 2. Methods

### 2.1. Subjects

Young adult (2 months of age at the start of testing) male Brown Norway rats ( $N = 52$ ) were obtained from Charles River Laboratories and housed individually in the AAALAC-accredited vivarium facility at the University of Florida McKnight Brain Institute. The vivarium was maintained at 25° C with a 12 h reversed light/dark cycle (lights on at 1900). Rats had free access to food and water at all times unless noted otherwise below. Animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee and followed National Institutes of Health guidelines.

### 2.2. Behavioral testing

#### 2.2.1. Behavioral testing apparatus

Rats were tested in eight identical touchscreen operant chambers housed in sound-attenuating cabinets (Lafayette Instrument, Lafayette, IN). These touchscreen chambers allow for a wide range of visual stimuli and permit within-subject experimental designs that are ideal for assessing cognition (Mar et al., 2013). Each chamber ( $33 \times 24 \times 30$  cm) consisted of black Perspex walls that formed an isosceles trapezoid-shaped floor, with a transparent lid and a grated metal floor. A food delivery trough, which could be illuminated by a small lightbulb, was located at the narrow end of the chamber. A single 45 mg food pellet reward (AIN-76A, Test Diet, Richmond, IN) was delivered into the food trough following correct responses. A 30.7 cm touch-sensitive video screen (resolution:  $800 \times 600$  px) was located on the chamber wall opposite from the food trough. A black plastic mask covered the screen, with two square ( $10 \text{ cm} \times 10 \text{ cm}$ ) cutouts that formed response windows that allowed rats to interact with the screen. Additionally, a black plastic shelf with a spring hinge mechanism was placed in front of the mask to facilitate rats' access to the response windows. Infrared beams used to assess activity crossed the touchscreen vertically as well as the long axis of the chamber in several locations. Movement in the center of the chamber, along with responses at the touchscreen and the food trough, were recorded as breaks of the beams crossing the locations of interest. A house light mounted in the ceiling of the chamber was used as a mild aversive stimulus delivered during "time out" periods following incorrect responses in some stages of training and testing (see below). Stimulus presentation and data collection were controlled by computers running ABET II Touch software (Campden Instruments Ltd, Lafayette, IN) and Whisker Server (Cardinal & Aitken, 2010).

#### 2.2.2. Shaping of touchscreen operant procedures

Rats were acclimated for at least 3 days after arrival in the vivarium before any procedures were initiated. Prior to the start of behavioral testing, rats were food restricted to 85% of their initial free-feeding weight, with a 5 g/week increase in their target weight to account for growth. Food restriction continued throughout the duration of behavioral testing. On the day before the first stage of shaping, rats received eight 45 mg food pellets in their home cage to attenuate neophobia to the food used in the behavioral task. Shaping procedures were performed in the touchscreen operant chambers and consisted of multiple stages designed to train rats to respond to visual stimuli presented on the touchscreen. All shaping sessions were 1 h in duration. Shaping stage 1 consisted of magazine training, wherein a single food pellet was delivered into the food trough on a variable interval schedule (15, 30, 45, or 60 s) throughout the session. During shaping stage 2, images were

displayed within both response windows, and rats were reinforced with a single food pellet for touches at either window, followed by a 5 s inter-trial interval (ITI). In shaping stage 3, an image was displayed in only one of the two response windows, and rats were reinforced only for touching the window with the image present, followed by a 5 s ITI. Touching the blank screen with no image present had no programmed consequences. In shaping stage 4, rats were trained to initiate stimulus presentation on the touchscreen. During this stage, the food trough light was activated at the start of each trial. When the rat triggered the photobeam in the food trough via a nosepoke, the food trough light was extinguished, and an image was displayed on the touchscreen in one of the two response windows. A touch on the window with the image present was reinforced with a food reward. Collection of the food reward initiated a 5 s ITI, after which the trough light was illuminated again to signal the start of the next trial. Shaping stage 5 was similar to stage 4 but was designed to punish incorrect responses. Once the image was presented on the touchscreen, incorrect responses (touches in the window without the image) caused the overhead house light to illuminate for 5 s and no reward was delivered. At the end of the 5 s time out period, the house light was extinguished, and the food trough light was activated to signal that the rat could initiate another trial.

### 2.2.3. Pairwise discrimination learning

After completion of the touchscreen shaping procedures, rats were trained on a pairwise discrimination learning task, which consisted of 1 h sessions wherein they learned to discriminate between a simultaneously displayed pair of distinct visual stimuli (S+, S-) presented within the two windows on the touchscreen. The position of each image on the touchscreen (left versus right) was randomized across trials. During each stimulus pair presentation, touching the correct (+) image yielded a food reward, whereas touching the incorrect (-) image resulted in no food reward and triggered a 5 s illumination of the house light. Rats were trained on a single pairwise discrimination problem until they reached criterion performance of at least 100 trials/session at  $\geq 80\%$  accuracy for two consecutive sessions. Training on the reversal

learning task began in the session immediately following.

### 2.2.4. Reversal learning task

The reversal learning task consisted of an initial acquisition phase and a reversal learning phase (Fig. 1A). All sessions were 1 h in duration. The initial acquisition phase began with sessions in which rats were trained sequentially on two pairwise discrimination problems (A+, B- and C+, D-) in a manner identical to the pairwise discrimination learning described above. On each trial, a stimulus pair was presented on the touchscreen. A response on the correct image resulted in food delivery, whereas a response on the incorrect image resulted in no food delivery and activation of the house light for 5 s. Rats were trained on one of the two stimulus pairs (A+, B- or C+, D-) until they reached criterion performance of 10 consecutive correct trials in a session. Once training with one stimulus pair was completed, rats were then trained on the second stimulus pair to the same criterion. The final step of acquisition consisted of sessions in which both the (A+, B-) and (C+, D-) stimulus pairs were presented in the same session intermixed in a pseudorandom order, until rats reached criterion performance of 10 consecutive correct trials in a session. Rats were then tested in one additional session with both stimulus pairs before moving on to the reversal learning phase of the task. The order in which the pairwise discrimination problems were presented, the image identities, and the reward contingencies were randomized across rats in each experiment.

After the initial acquisition phase, rats proceeded to the reversal learning phase of the task (Fig. 1A). This phase was conducted identically to the final session of the acquisition phase, wherein rats were required to discriminate between pairs of simultaneously presented visual images on the touchscreen, but the reinforcement contingencies of one of the two discrimination problems were switched. Thus, (A+, B-) became (A-, B+) whereas the reinforcement contingencies of the second discrimination problem remained constant; i.e. (C+, D-) remained (C+, D-). Rats were tested in the reversal phase until they reached criterion performance of  $\geq 80\%$  correct on both problems and a minimum of 720 (Experiment 1) or 900 (Experiments 2 and 3) trials completed. As with the initial learning phase, the identity of the reversed stimulus pair (A, B vs. C, D) was randomized across rats.

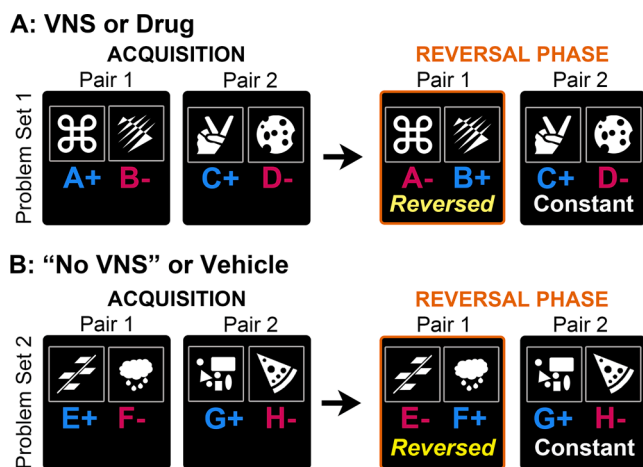
### 2.3. Experiment 1: Effects of baclofen on reversal learning

To validate the utility of the reversal learning task for assessing and detecting improvements in cognitive flexibility, we tested the effects of the GABA(B) receptor agonist baclofen, which has previously been shown to enhance cognitive flexibility in an attentional set shifting task (Beas, McQuail, Banelos, Setlow, & Bizon, 2017; Beas, Setlow, & Bizon, 2016). Rats ( $n = 7$ ) received intraperitoneal (i.p., 1.0 ml/kg) injections of baclofen (2.5 mg/kg) or vehicle (0.9% saline) 30 min prior to the start of test sessions in the reversal phase of the task.

Testing of baclofen effects on reversal learning was conducted using a within-subjects design. Hence, the experiment involved two rounds of acquisition and reversal (using novel stimuli for each), one with baclofen (Fig. 1A) and one with vehicle (Fig. 1B). The order of baclofen vs. vehicle delivery (on the first or second round of testing) was randomized, and rats were tested on the reversal phase (under both baclofen and vehicle conditions) until they reached criterion performance. Note that because rats required multiple sessions to reach criterion, baclofen or vehicle was administered prior to each reversal learning test session.

### 2.4. Experiment 2: Vagus nerve stimulation

Rats were initially trained on a pairwise visual discrimination problem as described above, followed by surgery to implant a cuff electrode on the left vagus nerve. After a 2-week recovery period, rats were tested on the reversal learning task under VNS or no-VNS (control) conditions, using a randomized, within-subjects design as in Experiment. 1. Hence, each experiment involved two rounds of initial



**Fig. 1.** Visual discrimination reversal learning task performed in touchscreen operant chambers. The task consisted of an initial Acquisition phase and a Reversal phase. Rats initially learned to discriminate between two distinct pairs of visual stimuli (Pair 1: A+, B-, and Pair 2: C+, D-). On each trial, touching the correct stimulus (+) in each pair yielded a food reward, whereas touching the incorrect stimulus (-) resulted in a 5 s “time out”. After learning each discrimination to criterion, the reward contingencies of one stimulus pair were reversed (Reversed problem: A-, B+), whereas the contingencies on the other stimulus pair remained unchanged (Constant problem: C+, D-). Each experiment involved two rounds of acquisition and reversal: one in which rats received VNS or drug (panel A), and one in which rats were tethered but no VNS was delivered, or vehicle was administered (panel B). Novel, counter-balanced stimuli were used for each experiment.

acquisition followed by reversal, one in which VNS was delivered during the reversal phase of the task (Fig. 1A), and one in which rats were tethered but no VNS was delivered (Fig. 1B). For each of the VNS experiments, the order of VNS delivery vs. no-VNS control condition (on the first or second round of the task) was randomized. Rats were tested in the reversal phase (in both the presence and absence of VNS) until they reached criterion performance.

Rats were tested under multiple VNS conditions in separate experiments. Experiment 2.1 evaluated the effects of 30 Hz VNS on reversal learning. In this experiment, 30 Hz VNS was delivered concurrently with presentation of the reversed problem (concurrently with the onset of presentation of the images on the screen) to determine whether it affected learning of this “reversed” problem. The “constant” (non-reversed) problem, which provided a within-session control to determine whether VNS affected performance of a well-learned discrimination problem, was presented on intermixed trials in the same session, but in the absence of VNS. In Experiment 2.2, VNS was delivered during presentation of the “constant” problem, to determine whether reversal learning is affected by VNS delivery during the same session despite being unpaired with the reversed problem. Experiment 2.3 determined how VNS frequencies higher or lower than 30 Hz influenced cognitive performance. The design of this experiment was identical to Experiment 2.1, with VNS delivered concurrently with presentation of the reversed problem, but the stimulation frequency was either 10 Hz or 50 Hz. Experiment 2.4 evaluated how 8 consecutive days of VNS at 30 Hz affected various off-target measures. Note that some rats in Experiments 2.1, 2.2, and 2.3 were used for more than one experiment.

#### 2.4.1. Vagus nerve cuff design

The vagus nerve cuff (Model 2600, Qualia Labs Inc., Dallas, TX) consisted of four 0.39 mm<sup>2</sup> titanium nitride-coated gold electrodes positioned on a planar thiolene/acrylate shape memory polymer substrate (Fig. 2A). Four insulated wires (~6 cm in length) connected to the four electrode contacts on the cuff were soldered to gold-plated

Amphenol pins seated in a 6-channel Delrin pedestal (P1 Technologies, Roanoke, VA) and secured by dental cement. A fifth gold-plated pin inside the Delrin pedestal was soldered to a separate non-insulated wire that could be wrapped around a skull anchor screw to serve as an electrical ground. Prior to implantation, electrode impedance was measured by electrochemical impedance spectroscopy. Cuffs containing electrode sites with impedance measured over 3 kΩ at 1 kHz were excluded from this study.

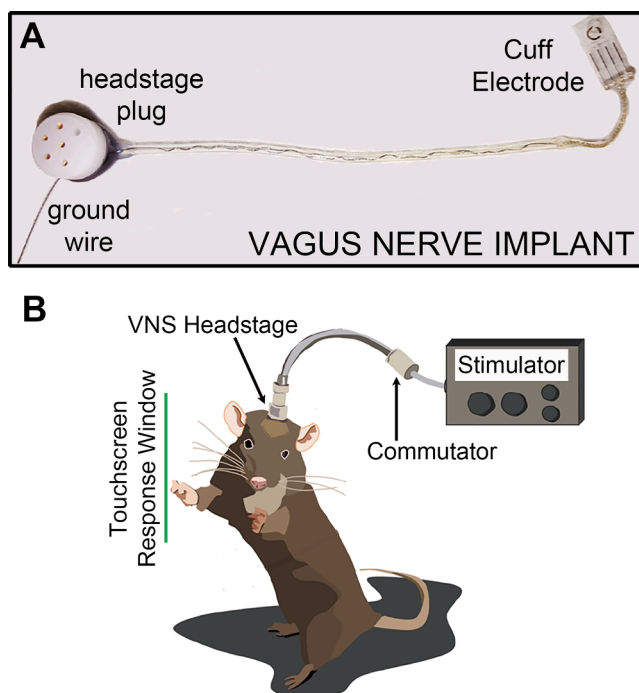
#### 2.4.2. Surgical procedures

Vagus nerve cuffs were sterilized with ethylene oxide gas prior to implantation. Rats were anaesthetized with 2–5% isoflurane in oxygen, and the surgical areas on the head and neck were shaved and disinfected with alcohol and chlorhexidine. Body temperature was maintained at ~37° C during surgery. The rat was secured in a stereotaxic apparatus and a midline incision was made to expose the skull. Four stainless steel anchor screws were inserted into the skull (two on either side of the sagittal suture anterior to bregma and two anterior to the interaural line) to stabilize the head stage. Once anchor screws were in place, a damp gauze soaked in warm sterile saline and antimicrobial hydrogel (Vet-ericyn, Rialto, CA) was placed on the surgical site to maintain hydration, and the rat was removed from the stereotaxic frame to prepare for cuff implantation on the left vagus nerve.

The rat was positioned in dorsal recumbency and a ~1.5 cm skin incision was made on the ventral neck, slightly left of midline, using the carotid pulse as reference. The skin was retracted to expose the underlying muscles, and blunt-tipped Mayo scissors were used to gently separate the muscle fibers within the incision site, avoiding damage to lymph nodes and blood vessels. Once the carotid artery was exposed, a small retractor was placed in the incision site to allow unimpeded access to the vagus nerve. Warm sterile saline was used as necessary to keep tissue within the surgical site hydrated. A blunt-tipped glass micro-probe was used to carefully separate a ~5 mm section of the vagus nerve from the carotid artery to allow placement of the cuff electrode. The cuff electrode was inserted under and wrapped around the isolated section of the vagus nerve. A 1 s train of electrical pulses (400 μA, 100 μs/phase biphasic pulse width, 50 Hz) was delivered to the electrode with the intent of eliciting a visually confirmed transient respiratory pause induced by the Hering-Breuer reflex to confirm successful cuff placement and functionality. Once the cuff electrode was confirmed to be functional, it was secured in place with cyanoacrylate adhesive (Gluture, World Precision Instruments, Sarasota, FL). A bio-compatible silicone adhesive (Kwik-Sil, World Precision Instruments, Sarasota, FL) was used to insulate the edges of the cuff to minimize irritation to surrounding tissue.

After placement of the cuff around the nerve, the muscle tissue around the cuff was sutured, and the electrode wires were tunneled subcutaneously from the neck incision site to the incision over the skull. The pedestal containing the Amphenol pin contacts was positioned in the center of the skull and the exposed wire wrapped around the anterior left screw to form a stable ground. The pedestal was fixed to the skull using surgical glue, and dental cement was placed around the anchor screws and pedestal assembly to form a stable head stage. The skin incisions were closed using non-absorbable 4/0 suture. Rats were monitored and temperature was maintained until rats were observed to be ambulatory and eating or drinking.

Post-operative weights were recorded daily, and saline was administered subcutaneously as necessary. Rats were provided with soft Transgenic Dough Diet (Bio-Serv, Flemington, NJ) and breeder food pellets (Teklad 2919 Standard Rodent Breeding Diet, Envigo, Tampa, FL) that were soaked in warm water to provide additional hydration. Single doses of Carprofen (5 mg/kg s.c.) and buprenorphine (0.05 mg/kg s.c.) were administered for 48 h post-surgery. Rats were allowed 2 weeks of post-operative recovery before behavioral testing began.



**Fig. 2.** Vagus nerve implant and experimental setup used during behavioral testing. A) Image of vagus nerve stimulating electrode that was implanted around the left vagus nerve. B) Illustration showing experimental setup for *in vivo* stimulation of the vagus nerve performed in touchscreen operant chambers.

#### 2.4.3. Vagus nerve stimulation during behavioral testing

During behavioral testing, rats were tethered via a flexible lead to 6-channel commutators (P1 Technologies, Roanoke VA) mounted in the ceiling of the operant chambers, which allowed free movement throughout the chambers (Fig. 2B). The commutators were connected to an 8-channel programmable constant current stimulator (STG 4000, Multi-Channel Systems, Reutlingen, Germany), which in turn was controlled by the ABET II Touch software (Campden Instruments Ltd) to allow VNS delivery at times determined by behavioral task performance of each rat. The VNS parameters used in Experiments 2.1, 2.2, and 2.4, (biphasic 0.8 s pulse train, 60  $\mu$ s per phase, 700  $\mu$ A, 30 Hz) were based on those previously shown to enhance cortical plasticity and extinction learning in rats (Engineer et al., 2011; Loerwald, Borland, Rennaker, Hays, & Kilgard, 2018; Peña et al., 2013). To determine the effects of stimulus frequency on VNS efficacy, the frequency was altered to 10 Hz and 50 Hz in Experiment 2.3.

#### 2.4.4. Vagus nerve cuff electrode testing

At the completion of each experiment, cuff electrodes were tested as previously described (Peña et al., 2013). Only data from lead combinations that were verified to be functional at the completion of an experiment were used for analyses. Briefly, rats were placed under light isoflurane anesthesia to induce a consistent respiratory rhythm, and the head stage was connected to a constant current stimulator. Respiratory rate was monitored, and a 1 s stimulus train (400  $\mu$ A, biphasic, 100  $\mu$ s pulse width/phase, 50 Hz) was delivered concurrent with the exhalation phase to elicit a transient pause in respiration. The electrode was considered to be functional if a brief pause in respiration was observed (i.e., the same approach used during surgery). If a respiratory pause was not observed, the stimulus pulse width was increased to 260  $\mu$ s and the respiratory pause was tested again. If cessation was not observed at a pulse width of 260  $\mu$ s, the pulse width was increased to 500  $\mu$ s, and the test was repeated. In rare circumstances in which a respiratory pause was not observed at these parameters, the stimulus amplitude was increased by 200  $\mu$ A and the pulse width was reset to 100  $\mu$ s. This pattern was followed until a maximum parameter set of 1.0 mA, 500  $\mu$ s pulse width, 50 Hz was reached. If a respiratory pause was not observed at any of these parameters, that electrode lead combination was considered to be nonfunctional and not used further, and any data collected with that lead combination were discarded.

#### 2.4.5. Effects of repeated VNS on physiological measures

Experiment 2.4 evaluated whether the VNS parameters and regimens used in the reversal learning task produced “off-target” effects. Rats were evaluated on a 12-day protocol that involved assessments of locomotor activity, food intake and body weight. Rats were implanted with a cuff electrode on the left vagus nerve and allowed to recover for 2 weeks post-surgery prior to the start of testing. On day 1 of the protocol, the rats were weighed and habituated to being tethered in the operant chamber for 30 min. On day 2, rats were habituated to tethering in the chamber for 1 h. On days 3 through 10, rats received 1-h sessions of VNS in the operant chambers or were tethered in the chambers for the same amount of time in the absence of VNS. No visual stimuli were presented in the chambers during these sessions. In each session, rats in the VNS condition received 100 VNS trains (biphasic 0.8 s pulse train, 60  $\mu$ s per phase, 700  $\mu$ A, 30 Hz) with a 36 s ITI. These parameters (including the number of VNS trains/day and the number of days of VNS) were designed to match as closely as possible the parameters experienced by the rats in Experiment 2.1 (which were effective in enhancing reversal learning). VNS cuffs were tested on day 10 to ensure that the leads remained viable throughout the course of VNS. Final body weights were obtained on day 12, followed by euthanasia. Note that although a total of  $n = 14$  VNS and  $n = 11$  control rats were tested on this protocol, not all rats were assessed on all measures.

#### 2.5. Experiment 3: Effects of atomoxetine on reversal learning

Electrical stimulation of the vagus nerve activates the LC and stimulates forebrain norepinephrine release (Groves, Bowman, & Brown, 2005; Hulse et al., 2017; Roosevelt et al., 2006; Shen et al., 2012). To determine whether an increase in norepinephrine availability could contribute to the enhancing effects of VNS on reversal learning, rats ( $n = 10$ ) received intraperitoneal injections (1.0 ml/kg) of the norepinephrine reuptake inhibitor atomoxetine (Tocris Bioscience, 1.0 mg/kg) or vehicle (50:50 dimethyl sulfoxide in 0.9% saline) 15 min prior to the start of reversal learning sessions. This dose of atomoxetine was chosen based on previous work demonstrating its efficacy in enhancing multiple forms of cognitive flexibility in rats (Seu et al., 2009; Totah, Logothetis, & Eschenko, 2015). The experimental design was otherwise identical to that of Experiment 1.

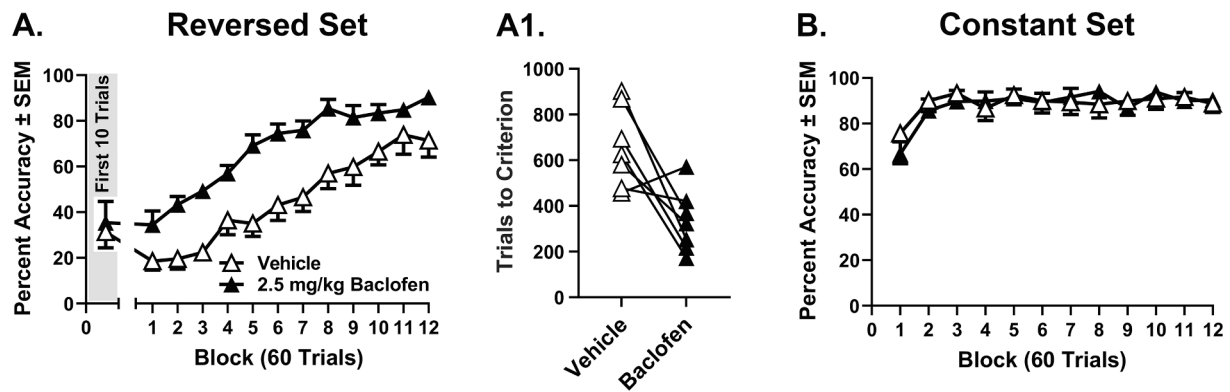
#### 2.6. Data analyses

For each experiment (with the exception of Experiment 2.4), rats were tested twice on the reversal learning task: once under drug or VNS conditions (Fig. 1A), and once under vehicle or no-VNS control conditions (Fig. 1B), in a randomized order with unique visual stimuli for each test. The primary measure of interest was performance accuracy (% correct) on each problem (both the reversed and the constant) during the reversal phase. Data were organized into 60-trial blocks. Accuracy under experimental and control conditions was compared using a two-factor repeated measures ANOVA, with both experimental condition (2 levels) and block (12 or 15 levels) as within-subjects variables. Greenhouse-Geisser corrections were applied in cases of violations of sphericity. Additional performance measures were also recorded, including the number of trials required to reach criterion performance (10 consecutive correct trials), the number of trials completed per session, trial initiation latency (the time from illumination of the trough light to a response in the trough) and locomotor activity (breaks of the infrared beams crossing the center of the chambers). These additional performance measures were compared using paired t-tests. Analyses were conducted using GraphPad Prism 8 (GraphPad Software, San Diego, CA), and  $p$  values  $< 0.05$  were considered statistically significant.

### 3. Results

#### 3.1. Experiment 1: Effects of baclofen on reversal learning

Previous work showed that acute systemic administration of the GABA(B) receptor agonist baclofen enhances cognitive flexibility in a set-shifting task (Beas et al., 2016). To determine whether performance on the novel reversal learning task was similarly sensitive to baclofen, the drug was administered prior to reversal learning test sessions. Analysis of performance during the reversal learning phase of the task revealed that rats learned the reversed discrimination problem under both baclofen and vehicle control conditions, as indicated by increased accuracy across blocks of trials [ $n = 7$ , Fig. 3A,  $F(11,66) = 35.55$ ,  $p < 0.001$ ]. Importantly, there was a main effect of drug condition, such that accuracy was greater under baclofen compared to vehicle conditions [ $F(1,6) = 26.43$ ,  $p < 0.001$ ], although there was no interaction between the two variables [ $F(11,66) = 1.45$ ,  $p = .25$ ]. Enhanced performance accuracy was also evident on the trials to criterion measure, which revealed that baclofen reduced the number of trials required to reach criterion performance [Fig. 3A1,  $t(6) = 3.16$ ,  $p = .02$ ]. Interestingly, inspection of the data showed that performance on the first block of 60 trials differed between baclofen and vehicle conditions, with accuracy being greater under baclofen than vehicle conditions [ $t(6) = 3.35$ ,  $p = .02$ ]. To determine whether this enhanced performance was present from the start of testing (vs. a consequence of more rapid learning), we compared performance between baclofen and vehicle conditions during the first block of 10 trials. This comparison (shaded region, block “0” in



**Fig. 3.** Effects of systemic baclofen on reversal learning. A) Baclofen enhanced accuracy of reversal learning compared to the vehicle control condition. A1) Baclofen produced a significant reduction in the number of trials required for rats to reach criterion performance on the reversed problem set. B) Baclofen did not affect performance accuracy on the constant problem set. In all graphs, error bars represent standard error of the mean (SEM).

Fig. 3A) revealed no difference between drug conditions [ $t(6) = 0.44$ ,  $p = 0.67$ ], suggesting that the enhanced performance under baclofen observed in the first block of 60 trials was not due to pre-existing or chance differences between conditions. In contrast to the enhanced performance on the reversed problem set, baclofen had no detectable effect on performance of the constant problem set [Fig. 3B, main effect of block,  $F(11,66) = 4.50$ ,  $p = .04$ ; main effect of drug,  $F(1,6) = 0.10$ ,  $p = 0.77$ , block  $\times$  drug interaction,  $F(11,66) = 0.70$ ,  $p = .55$ ], indicating that enhancements in reversal learning produced by baclofen were not due to impaired recall of the original reward contingencies (Beas et al., 2016).

Although acute systemic administration of baclofen effectively enhanced reversal learning, it also caused disruptions in a number of other measures of task performance (Table 1). Compared to vehicle conditions, baclofen caused a significant reduction in both the number of trials completed per session [ $t(6) = 10.58$ ,  $p < .001$ ] and locomotor activity [ $t(4) = 5.40$ ,  $p = .006$  – data from two rats were lost due to equipment malfunction], as well as an increase in latency to initiate trials [ $t(6) = 5.53$ ,  $p = .002$ ]. Considered together, these data show that acute systemic administration of baclofen effectively enhanced performance in the reversal learning task, but concurrently caused off-target

**Table 1**

Off-target behavioral measures (mean  $\pm$  SEM) recorded during the touchscreen reversal learning task. Comparisons of the number of trials per session, locomotor activity as measured by beam breaks, and trial initiation latency, between experimental (VNS or Drug) and control (No VNS or Vehicle) conditions. \* $p < .05$ ; \*\* $p < .01$ ; \*\*\* $p < .001$  compared to Vehicle or No VNS conditions.

Experimental Condition	Trials per Session	Locomotor Activity	Trial Initiation Latency
<i>Experiment 1: Baclofen</i>			
Drug	121.0 $\pm$ 10.8***	763.5 $\pm$ 68.49**	4.11 $\pm$ 1.13**
Vehicle	236.8 $\pm$ 7.8	1083.0 $\pm$ 58.27	1.53 $\pm$ 0.31
<i>Experiment 2.1: 30 Hz VNS on Reversed Set</i>			
VNS	156.7 $\pm$ 14.9	714.1 $\pm$ 98.52	3.54 $\pm$ 0.80
No VNS	151.3 $\pm$ 9.2	685.7 $\pm$ 93.85	3.24 $\pm$ 0.78
<i>Experiment 2.2: 30 Hz VNS on Constant Set</i>			
VNS	184.1 $\pm$ 9.7	883.9 $\pm$ 66.89	2.25 $\pm$ 0.20
No VNS	182.1 $\pm$ 6.9	914.6 $\pm$ 86.98	2.45 $\pm$ 0.38
<i>Experiment 2.3a: 10 Hz VNS on Reversed Set</i>			
VNS	171.4 $\pm$ 14.3*	839.3 $\pm$ 115.9*	3.14 $\pm$ 0.50*
No VNS	193.2 $\pm$ 11.6	925.7 $\pm$ 98.73	2.08 $\pm$ 0.27
<i>Experiment 2.3b: 50 Hz VNS on Reversed Set</i>			
VNS	218.5 $\pm$ 15.0	985.4 $\pm$ 68.7	1.90 $\pm$ 0.38
No VNS	223.5 $\pm$ 11.5	1053.0 $\pm$ 89.38	1.89 $\pm$ 0.13
<i>Experiment 3: Atomoxetine</i>			
Drug	142.4 $\pm$ 11.4***	636.8 $\pm$ 42.44**	3.37 $\pm$ 0.69*
Vehicle	202.0 $\pm$ 10.5	857.6 $\pm$ 74.18	1.90 $\pm$ 0.23

behavioral effects. Importantly, these effects of baclofen were similar to those in a set-shifting task, indicating a role for GABA(B) signaling across multiple forms of cognitive flexibility.

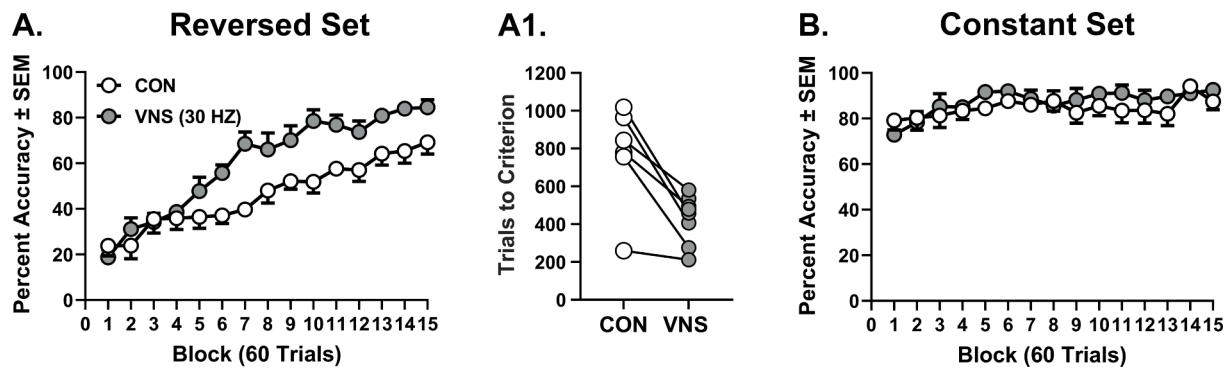
### 3.2. Experiment 2: Effects of vagus nerve stimulation on reversal learning

#### 3.2.1. Experiment 2.1: Effects of VNS delivered concurrently with the reversed problem

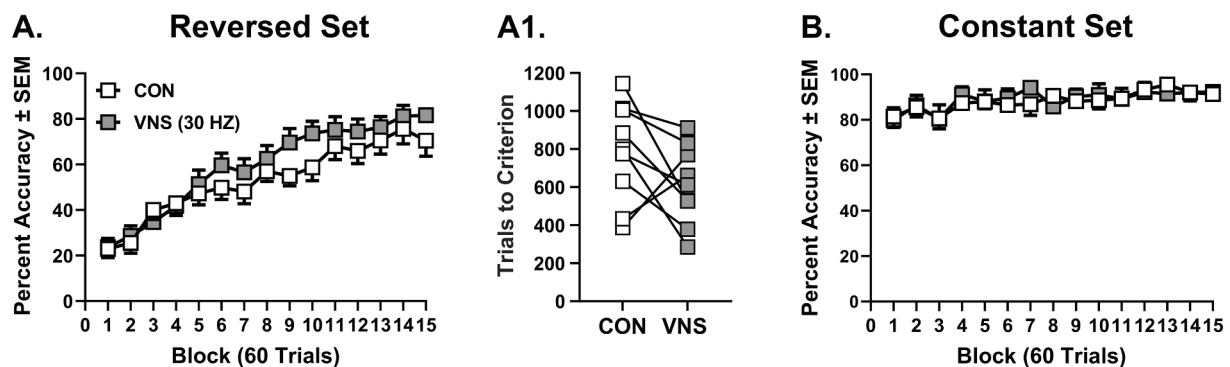
A within-subjects design was used to compare the effects of VNS (using parameters shown previously to enhance learning, (Buell et al., 2019; Buell et al., 2018)) to no-VNS control conditions. Under both VNS and no-VNS conditions, rats learned the reversed discrimination problem as indicated by increased accuracy across blocks of trials [ $n = 8$ , Fig. 4A,  $F(14,98) = 46.88$ ,  $p < .001$ ]. Importantly, there was both a main effect of VNS condition [ $F(1,7) = 9.37$ ,  $p = .02$ ], and an interaction between VNS condition and block [ $F(14,98) = 4.177$ ,  $p = .008$ ], indicating that VNS delivered concurrently with presentation of the reversed problem significantly enhanced reversal learning accuracy compared to control conditions. Enhanced performance on the reversed problem was also evident on the trials to criterion measure, which revealed that VNS significantly reduced the number of trials required to reach criterion performance [Fig. 4A1,  $t(7) = 5.25$ ,  $p = .001$ ]. As was the case with baclofen in Experiment 1, VNS delivered concurrently with the reversed problem had no effect on performance of the constant problem (Fig. 4B, main effect of block,  $F(14,98) = 4.15$ ,  $p = .01$ ; main effect of VNS condition,  $F(1,7) = 2.94$ ,  $p = .13$ ; block  $\times$  VNS condition interaction,  $F(14,98) = 0.87$ ,  $p = .47$ ). There were also no effects of VNS on measures of off-target effects (Table 1) including the number of trials completed per session [ $t(7) = 0.42$ ,  $p = .69$ ], locomotor activity [ $t(6) = 0.63$ ,  $p = .55$ ], and latency to initiate trials [ $t(7) = 1.14$ ,  $p = .29$ ], indicating that, unlike baclofen, the enhancing effects of VNS on reversal learning were not accompanied by motor or motivational alterations.

#### 3.2.2. Experiment 2.2: Effects of VNS delivered concurrently with the constant problem

To determine whether the timing of VNS delivery relative to the to-be-learned information is critical for its enhancing effects, a separate experiment examined the effects of 30 Hz VNS delivered concurrently with presentation of the constant problem set, on which rats already performed with a high degree of accuracy. In contrast to the enhanced accuracy on the reversed problem observed in Experiment 2.1, 30 Hz VNS delivered concurrently with presentation of the constant problem had no significant effects on reversal learning compared to the no-VNS control condition [Fig. 5A,  $n = 9$ , main effect of block,  $F(14,112) = 37.26$ ,  $p < .001$ ; main effect of VNS condition,  $F(1,8) = 1.64$ ,  $p = .24$ ; block  $\times$  VNS condition interaction,  $F(14,112) = 1.30$ ,  $p = .29$ ], nor was there an effect on the number of trials required to reach a criterion of 10



**Fig. 4.** Effects on reversal learning of 30 Hz VNS delivered during presentation of the reversed problem set. A) VNS (30 Hz) significantly enhanced reversal learning accuracy compared to no stimulation control (CON) conditions on the reversed problem set. A1) Under these conditions, VNS produced a significant reduction in the number of trials required to reach criterion performance. B) There was no effect of VNS on accuracy of the constant problem set. In all graphs, error bars represent standard error of the mean (SEM).



**Fig. 5.** Effects on reversal learning of 30 Hz VNS delivered during presentation of the constant problem set. A) VNS (30 Hz) administered concurrently with the constant problem set did not affect reversal learning accuracy compared to no stimulation control (CON) conditions. A1) Under these conditions, VNS had no effect on the number of trials to reach criterion performance. B) There was no effect of VNS on accuracy of the constant problem set. In all graphs, error bars represent standard error of the mean (SEM).

consecutive correct trials [Fig. 5A1,  $t(8) = 1.63$ ,  $p = .14$ ]. There was further no effect on accuracy on the constant problem [Fig. 5B, main effect of block,  $F(14,112) = 3.59$ ,  $p = .01$ ; main effect of VNS condition,  $F(1,8) = 0.08$ ,  $p = .78$ ; block  $\times$  VNS condition interaction,  $F(14,112) = 0.53$ ,  $p = .70$ ], indicating that VNS delivery does not affect recall or performance of well-learned discrimination problems. Considered together with the results of Experiment 2.1, these data suggest that the timing of VNS delivery relative to the to-be-learned information is critical for its enhancing effects on reversal learning. Moreover, as in Experiment 2.1, there were no differences between VNS and no-VNS conditions in the number of trials completed per session [ $t(8) = 0.22$ ,  $p = .83$ ], locomotor activity [ $t(8) = 0.76$ ,  $p = .47$ ], or latency to initiate trials [ $t(8) = 0.75$ ,  $p = .47$ ] (Table 1).

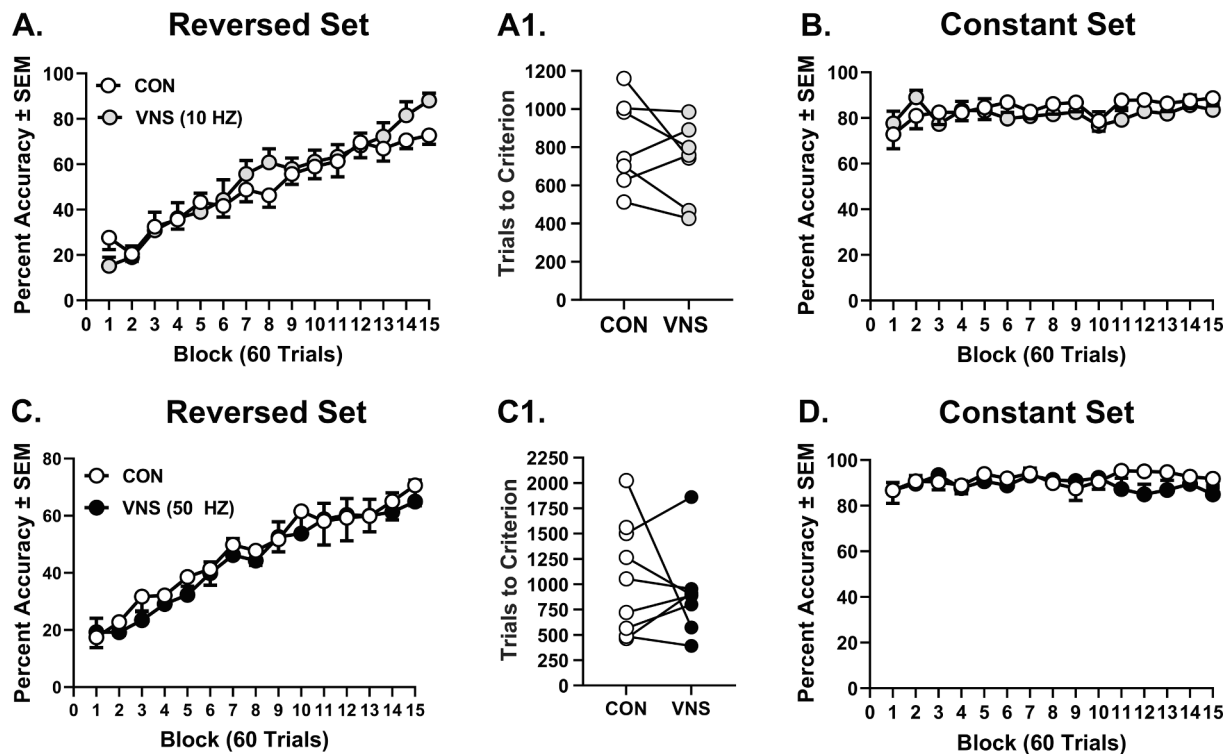
### 3.2.3. Experiment 2.3: Effects of different VNS frequencies on reversal learning

Previous studies have suggested that 30 Hz is an optimal frequency for the enhancing effects of VNS on cortical plasticity and motor skill learning (Buell et al., 2018; Engineer et al., 2011; Loerwald et al., 2018; Peña et al., 2013; Porter et al., 2012). To determine whether this frequency-dependent effect of VNS is also evident with reversal learning, separate experiments were conducted to evaluate VNS at lower (10 Hz) and higher (50 Hz) frequencies. The design of these experiments was identical to that in Experiment 2.1, with VNS administered concurrently during presentation of the reversed problem set.

In contrast to the enhancing effects of VNS delivered at 30 Hz, VNS at 10 Hz had no effect on accuracy of the reversed problem [Fig. 6A,  $n = 7$ ; main effect of block,  $F(14,84) = 40.69$ ,  $p < .001$ ; main effect of VNS

condition,  $F(1,6) = 0.39$ ,  $p = .56$ ; block  $\times$  VNS condition interaction,  $F(14,84) = 1.66$ ,  $p = .21$ ], nor were there effects of VNS on the number of trials required to reach criterion [Fig. 6A1,  $t(6) = 1.23$ ,  $p = .27$ ]. There were also no effects of 10 Hz VNS on performance of the constant problem set [Fig. 6B, main effect of block,  $F(14,84) = 1.72$ ,  $p = .18$ ; main effect of VNS condition,  $F(1,6) = 4.91$ ,  $p = .07$ ; block  $\times$  VNS condition interaction,  $F(14,84) = 0.77$ ,  $p = .54$ ]. Despite the absence of effects on discrimination performance, however, VNS at 10 Hz caused small but significant decreases in the number of trials completed [ $t(6) = 3.32$ ,  $p = .02$ ] and locomotor activity [ $t(6) = 3.31$ ,  $p = .02$ ], as well as a significant increase in latency to initiate trials [ $t(6) = 3.09$ ,  $p = .02$ ] (Table 1).

Similar to 10 Hz VNS, 50 Hz VNS delivered concurrently with the reversed problem also failed to affect reversal learning accuracy compared to no-VNS conditions (Fig. 6C,  $n = 9$ , main effect of block,  $F(14,112) = 23.83$ ,  $p < .001$ ; main effect of VNS condition,  $F(1,8) = 0.28$ ,  $p = .61$ ; block  $\times$  VNS condition interaction,  $F(14,112) = 0.59$ ,  $p = .72$ ], nor were there effects of VNS on the number of trials required to reach criterion [Fig. 6C1,  $t(8) = 0.02$ ,  $p = .99$ ]. There were also no effects of 50 Hz VNS on performance of the constant problem set [Fig. 6D, main effect of block,  $F(14,112) = 0.94$ ,  $p = .46$ ; main effect of VNS condition,  $F(1,8) = 1.25$ ,  $p = .30$ ; block  $\times$  VNS condition interaction,  $F(14,112) = 1.454$ ,  $p = .21$ ], nor were there differences between VNS and no-VNS conditions in the number of trials completed per session [ $t(8) = 0.52$ ,  $p = .61$ ], locomotor activity [ $t(8) = 1.35$ ,  $p = .21$ ], or latency to initiate trials [ $t(8) = 0.02$ ,  $p = .99$ ] (Table 1). Considered together, the results from experiments utilizing both 10 Hz and 50 Hz VNS parameters suggest that VNS frequency is a critical factor for its enhancing effects on



**Fig. 6.** Effects on reversal learning of 10 and 50 Hz VNS delivered during presentation of the reversed problem set. There were no effects of VNS compared to control (CON) conditions on the measures of reversal learning when delivered at either 10 Hz (A, A1) or 50 Hz (C, C1). There were similarly no effects of VNS on performance of the constant problem set when delivered at either 10 Hz (B) or 50 Hz (D). In all graphs, error bars represent standard error of the mean (SEM).

reversal learning, and that, as observed with other aspects of cognition and neural plasticity, 30 Hz is optimal.

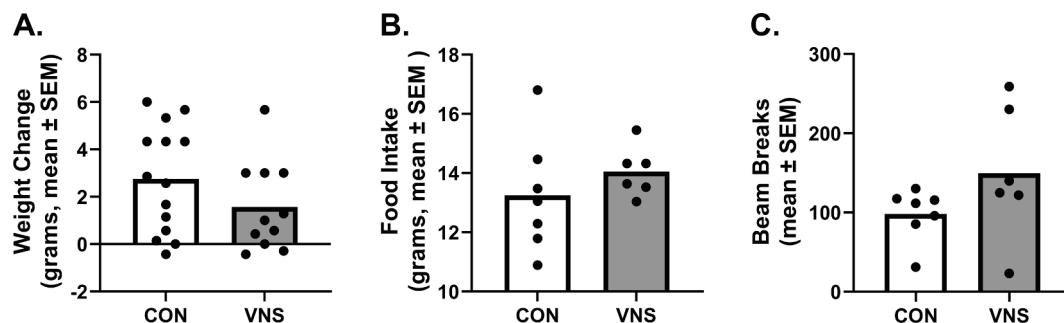
### 3.2.4. Experiment 2.4: Evaluation of “off-target” effects of repeated VNS

This experiment was designed to determine whether a VNS regimen (at 30 Hz) that modeled the maximum exposure experienced in the reversal learning task (8 daily sessions, 100 VNS trains/day) had effects on off-target measures beyond those assessed during task performance. Comparisons between VNS and no-VNS groups using unpaired t-tests revealed no differences in body weight change (Fig. 7A,  $t(23) = 1.40$ ,  $p = .18$ ), food intake (Fig. 7B,  $t(11) = 0.93$ ,  $p = .37$ ), or locomotor activity as measured by photobeam breaks in the chambers during VNS or no-VNS sessions (Fig. 7C,  $t(11) = 1.49$ ,  $p = 0.16$ ). Thus, a daily VNS regimen that produced a significant improvement in cognitive flexibility did not produce notable off-target physiological or behavioral effects.

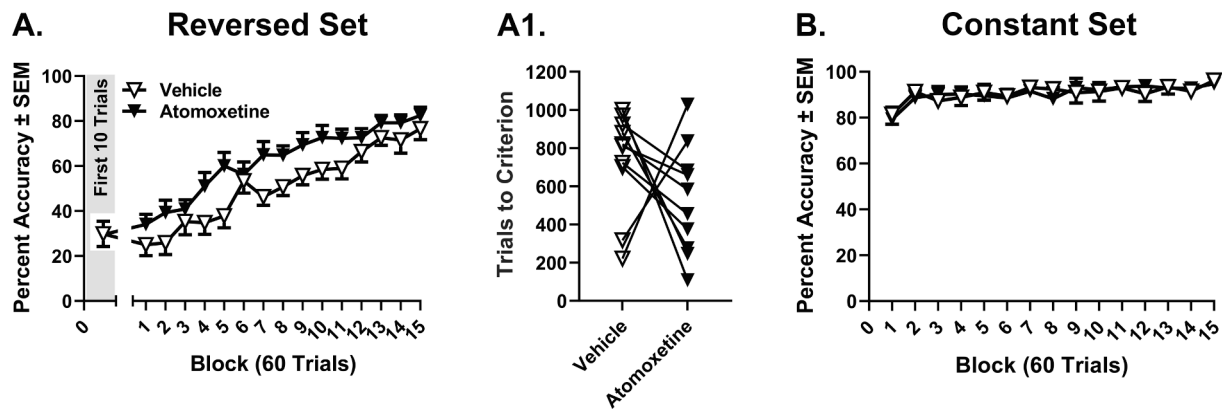
### 3.3. Experiment 3: Atomoxetine effects on reversal learning

Experiment 3 evaluated whether pharmacological enhancement of

central norepinephrine (which is stimulated by VNS and critical for some of the neuroplasticity-enhancing effects of VNS (Hulsey, Shedd, Sarker, Kilgard, & Hays, 2019)) enhanced performance on the reversal learning task. As with Experiment 1, atomoxetine was administered prior to the reversal phase of the task, in a randomized order across rats. A two-factor, repeated measures ANOVA revealed that rats learned the reversed discrimination problem under both atomoxetine and vehicle conditions [Fig. 8A,  $n = 10$ ,  $F(14,126) = 34.30$ ,  $p < .001$ ]. Importantly, there was a main effect of drug condition, such that accuracy was greater under atomoxetine than vehicle conditions [ $F(1,9) = 10.65$ ,  $p = .01$ ], although the interaction between trial block and drug condition did not reach statistical significance [ $F(14,126) = 2.01$ ,  $p = .10$ ]. In addition, the trials to criterion measure did not differ between drug conditions [Fig. 8A1,  $t(9) = 1.28$ ,  $p = .23$ ]. As with baclofen, performance in the first block of trials differed between atomoxetine and vehicle conditions [ $t(9) = 2.67$ ,  $p = .03$ ], raising the possibility of pre-existing differences between drug conditions. To determine whether this enhanced performance was present from the start of testing, we compared performance between atomoxetine and vehicle conditions during the first 10 trials.



**Fig. 7.** Effects of 30 Hz VNS on off-target measures. Compared to no-VNS control (CON) conditions, delivery of 30 Hz VNS for 8 consecutive days (1 h/day) had no effect on body weight (A), food intake (B), or locomotor activity (C). In all graphs, circles represent values for individual rats and bars represent group means.



**Fig. 8.** Effects of systemic atomoxetine on reversal learning. A) Atomoxetine enhanced accuracy of reversal learning compared to the vehicle control condition. A1) Atomoxetine had no significant effect on the number of trials required to reach criterion performance. B) Atomoxetine did not affect performance accuracy on the constant problem set. In all graphs, error bars represent standard error of the mean (SEM).

This comparison (shaded region, block “0” in Fig. 8A) revealed no difference between drug conditions [ $t(9) = 0.05$ ,  $p = 0.96$ ], suggesting that the enhanced performance under atomoxetine conditions observed in the first block of 60 trials was due to more rapid learning. In contrast to the enhanced performance on the reversed problem, atomoxetine did not affect accuracy on the constant problem [Fig. 8B, main effect of block,  $F(14,126) = 3.87$ ,  $p = .01$ ; main effect of drug,  $F(1,9) = 0.01$ ,  $p = 0.98$ ; block  $\times$  drug interaction,  $F(14,126) = 0.48$ ,  $p = .73$ ], indicating that enhancements in reversal learning produced by atomoxetine did not come at the expense of well-learned performance. Finally, similar to baclofen, atomoxetine decreased both the number of trials completed per session [ $t(9) = 5.65$ ,  $p < 0.001$ ] and locomotor activity [ $t(9) = 3.98$ ,  $p = 0.003$ ], and increased trial initiation latencies [ $t(9) = 2.58$ ,  $p = .03$ ] (Table 1). These data are consistent with the idea that VNS (at 30 Hz) enhances reversal learning through activation of noradrenergic signaling, although unlike VNS, atomoxetine produced significant off-target motor/motivational effects.

#### 4. Discussion

VNS has been shown previously to produce beneficial effects on neuroplasticity and cognition in a variety of contexts and species. To determine whether VNS is similarly effective for enhancing cognitive flexibility, we used a novel behavioral task design in rats to show that VNS enhances reversal learning in a manner dependent on both the timing of its delivery and the frequency of stimulation. This enhancement was evident without adverse, off-target effects, in contrast to several pharmacological manipulations (the GABA(B) receptor agonist baclofen and the selective norepinephrine reuptake inhibitor atomoxetine) that facilitated reversal learning but also caused motor and/or motivational deficits. These results expand the range of learning tasks in which VNS improves performance and support the utility of VNS for cognitive enhancement.

##### 4.1. A novel reversal learning task suitable for within-subjects experimental designs

Reversal learning is a form of cognitive flexibility in which a previously-learned set of stimulus-outcome or response-outcome contingencies is switched, and a subject must learn the new set of contingencies (e.g., A+, B- becomes A-, B+). Rats are adept at reversal learning, but most cognitive testing in rodents is conducted with a limited set of stimuli, such as auditory cues, response levers, or arms of a maze (Ghods-Sharifi, Haluk, & Floresco, 2008; Grospe, Baker, & Ragozzino, 2018; Hernandez et al., 2020; Hergiv, Piilgaard, Božič, Alsiö, & Robbins, 2020; Jenni, Li, & Floresco, 2021; McAlonan & Brown,

2003). Touchscreen operant chambers allow for an effectively unlimited number of visual stimuli, which facilitates experimental designs involving repeated testing of the same subjects (Brigman et al., 2010; Savolainen, Ihalainen, Hämäläinen, Tanila, & Forsberg, 2021) (note that olfactory stimuli can also be used for such designs (e.g., (Schoenbaum, Setlow, Nugent, Saddoris, & Gallagher, 2003; Setlow, Schoenbaum, & Gallagher, 2003)). Having numerous stimuli enables within-subjects comparisons of learning under different circumstances, such as different drug (Experiments 1 and 3) or VNS (Experiments 2.1–2.3) conditions, which both increases statistical power and reduces the number of subjects required. In the context of the reversal learning task used here, the availability of multiple stimuli also allows within-session comparisons of the effects of drugs or VNS on learning vs. well-learned performance. In theory, better performance on a reversal learning problem could arise from either enhanced cognitive flexibility or impaired recall of the original contingencies (see (Beas et al., 2016) for further discussion of this issue). The “constant problem” presented alongside the reversed problem (in which the originally learned contingencies were presented unchanged) provides a within-session test of the effects of drugs and VNS on recall. As performance on the constant problem was unaffected by either pharmacological or VNS manipulations, it seems unlikely that impaired recall accounts for their enhancing effects on reversal learning.

Previous work from our labs showed that acute administration of baclofen enhances rats’ cognitive flexibility in a set-shifting task that requires a shift in attention from one response rule to another (Beas, McQuail, Banuelos, Setlow, & Bizon, 2017; Beas, Setlow, & Bizon, 2016; Floresco, Block, & Tse, 2008). Baclofen had a similar enhancing effect on reversal learning in the present study, suggesting that activation of GABA(B) receptors broadly facilitates cognitive flexibility (indeed, in the set-shifting task, greater prefrontal cortical GABA(B) receptor expression is associated with better performance (Beas, McQuail, Banuelos, Setlow, & Bizon, 2017)). In addition, the current data demonstrate that the novel reversal learning task is suitable for within-subjects experimental designs, and that there is sufficient parametric space in which to detect enhanced performance, rendering it a useful context in which to assess effects of VNS.

##### 4.2. Effects of VNS on reversal learning

VNS can exert a range of physiological and behavioral effects, including facilitation of learning and plasticity across multiple contexts and species. In the present study, VNS enhanced performance on the reversal learning task, but only when delivered in conjunction with the to-be-learned (reversed) discrimination problem, and not in conjunction with the well-learned (constant) discrimination problem. The fact that

the efficacy of VNS for enhancing reversal learning depended upon the timing of its delivery is consistent with some previous work showing that VNS most effectively facilitates sensory/motor plasticity when delivered concurrently with an event such as an auditory cue or a specific limb movement (Engineer et al., 2011; Hays et al., 2014; Khodaparast et al., 2016). Such temporal specificity is certainly not necessary for all effects of VNS on behavioral outcomes, however. For example, VNS attenuates reinstatement of cocaine-seeking in rats, whether it is delivered contingent or non-contingent upon lever presses during extinction training (Childs, DeLeon, Nickel, & Kroener, 2017). Acute VNS delivery can also produce anxiolytic effects (Mathew et al., 2020), although there is no evidence that VNS alone is rewarding or reinforcing (Noble, Chuah, Callahan, Souza, & McIntyre, 2019), suggesting that its enhancing effects on reversal learning in the present study were not secondary to changes in appetitive or motivational properties of the stimuli used in the task.

In addition to depending upon the timing of its delivery, the enhancing effects of VNS on reversal learning were also frequency specific. The 30 Hz frequency that was effective in the reversal learning task is based on data showing that it is more effective than higher or lower frequencies for facilitating sensory cortical plasticity (Buell et al., 2018, 2019), as well as on data indicating that this frequency range is optimal for suppression of experimentally-induced seizures, recovery of function after injury, and enhancement of memory consolidation (Sanders et al., 2019; Smith et al., 2005; Zabara, 1992). The reasons that VNS at 30 Hz but not at 10 or 50 Hz enhanced reversal learning are not entirely clear, but may be related to its ability to activate ascending neuromodulatory systems, including norepinephrine, acetylcholine, and serotonin (Détári, Juhász, & Kukorelli, 1983; Hassert et al., 2004; Manta, Dong, Debonnel, & Blier, 2009; Morais et al., 2020; Roosevelt et al., 2006). In particular, VNS activates neurons of the locus coeruleus in a frequency-dependent manner, such that higher frequencies elicit more firing (Hulsey et al., 2017). Although this relationship is monotonic over the frequency range tested, the relationship between forebrain noradrenergic signaling and enhancements in neuroplasticity and cognition follows an inverted U-shaped function, such that only moderate levels of noradrenergic activity are beneficial (Datta et al., 2019; Giustino & Maren, 2018; Introini-Collison, Castellano, & McGaugh, 1994). In addition, the facilitating effects of VNS on cortical plasticity are dependent upon central noradrenergic (as well as cholinergic) signaling (Hulsey et al., 2019; Hulsey et al., 2016), suggesting a critical role for these ascending neuromodulatory systems in the cognitively enhancing effects of VNS. In support of such a role, Experiment 3 showed that acute administration of atomoxetine enhanced reversal learning in a manner similar to VNS, indicating that an increase in norepinephrine availability is sufficient to facilitate cognitive flexibility.

VNS has been used clinically for decades to attenuate seizure activity (Dibué-Adjei, Kamp, et al., 2019), and some studies suggest that VNS increases GABA availability and facilitates inhibitory neurotransmission (Ben-Menachem et al., 1995; Marrosu et al., 2003; Zhang, 2002). Given that activation of GABA(B) receptors by baclofen (either systemically or within prefrontal cortex) produces enhancements in behavioral flexibility (Experiment 1 in this study and as reported previously (Beas, McQuail, Banuelos, Setlow, & Bizon, 2017; Beas, Setlow, & Bizon, 2016)), it is possible that increases in GABAergic transmission are responsible for the enhancing effects of VNS on reversal learning. Stimulation of norepinephrine release (or application of adrenoceptor agonists) also facilitates inhibitory transmission and GABA release, including in prefrontal cortex (Luo, Zheng, Sun, & Tang, 2017; Toussay, Basu, Lacoste, & Hamel, 2013; Wang et al., 2011). Hence, it is possible that both VNS and atomoxetine enhance reversal learning through a common mechanism of increased GABAergic transmission.

Despite the fact that all three manipulations (Experiments 1, 2.1, and 3) enhanced reversal learning, there was a notable difference in the pattern of enhancement between the drugs and VNS. Whereas enhancing effects of VNS delivered during the reversed problems were

only evident after several blocks of trials, enhancing effects of baclofen and atomoxetine were present within the first block of 60 trials (although not within the first 10 trials). There are several possibilities that could account for this difference in the patterns of facilitated reversal learning. One is that the actions of the drugs across all trials (both reversed and constant) enhanced the distinction between the two trial types, which facilitated learning about the altered contingencies on the reversed problem. A second possibility (not mutually exclusive with the first) is that administration of the drugs 15 and 30 min prior to the test session enabled rapid actions on cognitive performance, whereas the effects of VNS on brain neurochemistry may take longer (and/or more trials) to reach an effective threshold. Finally, given that the effects of VNS on reversal learning emerged only on blocks of trials after the first test session, it is possible that its actions were limited to memory consolidation processes that took time to emerge, whereas the drugs acted more directly on learning processes (instead of, or in addition to, consolidation). This explanation would be consistent with evidence across species that VNS can enhance memory through actions specifically on consolidation (Clark et al., 1999; Clark, Krahl, Smith, & Jensen, 1995). The fact that VNS delivered during the constant problem failed to significantly enhance reversal learning, however, could argue against this possibility, as the actions of VNS (or other manipulations that enhance memory consolidation) are not usually viewed as having this degree of temporal specificity. Future studies that explicitly assess the effects of VNS on learning/performance vs. consolidation will be useful for addressing these issues.

In the present studies, the effects of VNS were tested only during the reversal learning phase of the task. It is unclear, however, whether the enhancing effects of VNS on task performance were specific to reversal of the previously learned discrimination problems (i.e., enhanced cognitive flexibility), or whether they influenced learning processes more generally, which facilitated acquisition of the new (reversed) stimulus-outcome contingencies. In support of the former possibility, VNS enhances extinction of previously learned cue-shock associations (fear conditioning), which can be viewed as a form of cognitive flexibility (Noble, Meruva, et al., 2019; Peña et al., 2013). In addition, acute baclofen administration (which had effects on reversal learning similar to those of VNS) enhances performance during the “shift” phase of a set-shifting task, but has no effect on discrimination learning prior to the shift (Beas et al., 2016). In contrast, VNS can enhance performance across a wide range of learning tasks in both rodents and humans (Clark et al., 1995; Noble et al., 2017; Sanders et al., 2019; Thakkar, Engelhart, Khodaparast, Abadzi, & Centanni, 2020; Vázquez-Oliver et al., 2020). Future studies will be required to evaluate whether VNS similarly enhances initial acquisition of the pairwise visual discrimination problems used in the reversal learning task.

A limitation of this study is that the effects of VNS (as well as baclofen and atomoxetine) on reversal learning were only evaluated in male rats. There is evidence across species for sex differences in reversal learning (e.g., (Aguirre et al., 2020; Bissonette, Lande, Martins, & Powell, 2012; Evans & Hampson, 2015; LaClair et al., 2019; Westbrook, Hankosky, Dwyer, & Gulley, 2018), but data are mixed as to the nature of these differences (e.g., whether males or females learn reversals more rapidly). There is also evidence for sex differences in vagus nerve anatomy and function (e.g., (Li et al., 2015; Moriyama, Hayashi, Inoue, Itoh, & Otsuka, 2016; Yaghouby, Jang, Hoang, Asgari, & Vasudevan, 2020), as well as in ascending neuromodulatory systems activated by VNS (Bangasser, Zhang, Garachh, Hanhauser, & Valentino, 2011; Luque, de Blas, Segovia, & Guillamón, 1992; Takase, Kimura, Yagami, & Mitsuhashi, 2009). To our knowledge, there has been only one side-by-side comparison in males and females of the effects of VNS on neuroplasticity or cognition, which found no sex difference in the efficacy of VNS for promoting motor cortical reorganization (Tseng, Brougher, Gauding, Hassan, & Thorn, 2020). Hence, although it is difficult to predict how sex would influence the effects of VNS on cognitive performance, this will be an important avenue for future research given the robust

evidence for qualitative and quantitative sex differences in a range of neurocognitive variables (Shansky & Murphy, 2021).

#### 4.3. Non-cognitive effects of VNS

The primary focus of these studies was on performance accuracy in the reversal learning task, but several additional measures were assessed concurrently, including the number of trials completed, latency to initiate trials, and locomotor activity within the operant chamber. Both baclofen and atomoxetine had effects on these measures consistent with motor and/or motivational deficits, including a decrease in locomotor activity and the number of completed trials, and an increase in trial initiation latency. In contrast, only 10 Hz VNS, which did not affect reversal learning, affected these “off-target” measures, in the same direction as baclofen and atomoxetine. In combination, these data suggest that motor/motivational effects of the drugs and 30 Hz VNS were broadly independent of their effects on reversal learning accuracy, and that VNS might yield cognitive benefits with fewer off-target effects than pharmacological approaches. Experiment 2.4 investigated additional potential off-target effects using a 30 Hz VNS regimen that matched that used during the reversal learning task, but outside the context of cognitive performance. As during the task, there were no effects on locomotor activity, nor did the VNS regimen produce alterations in body weight or food intake, which can be affected by VNS under some conditions (Madden, Santos da Conceicao, & Morrison, 2017; Pardo et al., 2008; Pavlov & Tracey, 2012; Székely, 2000; Vijgen et al., 2013).

#### 4.4. Potential therapeutic implications

Impairments in cognitive and behavioral flexibility accompany a range of neuropsychiatric disorders, including schizophrenia, substance use disorders, anorexia nervosa, and autism spectrum disorders. As such, approaches that remediate such impairments could be useful for treating these disorders. Several of these disorders are also accompanied by dysregulated GABAergic signaling, and in some cases, strategies that facilitate inhibitory signaling have shown therapeutic promise (Silverman et al., 2015; Stoppel et al., 2018; Veenstra-VanderWeele et al., 2017). The current results suggest that VNS might provide utility in these conditions as well, and potentially without the side-effect profile that can accompany pharmacological approaches. Implantable VNS devices have been used in almost 100,000 patients to date, and they have an excellent safety profile (Aaronson & Conway, 2018; Dibué-Adjei, Kamp, et al., 2019); however, VNS can also be applied non-invasively (transcutaneously), which further increases its accessibility (Yap et al., 2020). The fact that VNS can also have anti-inflammatory effects (Pavlov & Tracey, 2012) is an additional potential benefit, as elevated inflammatory markers can accompany neuropsychiatric disorders characterized by cognitive inflexibility and GABAergic dysregulation (Adams, Conigrave, Lewohl, Haber, & Morley, 2020; Comer, Carrier, Tremblay, & Cruz-Martín, 2020; Liu et al., 2020; Matta, Hill-Yardin, & Crack, 2019).

#### 4.5. Jim McGaugh, the vagus nerve, and cognition

This special issue of *Neurobiology of Learning and Memory* is dedicated to the legacy of Dr. Jim McGaugh, who has made pioneering contributions to our understanding of memory and memory consolidation (2004; LaLumiere, McGaugh, & McIntyre, 2017; McGaugh, 1973). Jim and his colleagues found that the vagus nerve and its projections to the nucleus of the solitary tract are an important route through which peripheral hormones induced by emotional arousal act to enhance memory consolidation (Gold & Van Buskirk, 1975; Gold, van Buskirk, & McGaugh, 1975; Miyashita & Williams, 2006; Nogueira, Tomaz, & Williams, 1994; Williams & McGaugh, 1993). These results formed the basis for subsequent work by Jim’s former postdoc Rob Jensen, who found that VNS can enhance memory consolidation in both rodents

(Clark et al., 1995, 1998) and humans (Clark et al., 1999). Those of us who conduct research on the vagus nerve and behavior owe a debt of gratitude to Jim and his work on the central role of the peripheral nervous system in cognition.

#### CRedit authorship contribution statement

**Lindsay K.-P. Altidor:** Investigation, Writing – original draft. **Matthew M. Bruner:** Investigation, Supervision, Formal analysis. **Josue F. Deslauriers:** Investigation, Writing – original draft. **Tyler S. Garman:** Investigation, Formal analysis. **Saúl Ramirez:** Investigation. **Elliott W. Dirr:** Investigation, Methodology, Validation. **Kaitlynn P. Olczak:** Investigation, Methodology, Validation. **Andrew P. Maurer:** Conceptualization. **Damon G. Lamb:** Conceptualization, Writing - review & editing. **Kevin J. Otto:** Conceptualization, Project administration, Writing - review & editing. **Sara N. Burke:** Conceptualization, Project administration. **Argyle V. Bumanglag:** Writing – original draft, Writing - review & editing. **Barry Setlow:** Conceptualization, Supervision, Project administration, Writing – original draft, Writing - review & editing. **Jennifer L. Bizon:** Conceptualization, Supervision, Project administration, Writing - review & editing.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

Supported by the Defense Advanced Research Projects Agency (DARPA) BTO under the auspices of Dr. Douglas Weber and Dr. Tristan McClure-Begley through the DARPA Contracts Management Office Grant No. HR0011-17-2-0019, and by the McKnight Brain Research Foundation to JLB. We thank Dr. Erica Dale for illustrations used in the manuscript, Ms. Bonnie McLaurin for conducting surgeries, and Ms. Vicky Kelley, Ms. Alyssa Finner, and Ms. Debora Calderon for assistance with behavioral testing.

#### References

- Aaronson, S. T., & Conway, C. R. (2018). Vagus nerve stimulation: Changing the paradigm for chronic severe depression? *Psychiatric Clinics of North America*, *41*, 409–418.
- Adams, C., Conigrave, J. H., Lewohl, J., Haber, P., & Morley, K. C. (2020). Alcohol use disorder and circulating cytokines: A systematic review and meta-analysis. *Brain, Behavior, and Immunity*, *89*, 501–512.
- Aguirre, C. G., Stolyarova, A., Das, K., Kolli, S., Marty, V., Ray, L., ... Izquierdo, A. (2020). Sex-dependent effects of chronic intermittent voluntary alcohol consumption on attentional, not motivational, measures during probabilistic learning and reversal. *PLoS ONE*, *15*, Article e0234729.
- Arnsten, A. F. (2011). Catecholamine influences on dorsolateral prefrontal cortical networks. *Biological Psychiatry*, *69*, e89–e99.
- Aston-Jones, G., & Waterhouse, B. (2016). Locus coeruleus: From global projection system to adaptive regulation of behavior. *Brain Research*, *1645*, 75–78.
- Bangasser, D. A., Zhang, X., Garachh, V., Hanhauser, E., & Valentino, R. J. (2011). Sexual dimorphism in locus coeruleus dendritic morphology: A structural basis for sex differences in emotional arousal. *Physiology & Behavior*, *103*, 342–351.
- Beas, B. S., McQuail, J. A., Bañuelos, C., Setlow, B., & Bizon, J. L. (2017). Prefrontal cortical GABAergic signaling and impaired behavioral flexibility in aged F344 rats. *Neuroscience*, *345*, 274–286.
- Beas, B. S., Setlow, B., & Bizon, J. L. (2013). Distinct manifestations of executive dysfunction in aged rats. *Neurobiology of Aging*, *34*, 2164–2174.
- Beas, B. S., Setlow, B., & Bizon, J. L. (2016). Effects of acute administration of the GABA (B) receptor agonist baclofen on behavioral flexibility in rats. *Psychopharmacology (Berl)*, *233*, 2787–2797.
- Ben-Menachem, E., Hamberger, A., Hedner, T., Hammond, E. J., Uthman, B. M., Slater, J., ... Wernicke, J. F. (1995). Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures. *Epilepsy Research*, *20*, 221–227.
- Birrell, J. M., & Brown, V. J. (2000). Medial frontal cortex mediates perceptual attentional set shifting in the rat. *Journal of Neuroscience*, *20*, 4320–4324.

- Bissonette, G. B., Lande, M. D., Martins, G. J., & Powell, E. M. (2012). Versatility of the mouse reversal/set-shifting test: Effects of topiramate and sex. *Physiology & Behavior*, *107*, 781–786.
- Bissonette, G. B., Martins, G. J., Franz, T. M., Harper, E. S., Schoenbaum, G., & Powell, E. M. (2008). Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. *Journal of Neuroscience*, *28*, 11124–11130.
- Bizon, J. L., Foster, T. C., Alexander, G. E., & Glisky, E. L. (2012). Characterizing cognitive aging of working memory and executive function in animal models. *Frontiers in Aging Neuroscience*, *4*, 19.
- Borodovitsyna, O., Flamini, M., & Chandler, D. (2017). Noradrenergic modulation of cognition in health and disease. *Neural Plast*, *2017*, 6031478.
- Brigman, J. L., Mathur, P., Harvey-White, J., Izquierdo, A., Saksida, L. M., Bussey, T. J., ... Holmes, A. (2010). Pharmacological or genetic inactivation of the serotonin transporter improves reversal learning in mice. *Cerebral Cortex*, *20*, 1955–1963.
- Buell, E. P., Borland, M. S., Loerwald, K. W., Chandler, C., Hays, S. A., Engineer, C. T., & Kilgard, M. P. (2019). Vagus nerve stimulation rate and duration determine whether sensory pairing produces neural plasticity. *Neuroscience*, *406*, 290–299.
- Buell, E. P., Loerwald, K. W., Engineer, C. T., Borland, M. S., Buell, J. M., Kelly, C. A., ... Kilgard, M. P. (2018). Cortical map plasticity as a function of vagus nerve stimulation rate. *Brain Stimulation*, *11*, 1218–1224.
- Cardinal, R. N., & Aitken, M. R. (2010). Whisker: a client-server high-performance multimedia research control system. *Behav Res Methods*, *42*(4), 1059–1071.
- Cain, R. E., Wasserman, M. C., Waterhouse, B. D., & McGaugh, J. A. (2011). Atomoxetine facilitates attentional set shifting in adolescent rats. *Developmental Cognitive Neuroscience*, *1*, 552–559.
- Chamberlain, S. R., & Robbins, T. W. (2013). Noradrenergic modulation of cognition: Therapeutic implications. *Journal of Psychopharmacology*, *27*, 694–718.
- Chandler, D., & Waterhouse, B. D. (2012). Evidence for broad versus segregated projections from cholinergic and noradrenergic nuclei to functionally and anatomically discrete subregions of prefrontal cortex. *Frontiers in Behavioral Neuroscience*, *6*, 20.
- Chandler, D. J., Gao, W. J., & Waterhouse, B. D. (2014). Heterogeneous organization of the locus coeruleus projections to prefrontal and motor cortices. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 6816–6821.
- Chandler, D. J., Lamperski, C. S., & Waterhouse, B. D. (2013). Identification and distribution of projections from monoaminergic and cholinergic nuclei to functionally differentiated subregions of prefrontal cortex. *Brain Research*, *1522*, 38–58.
- Childs, J. E., DeLeon, J., Nickel, E., & Kroener, S. (2017). Vagus nerve stimulation reduces cocaine seeking and alters plasticity in the extinction network. *Learning and Memory*, *24*, 35–42.
- Clark, K. B., Krahl, S. E., Smith, D. C., & Jensen, R. A. (1995). Post-training unilateral vagal stimulation enhances retention performance in the rat. *Neurobiology of Learning and Memory*, *63*, 213–216.
- Clark, K. B., Naritoku, D. K., Smith, D. C., Browning, R. A., & Jensen, R. A. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nature Neuroscience*, *2*, 94–98.
- Clark, K. B., Smith, D. C., Hassert, D. L., Browning, R. A., Naritoku, D. K., & Jensen, R. A. (1998). Posttraining electrical stimulation of vagal afferents with concomitant vagal efferent inactivation enhances memory storage processes in the rat. *Neurobiology of Learning and Memory*, *70*, 364–373.
- Comer, A. L., Carrier, M., Tremblay, M., & Cruz-Martín, A. (2020). The inflamed brain in schizophrenia: The convergence of genetic and environmental risk factors that lead to uncontrolled neuroinflammation. *Frontiers in Cellular Neuroscience*, *14*, 274.
- Cope, Z. A., Vazey, E. M., Floresco, S. B., & Aston Jones, G. S. (2019). DREADD-mediated modulation of locus coeruleus inputs to mPFC improves strategy set-shifting. *Neurobiology of Learning and Memory*, *161*, 1–11.
- Datta, D., Yang, S. T., Galvin, V. C., Solder, J., Luo, F., Morozov, Y. M., ... Wang, M. (2019). Noradrenergic  $\alpha$ -adrenoceptor actions in the primate dorsolateral prefrontal cortex. *Journal of Neuroscience*, *39*, 2722–2734.
- Desbèances Jodoin, V., Richer, F., Miron, J. P., Fournier-Gosselin, M. P., & Lespérance, P. (2018). Long-term sustained cognitive benefits of vagus nerve stimulation in refractory depression. *J ECT*, *34*, 283–290.
- Dias, R., Robbins, T. W., & Roberts, A. C. (1996). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature*, *380*, 69–72.
- Dibué-Adjei, M., Brigo, F., Yamamoto, T., Vonck, K., & Trinka, E. (2019a). Vagus nerve stimulation in refractory and super-refractory status epilepticus - A systematic review. *Brain Stimulation*, *12*, 1101–1110.
- Dibué-Adjei, M., Kamp, M. A., & Vonck, K. (2019b). 30 years of vagus nerve stimulation trials in epilepsy: Do we need neuromodulation-specific trial designs? *Epilepsy Research*, *153*, 71–75.
- Détári, L., Juhász, G., & Kukorelli, T. (1983). Effect of stimulation of vagal and radial nerves on neuronal activity in the basal forebrain area of anaesthetized cats. *Acta Physiologica Hungarica*, *61*, 147–154.
- Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., ... Kilgard, M. P. (2011). Reversing pathological neural activity using targeted plasticity. *Nature*, *470*, 101–104.
- Evans, K. L., & Hampson, E. (2015). Sex differences on prefrontally-dependent cognitive tasks. *Brain and Cognition*, *93*, 42–53.
- Floresco, S. B., Block, A. E., & Tse, M. T. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural Brain Research*, *190*, 85–96.
- Floresco, S. B., & Jentsch, J. D. (2011). Pharmacological enhancement of memory and executive functioning in laboratory animals. *Neuropsychopharmacology*, *36*, 227–250.
- Ghacibeh, G. A., Shenker, J. I., Shenal, B., Uthman, B. M., & Heilman, K. M. (2006). The influence of vagus nerve stimulation on memory. *Cognitive and Behavioral Neurology*, *19*, 119–122.
- Ghods-Sharifi, S., Haluk, D. M., & Floresco, S. B. (2008). Differential effects of inactivation of the orbitofrontal cortex on strategy set-shifting and reversal learning. *Neurobiology of Learning and Memory*, *89*, 567–573.
- Giustino, T. F., & Maren, S. (2018). Noradrenergic modulation of fear conditioning and extinction. *Frontiers in Behavioral Neuroscience*, *12*, 43.
- Glennon, E., Carcea, I., Martins, A. R. O., Multani, J., Shehu, I., Svirsky, M. A., & Froemke, R. C. (2019). Locus coeruleus activation accelerates perceptual learning. *Brain Research*, *1709*, 39–49.
- Gold, P. E., & Van Buskirk, R. B. (1975). Facilitation of time-dependent memory processes with posttrial epinephrine injections. *Behavioral Biology*, *13*, 145–153.
- Gold, P. E., van Buskirk, R. B., & McGaugh, J. L. (1975). Effects of hormones on time-dependent memory storage processes. *Progress in Brain Research*, *42*, 210–211.
- Groman, S. M., James, A. S., Seu, E., Crawford, M. A., Harpster, S. N., & Jentsch, J. D. (2013). Monoamine levels within the orbitofrontal cortex and putamen interact to predict reversal learning performance. *Biological Psychiatry*, *73*, 756–762.
- Grospe, G. M., Baker, P. M., & Ragozzino, M. E. (2018). Cognitive flexibility deficits following 6-OHDA lesions of the rat dorsomedial striatum. *Neuroscience*, *374*, 80–90.
- Groves, D. A., Bowman, E. M., & Brown, V. J. (2005). Recordings from the rat locus coeruleus during acute vagal nerve stimulation in the anaesthetized rat. *Neuroscience Letters*, *379*, 174–179.
- Hassert, D. L., Miyashita, T., & Williams, C. L. (2004). The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behavioral Neuroscience*, *118*, 79–88.
- Hays, S. A., Khodaparast, N., Ruiz, A., Sloan, A. M., Hulsey, D. R., Rennaker, R. L., & Kilgard, M. P. (2014). The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. *NeuroReport*, *25*, 676–682.
- Helmstaedt, C., Hoppe, C., & Elger, C. E. (2001). Memory alterations during acute high-intensity vagus nerve stimulation. *Epilepsy Research*, *47*, 37–42.
- Hernandez, C. M., Orsini, C. A., Blaes, S. L., Bizon, J. L., Febo, M., Bruijnzeel, A. W., & Setlow, B. (2020). Effects of repeated adolescent exposure to cannabis smoke on cognitive outcomes in adulthood. *Journal of Psychopharmacology*, *269881120965931*.
- Hervig, M. E., Piilgaard, L., Božič, T., Alsö, J., & Robbins, T. W. (2020). Glutamatergic and serotonergic modulation of rat medial and lateral orbitofrontal cortex in visual serial reversal learning. *Psychology & Neuroscience*, *13*, 438–458.
- Hulsey, D. R., Hays, S. A., Khodaparast, N., Ruiz, A., Das, P., Rennaker, R. L., & Kilgard, M. P. (2016). Reorganization of motor cortex by vagus nerve stimulation requires cholinergic innervation. *Brain Stimulation*, *9*, 174–181.
- Hulsey, D. R., Riley, J. R., Loerwald, K. W., Rennaker, R. L., Kilgard, M. P., & Hays, S. A. (2017). Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. *Experimental Neurology*, *289*, 21–30.
- Hulsey, D. R., Sadmaan, S., Abe, S., Hays, S., & Kilgard, M. P. (2018). Neuromodulatory pathways required for targeted plasticity therapy. *Society for Neuroscience Meeting Planner, Program No.*, 605, 22.
- Hulsey, D. R., Shedd, C. M., Sarker, S. F., Kilgard, M. P., & Hays, S. A. (2019). Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. *Experimental Neurology*, *320*, Article 112975.
- Hvoslef-Eide, M., Oomen, C. A., Fisher, B. M., Heath, C. J., Robbins, T. W., Saksida, L. M., & Bussey, T. J. (2015). Facilitation of spatial working memory performance following intra-prefrontal cortical administration of the adrenergic  $\alpha$ 1 agonist phenylephrine. *Psychopharmacology (Berl)*, *232*, 4005–4016.
- Introini-Collison, I. B., Castellano, C., & McGaugh, J. L. (1994). Interaction of GABAergic and beta-noradrenergic drugs in the regulation of memory storage. *Behavioral and Neural Biology*, *61*, 150–155.
- Izquierdo, A., & Jentsch, J. D. (2012). Reversal learning as a measure of impulsive and compulsive behavior in addictions. *Psychopharmacology (Berl)*, *219*, 607–620.
- Jacobs, H. I., Riphagen, J. M., Razat, C. M., Wiese, S., & Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiology of Aging*, *36*, 1860–1867.
- Janitzky, K., Lippert, M. T., Engelhorn, A., Tegmeier, J., Goldschmidt, J., Heinze, H. J., & Ohl, F. W. (2015). Optogenetic silencing of locus coeruleus activity in mice impairs cognitive flexibility in an attentional set-shifting task. *Frontiers in Behavioral Neuroscience*, *9*, 286.
- Jenni, N. L., Li, Y. T., & Floresco, S. B. (2021). Medial orbitofrontal cortex dopamine D. *Neuropsychopharmacology*.
- Khodaparast, N., Kilgard, M. P., Casavant, R., Ruiz, A., Qureshi, I., Ganzer, P. D., ... Hays, S. A. (2016). Vagus nerve stimulation during rehabilitative training improves forelimb recovery after chronic ischemic stroke in rats. *Neurorehabilitation and Neural Repair*, *30*, 676–684.
- Kim, C., Johnson, N. F., Cilles, S. E., & Gold, B. T. (2011). Common and distinct mechanisms of cognitive flexibility in prefrontal cortex. *Journal of Neuroscience*, *31*, 4771–4779.
- LaClair, M., Febo, M., Nephew, B., Gervais, N. J., Poirier, G., Workman, K., ... LaCrosse, A. (2019). Sex differences in cognitive flexibility and resting brain networks in middle-aged marmosets. *eNeuro*, *6*.
- LaLumière, R. T., McGaugh, J. L., & McIntyre, C. K. (2017). Emotional modulation of learning and memory: Pharmacological implications. *Pharmacological Reviews*, *69*, 236–255.
- Li, J. N., Li, X. L., He, J., Wang, J. X., Zhao, M., Liang, X. B., ... Li, B. Y. (2015). Sex- and afferent-specific differences in histamine receptor expression in vagal afferents of rats: A potential mechanism for sexual dimorphism in prevalence and severity of asthma. *Neuroscience*, *303*, 166–177.

- Liu, C. H., Yang, M. H., Zhang, G. Z., Wang, X. X., Li, B., Li, M., ... Wang, L. (2020). Neural networks and the anti-inflammatory effect of transcutaneous auricular vagus nerve stimulation in depression. *Journal of Neuroinflammation*, *17*, 54.
- Loerwald, K. W., Borland, M. S., Rennaker, R. L., Hays, S. A., & Kilgard, M. P. (2018). The interaction of pulse width and current intensity on the extent of cortical plasticity evoked by vagus nerve stimulation. *Brain Stimulation*, *11*, 271–277.
- Loerwald, K. W., Buell, E. P., Borland, M. S., Rennaker, R. L., Hays, S. A., & Kilgard, M. P. (2018). Varying stimulation parameters to improve cortical plasticity generated by VNS-tone pairing. *Neuroscience*, *388*, 239–247.
- Luo, F., Zheng, J., Sun, X., & Tang, H. (2017). Inward rectifier K. *Experimental Neurology*, *288*, 51–61.
- Luque, J. M., de Blas, M. R., Segovia, S., & Guillamón, A. (1992). Sexual dimorphism of the dopamine-beta-hydroxylase-immunoreactive neurons in the rat locus coeruleus. *Brain Research. Developmental Brain Research*, *67*, 211–215.
- Madden, C. J., Santos da Conceicao, E. P., & Morrison, S. F. (2017). Vagal afferent activation decreases brown adipose tissue (BAT) sympathetic nerve activity and BAT thermogenesis. *Temperature (Austin)*, *4*, 89–96.
- Manta, S., Dong, J., Debonnel, G., & Blier, P. (2009). Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *Journal of Psychiatry and Neuroscience*, *34*, 272–280.
- Mar, A. C., Horner, A. E., Nilsson, S. R., Alsö, J., Kent, B. A., Kim, C. H., ... Bussey, T. J. (2013). The touchscreen operant platform for assessing executive function in rats and mice. *Nature Protocols*, *8*, 1985–2005.
- Marrosu, F., Serra, A., Maleci, A., Puligheddu, M., Biggio, G., & Piga, M. (2003). Correlation between GABA(A) receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. *Epilepsy Research*, *55*, 59–70.
- Mathew, E., Tabet, M. N., Robertson, N. M., Hays, S. A., Rennaker, R. L., Kilgard, M. P., ... Souza, R. R. (2020). Vagus nerve stimulation produces immediate dose-dependent anxiolytic effect in rats. *Journal of Affective Disorders*, *265*, 552–557.
- Matta, S. M., Hill-Yardin, E. L., & Crack, P. J. (2019). The influence of neuroinflammation in Autism Spectrum Disorder. *Brain, Behavior, and Immunity*, *79*, 75–90.
- McAlonan, K., & Brown, V. J. (2003). Orbital prefrontal cortex mediates reversal learning and not attentional set shifting in the rat. *Behavioural Brain Research*, *146*, 97–103.
- McDonald, W. M. (2016). Neuromodulation treatments for geriatric mood and cognitive disorders. *The American Journal of Geriatric Psychiatry*, *24*, 1130–1141.
- McGaugh, J. L. (1973). Drug facilitation of learning and memory. *Annual Review of Pharmacology and Toxicology*, *13*, 229–241.
- McGaugh, J. L. (2004). The amygdala modulates the consolidation of memories of emotionally arousing experiences. *Annual Review of Neuroscience*, *27*, 1–28.
- Merrill, C. A., Jonsson, M. A., Minthorn, L., Egnell, H., C-son Silander, H., Blennow, K., ... Sjögren, M. J. (2006). Vagus nerve stimulation in patients with Alzheimer's disease: Additional follow-up results of a pilot study through 1 year. *Journal of Clinical Psychiatry*, *67*, 1171–1178.
- Miyashita, T., & Williams, C. L. (2006). Epinephrine administration increases neural impulses propagated along the vagus nerve: Role of peripheral beta-adrenergic receptors. *Neurobiology of Learning and Memory*, *85*, 116–124.
- Morais, A., Liu, T. T., Qin, T., Sadhegian, H., Ay, I., Yagmur, D., ... Ayata, C. (2020). Vagus nerve stimulation inhibits cortical spreading depression exclusively through central mechanisms. *Pain*, *161*, 1661–1669.
- Morgan, M. A., & LeDoux, J. E. (1995). Differential contribution of dorsal and ventral medial prefrontal cortex to the acquisition and extinction of conditioned fear in rats. *Behavioral Neuroscience*, *109*, 681–688.
- Morgan, M. A., Romanski, L. M., & LeDoux, J. E. (1993). Extinction of emotional learning: Contribution of medial prefrontal cortex. *Neuroscience Letters*, *163*, 109–113.
- Moriyama, H., Hayashi, S., Inoue, Y., Itoh, M., & Otsuka, N. (2016). Sex differences in morphometric aspects of the peripheral nerves and related diseases. *NeuroRehabilitation*, *39*, 413–422.
- Naritoku, D. K., Terry, W. J., & Helfert, R. H. (1995). Regional induction of fos immunoreactivity in the brain by anticonvulsant stimulation of the vagus nerve. *Epilepsy Research*, *22*, 53–62.
- Noble, L. J., Chuah, A., Callahan, K. K., Souza, R. R., & McIntyre, C. K. (2019). Peripheral effects of vagus nerve stimulation on anxiety and extinction of conditioned fear in rats. *Learning and Memory*, *26*, 245–251.
- Noble, L. J., Gonzalez, I. J., Meruva, V. B., Callahan, K. A., Belfort, B. D., Ramanathan, K. R., ... McIntyre, C. K. (2017). Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats. *Translational Psychiatry*, *7*, Article e1217.
- Noble, L. J., Meruva, V. B., Hays, S. A., Rennaker, R. L., Kilgard, M. P., & McIntyre, C. K. (2019). Vagus nerve stimulation promotes generalization of conditioned fear extinction and reduces anxiety in rats. *Brain Stimulation*, *12*, 9–18.
- Nogueira, P. J., Tomaz, C., & Williams, C. L. (1994). Contribution of the vagus nerve in mediating the memory-facilitating effects of substance P. *Behavioural Brain Research*, *62*, 165–169.
- Pardo, J. V., Sheikh, S. A., Schwindt, G. C., Lee, J. T., Kuskowski, M. A., Surerus, C., ... Rittberg, B. R. (2008). Chronic vagus nerve stimulation for treatment-resistant depression decreases resting ventromedial prefrontal glucose metabolism. *NeuroImage*, *42*, 879–889.
- Pavlov, V. A., & Tracey, K. J. (2012). The vagus nerve and the inflammatory reflex-linking immunity and metabolism. *Nature Reviews Endocrinology*, *8*, 743–754.
- Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning and Memory*, *16*, 279–288.
- Peña, D. F., Engineer, N. D., & McIntyre, C. K. (2013). Rapid remission of conditioned fear expression with extinction training paired with vagus nerve stimulation. *Biological Psychiatry*, *73*, 1071–1077.
- Poe, G. R., Foote, S., Eschenko, O., Johansen, J. P., Bouret, S., Aston-Jones, G., ... Sara, S. J. (2020). Locus coeruleus: A new look at the blue spot. *Nature Reviews Neuroscience*, *21*, 644–659.
- Porter, B. A., Khodaparast, N., Fayyaz, T., Cheung, R. J., Ahmed, S. S., Vrana, W. A., ... Kilgard, M. P. (2012). Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. *Cerebral Cortex*, *22*, 2365–2374.
- Prado, V. F., Janickova, H., Al-Onaizi, M. A., & Prado, M. A. (2017). Cholinergic circuits in cognitive flexibility. *Neuroscience*, *345*, 130–141.
- Quirk, G. J., Garcia, R., & González-Lima, F. (2006). Prefrontal mechanisms in extinction of conditioned fear. *Biological Psychiatry*, *60*, 337–343.
- Reuter, U., McClure, C., Liebler, E., & Pozo-Rosich, P. (2019). Non-invasive neuromodulation for migraine and cluster headache: A systematic review of clinical trials. *Journal of Neurology, Neurosurgery and Psychiatry*, *90*, 796–804.
- Roosevelt, R. W., Smith, D. C., Clough, R. W., Jensen, R. A., & Browning, R. A. (2006). Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. *Brain Research*, *1119*, 124–132.
- Rorabaugh, J. M., Chalermpanupap, T., Botz-Zapp, C. A., Fu, V. M., Lembeck, N. A., Cohen, R. M., & Weinshenker, D. (2017). Chemogenetic locus coeruleus activation restores reversal learning in a rat model of Alzheimer's disease. *Brain*, *140*, 3023–3038.
- Sadacca, B. F., Wikenheiser, A. M., & Schoenbaum, G. (2017). Toward a theoretical role for tonic norepinephrine in the orbitofrontal cortex in facilitating flexible learning. *Neuroscience*, *345*, 124–129.
- Samanez-Larkin, G. R., Buckholz, J. W., Cowan, R. L., Woodward, N. D., Li, R., Ansari, M. S., ... Zald, D. H. (2013). A thalamocorticostratial dopamine network for psychostimulant-enhanced human cognitive flexibility. *Biological Psychiatry*, *74*, 99–105.
- Sanders, T. H., Weiss, J., Hogewood, L., Chen, L., Paton, C., McMahan, R. L., & Sweatt, J. D. (2019). Cognition-enhancing vagus nerve stimulation alters the epigenetic landscape. *Journal of Neuroscience*, *39*, 3454–3469.
- Sara, S. J. (2009). The locus coeruleus and noradrenergic modulation of cognition. *Nature Reviews Neuroscience*, *10*, 211–223.
- Savolainen, K., Ihalainen, J., Hämäläinen, E., Tanila, H., & Forsberg, M. M. (2021). Phencyclidine-induced cognitive impairments in repeated touchscreen visual reversal learning tests in rats. *Behavioural Brain Research*, *404*, Article 113057.
- Schoenbaum, G., Setlow, B., Nugent, S. L., Sadoris, M. P., & Gallagher, M. (2003). Lesions of orbitofrontal cortex and basolateral amygdala complex disrupt acquisition of odor-guided discriminations and reversals. *Learning and Memory*, *10*, 129–140.
- Setlow, B., Schoenbaum, G., & Gallagher, M. (2003). Neural encoding in ventral striatum during olfactory discrimination learning. *Neuron*, *38*, 625–636.
- Seu, E., & Jentsch, J. D. (2009). Effect of acute and repeated treatment with desipramine or methylphenidate on serial reversal learning in rats. *Neuropharmacology*, *57*, 665–672.
- Seu, E., Lang, A., Rivera, R. J., & Jentsch, J. D. (2009). Inhibition of the norepinephrine transporter improves behavioral flexibility in rats and monkeys. *Psychopharmacology (Berl)*, *202*, 505–519.
- Shansky, R. M., & Murphy, A. Z. (2021). Considering sex as a biological variable will require a global shift in science culture. *Nature Neuroscience*, *24*, 457–464.
- Shen, H., Fuchino, Y., Miyamoto, D., Nomura, H., & Matsuki, N. (2012). Vagus nerve stimulation enhances perforant path-CA3 synaptic transmission via the activation of beta-adrenergic receptors and the locus coeruleus. *International Journal of Neuropsychopharmacology*, *15*, 523–530.
- Silverman, J. L., Pride, M. C., Hayes, J. E., Puhger, K. R., Butler-Struben, H. M., Baker, S., & Crawley, J. N. (2015). GABAB receptor agonist R-baclofen reverses social deficits and reduces repetitive behavior in two mouse models of autism. *Neuropsychopharmacology*, *40*, 2228–2239.
- Sjögren, M. J., Hellström, P. T., Jonsson, M. A., Runnerstam, M., Silander, H. C., & Ben-Menachem, E. (2002). Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's disease: A pilot study. *Journal of Clinical Psychiatry*, *63*, 972–980.
- Smith, D. C., Modglin, A. A., Roosevelt, R. W., Neese, S. L., Jensen, R. A., Browning, R. A., & Clough, R. W. (2005). Electrical stimulation of the vagus nerve enhances cognitive and motor recovery following moderate fluid percussion injury in the rat. *Journal of Neurotrauma*, *22*, 1485–1502.
- Smucny, J., Visani, A., & Tregellas, J. R. (2015). Could vagus nerve stimulation target hippocampal hyperactivity to improve cognition in schizophrenia? *Frontiers in Psychiatry*, *6*, 43.
- Stoppel, L. J., Kazdoba, T. M., Schaffler, M. D., Preza, A. R., Heynen, A., Crawley, J. N., & Bear, M. F. (2018). R-baclofen reverses cognitive deficits and improves social interactions in two lines of 16p11.2 deletion mice. *Neuropsychopharmacology*, *43*, 513–524.
- Stuchlik, A., & Sumiyoshi, T. (2014). Cognitive deficits in schizophrenia and other neuropsychiatric disorders: Convergence of preclinical and clinical evidence. *Frontiers in Behavioral Neuroscience*, *8*, 444.
- Sun, L., Peräkylä, J., Holm, K., Haapasalo, J., Lehtimäki, K., Ogawa, K. H., ... Hartikainen, K. M. (2017). Vagus nerve stimulation improves working memory performance. *Journal of Clinical and Experimental Neuropsychology*, *39*, 954–964.
- Székely, M. (2000). The vagus nerve in thermoregulation and energy metabolism. *Autonomic Neuroscience*, *85*, 26–38.
- Tait, D. S., Chase, E. A., & Brown, V. J. (2014). Attentional set-shifting in rodents: A review of behavioural methods and pharmacological results. *Current Pharmaceutical Design*, *20*, 5046–5059.
- Takase, K., Kimura, F., Yagami, T., & Mitsuhashi, D. (2009). Sex-specific 24-h acetylcholine release profile in the medial prefrontal cortex: Simultaneous measurement of spontaneous locomotor activity in behaving rats. *Neuroscience*, *159*, 7–15.

- Thakkar, V. J., Engelhart, A. S., Khodaparast, N., Abadzi, H., & Centanni, T. M. (2020). Transcutaneous auricular vagus nerve stimulation enhances learning of novel letter-sound relationships in adults. *Brain Stimulation*, *13*, 1813–1820.
- Totah, N. K., Logothetis, N. K., & Eschenko, O. (2015). Atomoxetine accelerates attentional set shifting without affecting learning rate in the rat. *Psychopharmacology (Berl)*, *232*, 3697–3707.
- Toussay, X., Basu, K., Lacoste, B., & Hamel, E. (2013). Locus coeruleus stimulation recruits a broad cortical neuronal network and increases cortical perfusion. *Journal of Neuroscience*, *33*, 3390–3401.
- Tseng, C. T., Brougher, J., Gaulding, S. J., Hassan, B. S., & Thorn, C. A. (2020). Vagus nerve stimulation promotes cortical reorganization and reduces task-dependent calorie intake in male and female rats. *Brain Research*, *1748*, Article 147099.
- van Hoorn, A., Carpenter, T., Oak, K., Laugharne, R., Ring, H., & Shankar, R. (2019). Neuromodulation of autism spectrum disorders using vagal nerve stimulation. *Journal of Clinical Neuroscience*, *63*, 8–12.
- Veenstra-VanderWeele, J., Cook, E. H., King, B. H., Zarevics, P., Cherubini, M., Walton-Bowen, K., ... Carpenter, R. L. (2017). Arbaclofen in children and adolescents with autism spectrum disorder: A randomized, controlled, phase 2 trial. *Neuropsychopharmacology*, *42*, 1390–1398.
- Vijgen, G. H., Bouvy, N. D., Leenen, L., Rijkers, K., Cornips, E., Majoie, M., ... van Marken Lichtenbelt, W. D. (2013). Vagus nerve stimulation increases energy expenditure: Relation to brown adipose tissue activity. *PLoS ONE*, *8*, Article e77221.
- Vázquez-Oliver, A., Brambilla-Pisoni, C., Domingo-Gainza, M., Maldonado, R., Ivorra, A., & Ozaita, A. (2020). Auricular transcutaneous vagus nerve stimulation improves memory persistence in naïve mice and in an intellectual disability mouse model. *Brain Stimulation*, *13*, 494–498.
- Wang, Y., Liu, J., Gui, Z. H., Ali, U., Fan, L. L., Hou, C., ... Li, Q. (2011).  $\alpha 2$ -Adrenoceptor regulates the spontaneous and the GABA/glutamate modulated firing activity of the rat medial prefrontal cortex pyramidal neurons. *Neuroscience*, *182*, 193–202.
- Waterhouse, B. D., Devilbiss, D., Fleischer, D., Sessler, F. M., & Simpson, K. L. (1998). New perspectives on the functional organization and postsynaptic influences of the locus ceruleus efferent projection system. *Advances in Pharmacology*, *42*, 749–754.
- Waterhouse, B. D., Lin, C. S., Burne, R. A., & Woodward, D. J. (1983). The distribution of neocortical projection neurons in the locus coeruleus. *The Journal of Comparative Neurology*, *217*, 418–431.
- Waterhouse, B. D., & Navarra, R. L. (2019). The locus coeruleus-norepinephrine system and sensory signal processing: A historical review and current perspectives. *Brain Research*, *1709*, 1–15.
- Westbrook, S. R., Hankosky, E. R., Dwyer, M. R., & Gulley, J. M. (2018). Age and sex differences in behavioral flexibility, sensitivity to reward value, and risky decision-making. *Behavioral Neuroscience*, *132*, 75–87.
- Williams, C. L., & McGaugh, J. L. (1993). Reversible lesions of the nucleus of the solitary tract attenuate the memory-modulating effects of posttraining epinephrine. *Behavioral Neuroscience*, *107*, 955–962.
- Yaghoubi, F., Jang, K., Hoang, U., Asgari, S., & Vasudevan, S. (2020). Sex differences in vagus nerve stimulation effects on rat cardiovascular and immune systems. *Frontiers in Neuroscience*, *14*, Article 560668.
- Yap, J. Y. Y., Keatch, C., Lambert, E., Woods, W., Stoddart, P. R., & Kameneva, T. (2020). Critical review of transcutaneous vagus nerve stimulation: Challenges for translation to clinical practice. *Frontiers in Neuroscience*, *14*, 284.
- Zabara, J. (1992). Inhibition of experimental seizures in canines by repetitive vagal stimulation. *Epilepsia*, *33*, 1005–1012.
- Zhang, J. (2002). The influence of vagus nerve stimulation on NMDAR1 mRNA and GABAAR alpha 1 mRNA in thalamic reticular nucleus of pentylenetetrazole-induced epileptic rats. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*, *19*, 566–568.