Bidirectional pharmacological perturbations of the noradrenergic system differentially affect tactile detection

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Abstract

The brain neuromodulatory systems heavily influence behavioral and cognitive processes. Previous work has shown that norepinephrine (NE), a classic neuromodulator mainly derived from the locus coeruleus (LC), enhances neuronal responses to sensory stimuli. However, the role of the LC-NE system in modulating perceptual task performance is not well understood. In addition, systemic perturbation of NE signaling has often been proposed to specifically target the LC in functional studies, yet the assumption that localized (specific) and systemic (nonspecific) perturbations of LC-NE have the same behavioral impact remains largely untested. In this study, we trained mice to perform a head-fixed, quantitative tactile detection task, and administered an α2 adrenergic receptor agonist or antagonist to pharmacologically down- or up-regulate LC-NE activity, respectively. We addressed the outstanding question of how bidirectional perturbations of LC-NE activity affect tactile detection, and tested whether localized and systemic drug treatments exert the same behavioral effects. We found that both localized and systemic suppression of LC-NE impaired tactile detection by reducing motivation. Surprisingly, while locally activating LC-NE enabled mice to perform in a near-optimal regime, systemic activation impaired

behavior by promoting impulsivity. Our results demonstrate that localized silencing and activation of LC-NE differentially affect tactile detection, and that localized and systemic NE activation induce distinct behavioral changes.

1. Introduction

The locus coeruleus (LC) is a major source of the neuromodulator norepinephrine (NE) in mammalian brains. With profuse projections across the central nervous system, this modulatory circuit has been hypothesized to be critical in mediating a variety of brain functions and behavior, including sleep-wake transition, perception, attention and learning. The dysfunction of the LC-NE circuit has also been thought to be involved in several neurological disorders (Arnsten, 2000; Berridge and Waterhouse, 2003; Aston-Jones and Cohen, 2005; Sara, 2009; Sara and Bouret, 2012; Waterhouse and Navarra, 2019).

We recently proposed that understanding how LC-NE modulates sensory perception offers a stepping stone toward unraveling its roles in higher cognitive functions (McBurney-Lin et al., 2019). LC neurons extensively innervate sensory cortical and subcortical regions, and LC-NE signaling modulates sensory neuron responses to external stimuli (e.g., (Foote et al., 1975; Waterhouse et al., 1980; Kasamatsu and Heggelund, 1982; Morrison and Foote, 1986; Simpson et al., 1997; Devilbiss and Waterhouse, 2004; Manella et al., 2017; Navarra et al., 2017; Rho et al., 2018)). LC-NE may also affect sensory perception through modulating motivation or attention (Berridge and Waterhouse, 2003; Lee and Dan, 2012; Sara and Bouret, 2012; Thiele and Bellgrove, 2018). To our knowledge, only a limited number of studies have examined LC-NE influence on perception-related behavior (Doucette et al., 2007; Escanilla et al., 2010; Martins and Froemke, 2015; Navarra et al., 2017; Rodenkirch et al., 2019). It remains poorly understood how bidirectional perturbations of LC-NE activity affect perceptual task performance.

To examine the causal role of a neural circuit, such as the LC-NE, in regulating behavior, one would perturb this system and assess the subsequent behavioral changes. Traditional lesion

approaches may induce compensatory plasticity changes (Acheson et al., 1980; Harik et al., 1981; Valentini et al., 2004) and mask the effects specific to LC-NE. More recent studies employed acute, reversible perturbations including pharmacological, electrical, chemogenetic, and optogenetic stimulations. Among these approaches, pharmacology facilitates translational comparison between animal and human studies. The inhibitory α2 adrenergic receptors (ARs) are highly expressed in the LC, but only sparsely expressed, if at all, in neighboring brainstem regions (McCune et al., 1993; Nicholas et al., 1993). Targeting α2 ARs is considered a specific manner to perturb LC-NE activity (e.g., (Neves et al., 2018)). Agonizing α2 ARs suppresses LC-NE signaling by hyperpolarizing LC neurons and reducing NE release in downstream areas (Cedarbaum and Aghajanian, 1977; Aghajanian and VanderMaelen, 1982; Abercrombie and Jacobs, 1987; Aghajanian and Wang, 1987; Adams and Foote, 1988; Berridge et al., 1993; Kalwani et al., 2014). Conversely, antagonists acting on α2 ARs increase LC neuron excitability and spiking response to stimuli as well as NE release (Cedarbaum and Aghajanian, 1976; Aghajanian and VanderMaelen, 1982; Raiteri et al., 1983; Rasmussen and Jacobs, 1986; Simson and Weiss, 1987; Adams and Foote, 1988; Herr et al., 2012).

Human studies have reported that systemically up- or down-regulating NE signaling (mainly through targeting α2 ARs) affected subjects performing perception-related tasks (Halliday et al., 1989; Turetsky and Fein, 2002; Gelbard-Sagiv et al., 2018). Targeting α2 ARs nonspecifically (e.g., intraperitoneal – i.p. or intracerebroventricular – i.c.v., hereafter referred to as “systemic”) or specifically (e.g., intra- or peri-LC, hereafter referred to as “localized”) exerts similar changes on LC activity (Aghajanian and VanderMaelen, 1982; Adams and Foote, 1988; Berridge et al., 1993). However, systemic perturbations of α2-ARs could induce physiological and behavioral effects that are different from localized perturbation. Systemic α2 perturbation would likely affect noradrenergic neurons in the nucleus of the solitary tract (Van Bockstaele et al., 1999; Kirouac, 2015), as well as many α2-expressing regions in the nervous system (McCune et al., 1993; Nicholas et al., 1993; Robertson et al., 2013). It should also be noted that α2-ARs are

expressed both presynaptically (auto-receptors) and postsynaptically in terminal fields. Agonizing or antagonizing presynaptic α2-ARs suppresses or enhances NE release, respectively, and the postsynaptic effects would depend on the specific types of postsynaptic adrenergic receptors that are activated in terminal fields. In contrast, agonizing or antagonizing postsynaptic α2-ARs exerts direct inhibitory or excitatory postsynaptic effects, respectively.

Head-fixed behavior facilitates stimulus control and movement measurement, and allows reliable quantification of different components of perceptual behavior, including detection, discrimination, impulsivity and motivation (Schwarz et al., 2010; Guo et al., 2014). To our knowledge, using well-controlled, quantitative perceptual behavior to examine the effects of localized (specific) and systemic (nonspecific) perturbations of LC-NE is lacking.

In the current study, we trained mice to perform a head-fixed, quantitative tactile detection task. We administered an α2 agonist or antagonist to pharmacologically down- or up-regulate LC activity, respectively. We addressed the outstanding question of how bidirectional perturbations of LC activity affect tactile detection, and tested whether localized and systemic drug treatments exert the same behavioral effects.

2. Methods

2.1 Mice

Both male and female mice were used in this study. All mice were C57BL/6J except 2 (out of 6) included in the localized clonidine treatment were of mixed B6J/129 background. Mice were housed with reversed light/dark cycle (9A – 9P dark, 9P – 9A light). Mice of 6-12 weeks were implanted with head posts and/or cannulae. Clonidine (an α2 agonist, Sigma-Aldrich) was administered locally in 6 mice and systemically in 3 mice. Yohimbine (an α2 antagonist, Sigma-Aldrich) was administered locally in 7 mice and systemically in 5 mice. Every mouse received corresponding localized or systemic saline injections as controls. Quantification of localized pharmacological effects on LC activity was performed by immunostaining for the immediate early

105 gene c-fos in a separate group of 11 mice. All procedures were approved by the UC Riverside
106 Animal Care and Use Committee.
107
108 *2.2 Surgery*
109 Head post surgeries were similar to previously published work (Yang et al., 2016). In brief, mice
110 were anesthetized (1-2% isoflurane) and affixed to a stereotaxic instrument (Kopf, RWD). Body
111 temperature was maintained with a heating blanket (Harvard Apparatus, RWD) throughout the
112 surgical procedures. The scalp over the dorsal surface of the skull was cleaned with betadine and
113 70% ethanol, and removed. The periosteum was removed and the skull scored with a dental drill.
114 Cyanoacrylate was applied to the border of the skull and scalp. The head post was placed and
115 secured with dental acrylic. A craniotomy of ~1 mm x 1 mm was made over the left hemisphere,
116 centered at 5.2 - 5.3 mm posterior to bregma and 0.9 - 1.0 mm lateral to midline. A guide cannula
117 (27G, 3.5 mm long, RWD) with dummy insert was advanced vertically into the brain until a depth
118 of 1.8 mm. Dental acrylic was used to secure the guide cannula and filled in the remaining
119 exposed skull surface. After surgery, mice were single housed and allowed to recover for at least
120 48 hours.
121
122 *2.3 Behavioral Task*
123 Following recovery from the surgery, mice were restricted to 1 mL/day water consumption for 7-
124 10 days before behavioral training. The behavior task was adapted from published work (Yang et
125 al., 2016). Briefly, mice were trained to perform a head-fixed, Go/NoGo single-whisker detection
126 task, in which mice reported whether they perceived a brief deflection (200 ms, 25 Hz, ~600 deg/s)
127 to the right C2 whisker by licking or withholding licking. Ambient white noise (1 - 40 kHz) was
128 played throughout the session. An auditory cue (8 kHz) was presented at the beginning of each
129 trial, 1.5 s prior to the time of possible stimulus onset. Trial outcomes comprised a mixture of

successful and failed stimulus detection (Hit and Miss), as well as successful and failed responses to stimulus absence (Correct Rejection and False Alarm). Trials were aborted if mice licked prematurely during the waiting period between auditory cue and the time of possible stimulus onset (Impulsive). Trials were also considered impulsive when mice licked within the first 100 ms window from stimulus onset (Mayrhofer et al., 2013; Yang et al., 2016). Mice performed one behavior session (300-500 trials) per day. Mice never achieved saturating performance in this task (Yang et al., 2016), indicating that detecting weak single-whisker deflection is perceptually demanding. All aspects of behavioral control were managed by custom Arduino-based hardware and software. Behavioral data were acquired with WaveSurfer (https://www.janelia.org/open-science/wavesurfer).

2.4 Pharmacology

All drugs were dissolved in physiological saline. Localized pharmacology was administered during behavior sessions. Drug or saline was loaded into a 1 µL Hamilton syringe, controlled by a syringe pump (Harvard Apparatus). Mice were placed in the behavior chamber, and injection cannula (33G, 5 mm long) inserted into the guide cannula. The infusion depth was 3.3 mm. Infusion was initiated within the first 20 behavior trials. 300 nL of drug or saline was infused at a rate of 60 nL/min. At the conclusion of a behavior session, injection cannula was removed and dummy insert replaced.

Systemic pharmacology was administered just prior to behavior sessions. Mice were briefly anesthetized (< 1 minute) with 2-3% isoflurane, during which 50 µL of drug or saline was injected via i.p.. Mice were allowed to recover for 5 minutes before starting the behavior session. During baseline behavioral sessions (one day before i.p. treatment), mice were also briefly anesthetized to account for any potential effects from anesthesia.

2.5 Histology

At the conclusion of behavioral experiments, mice with cannula implants received localized Fluoro-Gold infusion (0.1-1%, 300 nL) at a rate of 60 nL/min. 40-60 minutes later, mice were anesthetized with ketamine/xylazine and perfused intracardially with 4% paraformaldehyde, and the brains harvested and post fixed. 100 μM thick coronal sections were cut (Leica, VT1200s). Sections containing LC were incubated with rabbit anti-Tyrosine Hydroxylase (TH) antibody (Thermofisher OPA 1-04050, 1:1000), followed by goat anti-rabbit IgG Alexa Fluor 488 or 594 secondary antibody (Thermofisher A32731 or A32740, 1:1000), and mounted with DAPI mounting media (Vector labs). Co-localization of Fluoro-Gold and TH immunoactivity, as well as the cannula tract, were used to verify cannula placement.

The expression of an immediate early gene, c-fos, was examined to assess the impact of localized drug treatment on LC activity. Infusions were performed in the left LC, with the contralateral (right) LC serving as a control. Clonidine was infused in 4 awake mice. Yohimbine was infused in 5 mice, 2 of which received infusion under anesthesia, with the purpose to reduce basal LC activation and enhance the contrast between the injected side and the control side. The remaining 3 mice received infusion during wakefulness. Saline was infused in 2 awake mice. All mice were perfused 40-60 minutes post infusion. Coronal sections containing LC were first incubated with rabbit anti-c-fos antibody (Cell Signaling 2250S, 1:400), followed by secondary antibody (Thermofisher A32740, 1:400). Sections were then incubated with rabbit IgG isotype control (Thermofisher 31235, 1:17000) to quench nonspecific signals, and subsequently stained for TH. Z-stack images were acquired using a confocal microscope (Leica SPE II) and flattened using Fiji (Schindelin et al., 2012).

2.6 Data Analysis

Behavior data were analyzed off-line with MATLAB. To account for the fact that some mice did not immediately engage in the task, the initial 20-40 trials were removed from behavior analysis. In some sessions, trials toward session end were also removed from analysis when mice

appeared to be disengaged from the task (Hit rate dropped below 50%, typically after 300-400 trials). For sessions shown in Fig. 1, we included an additional 20-50 trials toward session end to demonstrate a near-complete cessation of task performance. Decision bias/criterion (c) and detection sensitivity (d') were calculated based on Hit rate (HR) and False Alarm rate (FAR): $c = z(HR) - z(FAR)$, $d' = -(z(HR) + z(FAR))/2$, where z is the normal inverse cumulative distribution function.

c-fos expression was analyzed using QuPath (Bankhead et al., 2017). Borders around the LC were manually drawn to identify regions of interest. For each mouse, 2-3 images with the greatest TH and c-fos expressions were used to determine the minimum and maximum cell sizes, as well as the fluorescent intensity threshold. Individual cells expressing supra-threshold TH or c-fos were detected. Results were manually verified for each image.

Data were reported as mean ± s.e.m. unless otherwise noted. Statistical tests were by two-tailed Wilcoxon signed rank unless otherwise noted.

3. Results

3.1 Mouse behavior fluctuates within single sessions

Mice were trained to perform a head-fixed, Go/NoGo single-whisker detection task, in which mice reported whether they perceived a brief deflection to the right C2 whisker by licking or withholding licking (Fig. 1a). The performance of well-trained mice fluctuated during single behavior sessions, as reported by others recently (Berditchevskaia et al., 2016). A typical behavior session started with mice licking indiscriminately, resulting in high Hit rate (fraction of Hit trials among Go trials), high Impulsive rate (IS rate, fraction of IS trials among all trials), and low Correct Rejection rate (CR rate, fraction of CR trials among NoGo trials). As the session proceeded, Hit rate remained high while mice better withheld licking in NoGo trials, increasing Correct Rejection rate. Towards session end, mice licked less in all trials, and Hit and Impulsive rates reached a minimum and Correct Rejection rate reached a maximum (Fig. 1b). Within sessions, the fluctuations of

Impulsive rate were positively correlated with Hit rate, and highly anti-correlated with Correct Rejection rate (Fig. S1). Using signal detection theory (Green and Swets, 1966), we found that decision bias/criterion (c) increased over time, while detection sensitivity/discriminability (d') exhibited an inverted-U profile (Fig. 1c). Toward session end, reaction time (RT, latency from stimulus onset to the time of first licking response) increased and lick frequency declined (Fig. 1e, Fig. S2). As demonstrated in previous work (e.g., (Dickinson and Balleine, 1994; Mayrhofer et al., 2013; Berditchevskaia et al., 2016)), these behavioral changes reflect a systematic shift of the motivational states of the mice. To illustrate this shift, we constructed a trajectory of motivational states based on Hit rate and Correct Rejection rate (Fig. 1g): mice started with an over-motivated/impulsive state (high Hit and Impulsive rates, low Correct Rejection rate and decision bias, and short reaction time), potentially due to being water restricted. As the behavior session progressed, their performance transitioned to a near-optimal regime (high Hit rate, intermediate Correct Rejection rate, high detection sensitivity, and short reaction time). Eventually, mice were much less motivated to perform the task and often disengaged (low Hit and Impulsive rates, high Correct Rejection rate and decision bias, and long reaction time), potentially due to satiety (Fig. 1f). The collective changes of Hit and Correct Rejection rates led to an inverted-U trajectory of overall performance (Fraction Correct, Fig. 1d), which peaked in the middle of a session and declined toward session start and session end. Interestingly, this inverted-U relationship resembles how LC-NE has been hypothesized to modulate task performance (Aston-Jones and Cohen, 2005).

3.2 Localized and systemic clonidine treatments similarly impair detection performance

To assess the behavioral effects of suppressing LC activity, we implanted drug delivery cannulae unilaterally in the left LC (contralateral to whisker stimulation) of 6 mice to locally infuse an α2 agonist clonidine (300 nL, 10 mM, 60 nL/min, Fig. 2a). Cannula placement was verified post-hoc to ensure targeted drug administration to the LC (Fig. 2b). Clonidine infusion suppressed LC

activity as it reduced c-fos expression in LC neurons (Fig. 2c). On average, c-fos expression was ~40% lower in the clonidine side compared with the contralateral control side (12.7% vs. 19.5%). This reduction was also significant in individual mice ($P < 0.01$ in 3 out 4 mice, permutation test. Table 1). Saline infusion did not significantly change c-fos expression in the LC ($P > 0.05$ in 2 mice, permutation test. Table S1). Drug spread was estimated to be ~400 μm (Fig. S3, (St. Peters et al., 2011)). Following clonidine treatments, mice licked less in all trials. As a result, Hit and Impulsive (IS) rates decreased and Correct Rejection (CR) rate increased (Fig. 2d, e). Later in the session, mice showed a tendency of behavioral recovery and re-engaged in the task (Fig. 2d, Fig. S4). Since a typical behavior session in our study lasts 40-50 minutes, this time course is consistent with diminished clonidine effects after ~30 minutes (Abercrombie and Jacobs, 1987; Adams and Foote, 1988; Kalwani et al., 2014). Saline infusion had no effects on behavior (Fig. S5, S6). In addition, in mice where drug infusion was outside of LC we observed minimal behavioral changes (Fig. S7). 5 mM clonidine did not have a significant influence on tactile detection, but the trend is consistent with a dose-dependent effect (Fig. S8). Overall, localized clonidine infusion decreased Hit rate, Impulsive rate and detection sensitivity (d'), elevated Correct Rejection rate, reaction time (RT) and decision bias (c), and impaired task performance (Fig. 2e-g, Fig. S5). Clonidine treated mice behaved as if they were at the end of normal behavior sessions (Fig. 2h). Decreased Impulsive rate, increased reaction time and increased decision bias (changes in c are greater than changes in d', 1.20 ± 0.15 vs. 0.61 ± 0.10, $P = 0.002$, $n = 10$) are all indicative of a motivational shift (Dickinson and Balleine, 1994; Schwarz et al., 2010; Mayrhofer et al., 2013; Berditchevskaia et al., 2016). Thus, we conclude that reduced motivation is the main factor underlying impaired behavior during localized clonidine treatment.

To compare the behavioral effects of localized and systemic drug treatments, we injected clonidine via i.p. (0.05-0.1 mg/kg, (Marzo et al., 2014; Devilbiss, 2019)) in an additional 3 mice. Although systemic drug treatment may affect other areas in the nervous system, the observed behavioral changes resembled localized infusion (reduced Hit rate, Impulsive rate and detection

sensitivity, elevated Correct Rejection rate, reaction time and decision bias, Fig. 3a-c). Saline injection did not affect behavior (Fig. S9).

To conclude, we found that both localized and systemic clonidine treatments (decreasing LC activity) impaired task performance in a similar fashion, i.e., by reducing motivation (Fig. 2h, Fig. 3d).

3.3 Localized and systemic yohimbine treatments differently affect detection performance

Next, to assess the behavioral effects of enhancing LC activity, we locally infused an α2 antagonist yohimbine (300 nL, 10 mM, 60 nL/min) in the left LC of 7 mice. Localized yohimbine administration enhanced LC activity as it increased c-fos expression in LC neurons (Fig. 4a). On average, c-fos expression was ~100% higher in the yohimbine side compared with the contralateral control side (38.6% vs. 19.9%). This effect was significant in individual mice ($P < 1e-5$ in all 5 mice, permutation test. Table 2). Interestingly, we did not observe any changes in Hit rate after yohimbine infusion, but Correct Rejection (CR) rate was significantly increased, accompanied with a reduction of Impulsive (IS) rate (Fig. 4b, c, Fig. S10). We note that later in the session Correct Rejection rate returned to baseline levels (after ~30 minutes, Fig. 4b), consistent with the time course of diminished yohimbine effects (Andén et al., 1982). However, it has also been reported that elevated LC baseline firing could be sustained up to 60 minutes upon yohimbine administration (Rasmussen and Jacobs, 1986). Saline treatment did not affect behavior (Fig. S10, S11). 20 mM yohimbine had a similar influence on behavior as 10 mM, and the trend is consistent with a dose-dependent effect (Fig. S12). However, 20 mM yohimbine appeared to induce transient behavioral arrests during the initial 50-100 trials (data not shown), implying that this dose over-activates LC (Carter et al., 2010). Overall, the primary behavioral effect of localized yohimbine treatment was an improvement of task performance as mice could better withhold licking in NoGo trials and were less prone to False Alarms (Fig. 4b-d, Fig. S10), resembling their peak performance in the middle of normal behavior sessions (Fig. 4f). Yohimbine

286 did not affect decision bias but significantly increased detection sensitivity (Fig. 4e, Fig. S10),
287 which suggests that the behavioral improvement is not simply a result of an overall increase of
288 arousal (which would be reflected by significant decreases in decision bias (Gelbard-Sagiv et al.,
289 2018)), but more specifically of enhanced sensory processing (e.g., increased signal-to-noise
290 ratio).

291 To compare the behavioral effects of localized and systemic drug treatments, we injected
292 yohimbine via i.p. in 5 mice (2 mg/kg, (Rasmussen and Jacobs, 1986)). Contrary to localized
293 infusion, systemically treated mice were less capable of withholding licks during the waiting
294 periods as well as in NoGo trials, resulting in increased Impulsive rate and reduced Correct
295 Rejection rate, decision bias and detection sensitivity (Fig. 5a-c). These behavioral changes are
296 consistent with an increase of impulsivity, and mice behaved as if they were at the beginning of
297 normal behavior sessions (Fig. 5d).

298 To conclude, we found that localized and systemic yohimbine treatments (increasing LC
299 activity) exerted opposing behavioral effects. Localized infusion improved tactile detection, and
300 mice achieved near-optimal performance (Fig. 4f). In contrast, systemic treatment impaired
301 performance by promoting impulsivity (Fig. 5d).

302

303 **4. Discussion**

304 The current study is one of the first to investigate how bidirectional perturbations of LC-NE affect
305 quantitative perceptual task performance. We found that localized and systemic pharmacological
306 suppression of LC-NE similarly impaired tactile detection (decreased Hit and Impulsive rates,
307 elevated Correct Rejection rate and decision bias, and prolonged reaction time), suggesting that
308 a major site of action during systemic clonidine treatment is the LC.

309 Our results support previous findings that suppressing LC-NE signaling decreases arousal,
310 promotes sleep, and slows down reaction time (Sarro et al., 1987; Halliday et al., 1989; Berridge
311 et al., 1993; Turetsky and Fein, 2002; Hou et al., 2005; Carter et al., 2010). Given that the main

effect of suppressing LC-NE is to reduce arousal/motivation, the behavioral impairment is likely to be task-independent. A recent study showed that systemic clonidine did not affect decision bias (Gelbard-Sagiv et al., 2018). In this study human subjects were instructed to adjust their preparedness before initiating a new trial, which possibly engaged other arousal-promoting circuits (e.g., the cholinergic system (McGaughy et al., 1996)) to compensate the clonidine-induced decline of arousal/motivation (Thiele and Bellgrove, 2018).

In terms of activation, we found that localized yohimbine infusion in the LC improved tactile detection (increased Correct Rejection rate and detection sensitivity, and reduced Impulsive rate), while systemic yohimbine treatment impaired behavior (elevated Impulsive rate, and decreased Correct Rejection rate, decision bias and detection sensitivity). Our findings are consistent with others showing that systemic yohimbine increased impulsivity (e.g., (Swann et al., 2005, 2013; Sun et al., 2010)). The different behavioral effects between localized and systemic treatments suggest that increased impulsivity is likely due to yohimbine acting on presynaptic and postsynaptic α2 ARs (Starke et al., 1975; Szemeredi et al., 1991; Arnsten and Cai, 1993) in "off-target" α2-expressing regions, such as noradrenergic neurons in the nucleus of the solitary tract (Van Bockstaele et al., 1999; Kirouac, 2015), and the prefrontal cortex (Solanto, 1998; Arnsten, 2000; Ramos and Arnsten, 2007; Sun et al., 2010; Janitzky et al., 2015). It should be noted that yohimbine also has pronounced affinity to 5-HT1 receptors and dopamine D2 receptors (Millan et al., 2000). In addition, activating LC via localized or systemic administration of corticotropin-releasing factors differently affected rats performing an attention set shifting task (Snyder et al., 2012). Together, these findings strongly suggest that systemic yohimbine treatment, or in general non-specific NE activation, cannot be interpreted as specific manipulation of the LC-NE circuit.

Importantly, whether systemic (non-specific) NE activation impairs or improves task performance likely depends on the brain regions, the receptors (adrenergic and non-adrenergic), and the type of behavior task involved. For example, during systemic administration of the psychostimulant methylphenidate (MPH, an NE-DA reuptake inhibitor), enhanced NE release

338 acting on α1 ARs in the prefrontal cortex was reasoned to underlie the dose-dependent changes
339 in rats performing a sustained attention task (Berridge et al., 2006, 2012; Andrzejewski et al.,
340 2014; Spencer et al., 2015; Berridge and Spencer, 2016). On the other hand, activation of the
341 prefrontal α2 ARs and dopamine D1 receptors during MPH administration contributed to the
342 improved performance in a spatial working memory task (e.g., (Arnsten and Dudley, 2005;
343 Berridge et al., 2006)).

344 Our study could have implications for several neurological disorders, including attention-
345 deficit hyperactivity disorder (ADHD), for which one of the major diagnostic criteria is impulsive
346 behavior (Castellanos and Tannock, 2002). In children performing a Go/NoGo learning task, those
347 diagnosed with ADHD had a higher False Alarm rate than controls (e.g., (Iaboni et al., 1995)).
348 Mice with ADHD-phenotypes also exhibited higher False Alarm and Impulsive rates during
349 Go/NoGo motor tests (Majdak et al., 2016). Interestingly, this impulsive/distractible response has
350 been linked to high tonic LC activity (Rajkowski et al., 1994; Usher et al., 1999; Aston-Jones and
351 Cohen, 2005). Consistent with these findings, clonidine, and possibly other α2 agonists, can
352 suppress LC activity and reduce impulsivity (Mangeot et al., 2001; Berridge and Waterhouse,
353 2003).

354 We found that unilateral LC perturbation (contralateral to whisker stimulation) is sufficient
355 to produce pronounced behavioral changes. Since unilateral LC suppression mainly reduced
356 arousal/motivation, it suggests that this manipulation affects arousal-related circuits downstream
357 of LC, such as the basal forebrain cholinergic system and the preoptic area of the hypothalamus
358 (Jones and Moore, 1977; España and Berridge, 2006). Thus, we anticipate that the behavioral
359 impairment is laterality-independent, i.e., suppressing the LC ipsilateral to whisker stimulation
360 would similarly reduce arousal/motivation. We found that unilateral LC activation improves tactile
361 detection. In our behavior task, the right C2 whisker was stimulated, and yohimbine was infused
362 in the left LC. In rodents, the ascending whisker information is fully crossed in somatosensory
363 thalamus and cortex (Diamond et al., 2008), which in turn receive extensive innervations from the

ipsilateral LC (Simpson et al., 1997). Since unilateral LC activation improves detection sensitivity (d') while leaving decision bias (c) unaffected, our results imply that activating the left LC enhances the representation of the contralateral (right) whisker stimulation to improve task performance, potentially through NE modulating the ipsilateral (left) somatosensory thalamus and/or somatosensory cortex. This interpretation is in line with previous results showing that enhanced LC-NE signaling improves sensory processing in somatosensation-related areas (e.g., (Lecas, 2004; Devilbiss et al., 2006; Hirata et al., 2006; Vazey et al., 2018)). We anticipate that stimulating the right LC (ipsilateral to whisker stimulation) would not produce similar behavioral effects, and that the behavioral improvement is laterality- and task-dependent (e.g., perceptual vs. non-perceptual). However, it remains a possibility that unilateral LC activation could enhance bilateral LC responses (Marzo et al., 2014), and stimulating the right LC could produce similar behavioral improvement. Future experiments are needed to test these hypotheses.

Our localized yohimbine results support two recent studies testing how activating LC-NE affects perceptual task performance. In one, LC was optogenetically activated in rats performing a tactile frequency discrimination task (Rodenkirch et al., 2019). In another, LC-NE signaling was enhanced by using a selective NE reuptake inhibitor in human subjects performing visual detection/discrimination tasks (Gelbard-Sagiv et al., 2018). Regardless of the differences in species and perturbation methods, activating LC-NE improves sensitivity (d') and performance, suggesting that the behavioral enhancement is more specific to LC-NE acting on sensory processing-related areas. Future work is needed to examine how LC projections in different somatosensory areas differentially contribute to tactile perception, and how perturbing LC-NE modulates other types of behavioral tasks.

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Conflict of interest

We declare there are no competing financial interests in relation to the work described.

Author contributions

J.M.L., Y.S. and H.Y. planned the project. J.M.L., Y.S., and L.S.T. performed experiments. Q.A.N. and S.H.Y. helped with c-fos staining and quantification. J.M.L. and H.Y. built apparatus, analyzed data and wrote the manuscript with comments from all authors.

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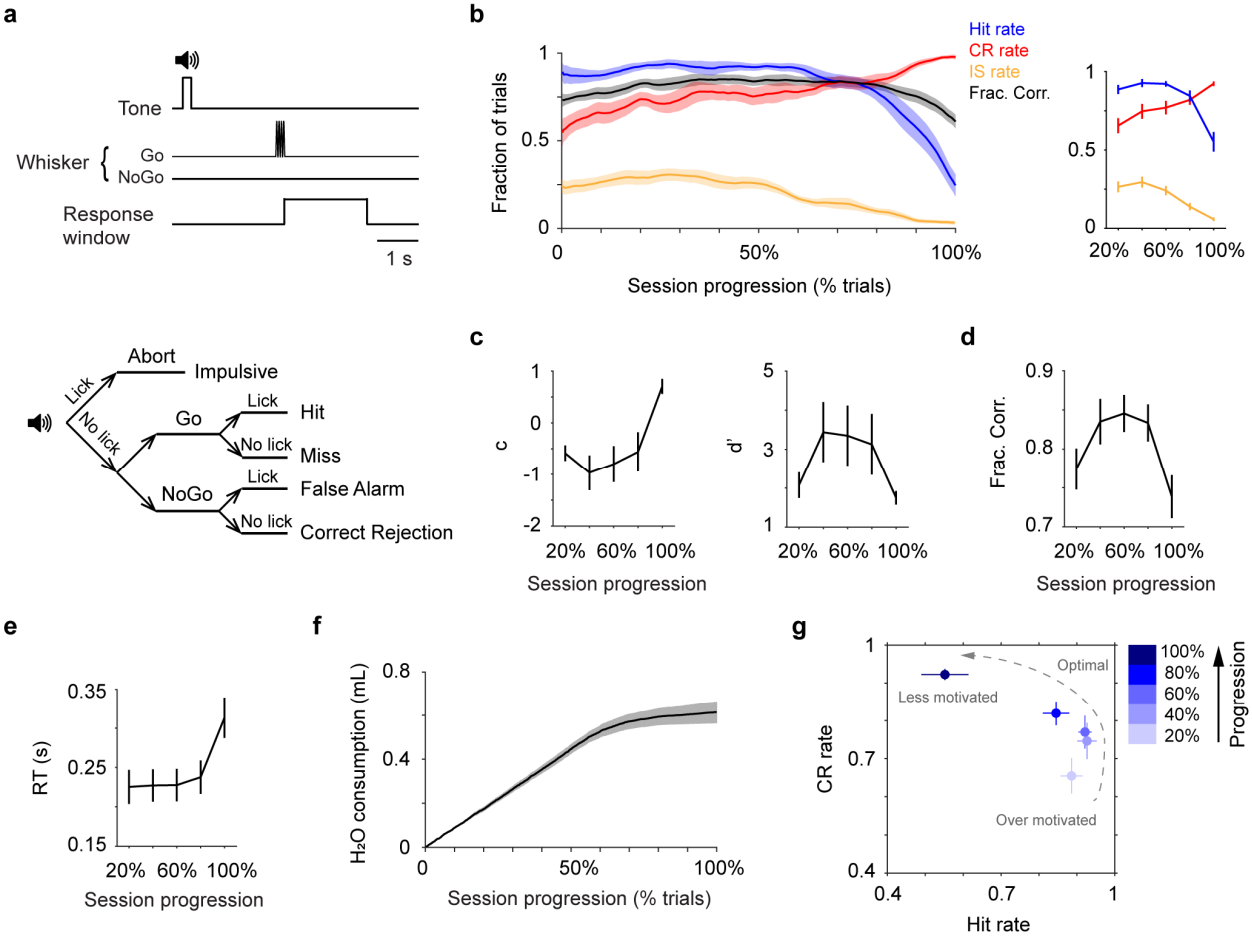

Figure 1. Mouse behavior fluctuates within single sessions. **a.** Trial structure (top) and the five possible trial types (bottom). **b.** Left: Mean single-session trajectories of Hit rate, CR rate, IS rate and overall performance (± s.e.m.). Behavioral sessions of different lengths (348 ± 20 trials, mean ± s.e.m., n = 13) are normalized using % total number of trials (session progression). Trajectories are smoothed using a moving window of 30 trials. Right: Trajectories of Hit, CR and IS rates averaged every 20% progression. **c.** Trajectories of decision bias (c) and detection sensitivity (d'), based on Hit and CR rates in **b**. **d.** Trajectory of overall performance (Fraction Correct shown in **b**, averaged every 20% progression) illustrates an inverted-U shape. **e**. Mean single-session trajectory of RT (± s.e.m.), averaged every 20% progression. **f.** Mean single-session trajectory of cumulated water consumption (± s.e.m.), based on an estimate of 5 µL dispense per Hit trial. **g.** CR rate vs. Hit rate trajectory, based on values in **b**. CR, Correct Rejection; IS, Impulsive; RT, Reaction Time.

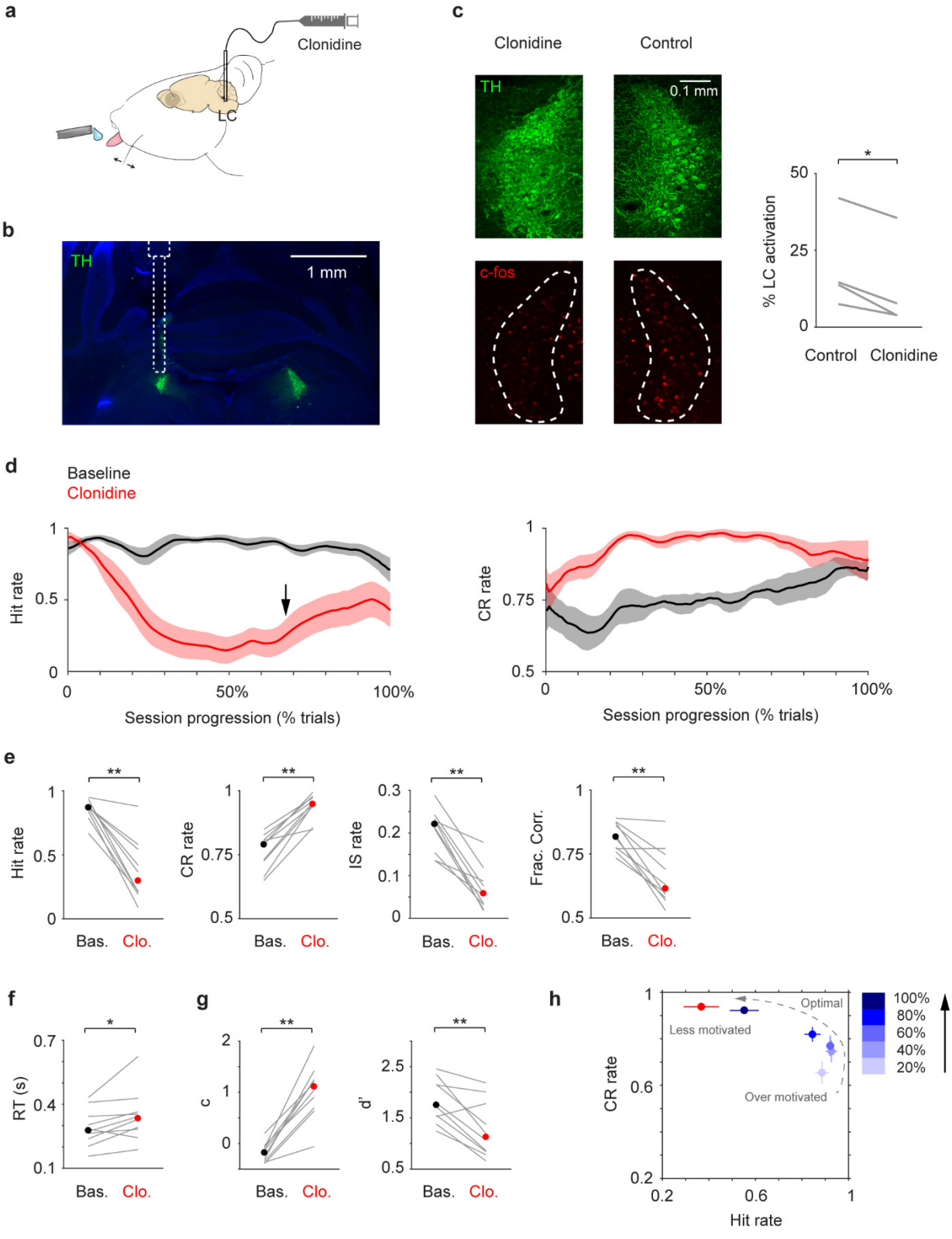

Figure 2. Localized clonidine infusion impairs tactile detection. **a.** Schematic of drug infusion setup. **b.** Histological section showing LC (green) and the tract of the infusion cannula, overlaid with an illustration of cannula placement. **c.** Left: Example c-fos expression (red) in the LC (green) after localized clonidine infusion. The contralateral LC serves as a basal level control. Right: c-fos expression was reduced upon clonidine infusion in 4 awake mice ($P = 0.014$, two-tailed paired t-test. Cell counts for individual mice are shown in Table 1). % LC activation was defined as the fraction of TH/c-fos double positive cells among TH positive cells. **d.** Mean single-session trajectories for Hit (left) and CR (right) rates during baseline and clonidine sessions (± s.e.m.). Baseline sessions were recorded one day before infusion. Black arrow indicates the onset of Hit rate recovery. **e-g.** Hit rate, CR rate, IS rate, Fraction Correct, RT, decision bias (c) and detection sensitivity (d') for baseline (black dot, median) and clonidine (red dot, median) sessions. Gray lines indicate individual consecutive two-day, baseline-clonidine pairs. Hit rate, $P = 0.002$, Signed rank = 55; CR rate, $P = 0.002$, Signed rank = 0; IS rate, $P = 0.002$, Signed rank = 55; Frac. Corr., $P = 0.0039$, Signed rank = 54; RT, $P = 0.019$, Signed rank = 5; c, $P = 0.002$, Signed rank = 0; d', $P = 0.0059$, Signed rank = 53. n = 10. **h.** CR rate vs. Hit rate trajectory showing clonidine reduces motivation (low Hit rate and high CR rate), which coincides with mouse behavior toward the end of normal baseline sessions. n.s., $P > 0.05$; * $P < 0.05$; ** $P < 0.01$.

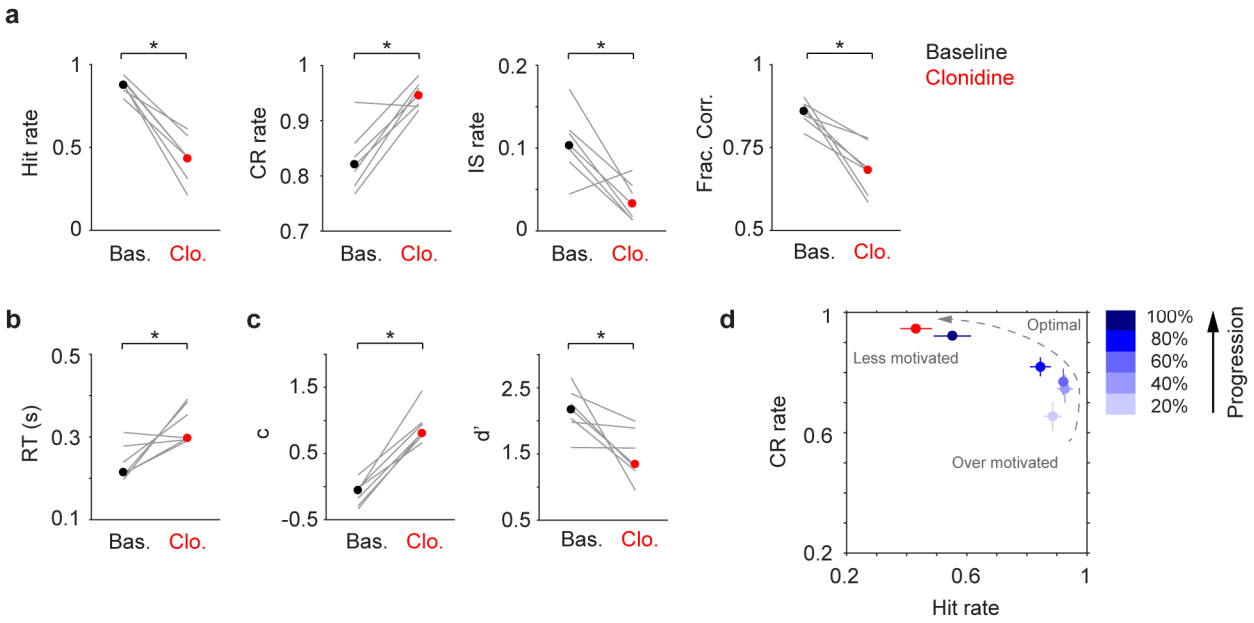

Figure 3. Systemic clonidine treatment impairs tactile detection. **a-c.** Hit rate, CR rate, IS rate, Fraction Correct, RT, decision bias (c) and detection sensitivity (d') for baseline (black dot, median) and clonidine (red dot, median) sessions. Gray lines indicate individual consecutive two-day, baseline-clonidine pairs. Hit rate, P = 0.016, Signed rank = 28; CR rate, P = 0.031, Signed rank = 1; IS rate, P = 0.031, Signed rank = 27; Frac. Corr., P = 0.016, Signed rank = 28; RT, P = 0.031, Signed rank = 1; c, P = 0.016, Signed rank = 0; d', P = 0.016, Signed rank = 28. n = 7. **d.** CR rate vs. Hit rate trajectory showing clonidine reduces motivation (low Hit rate and high CR rate), similar to localized infusion in **Fig. 2h**. * P < 0.05.

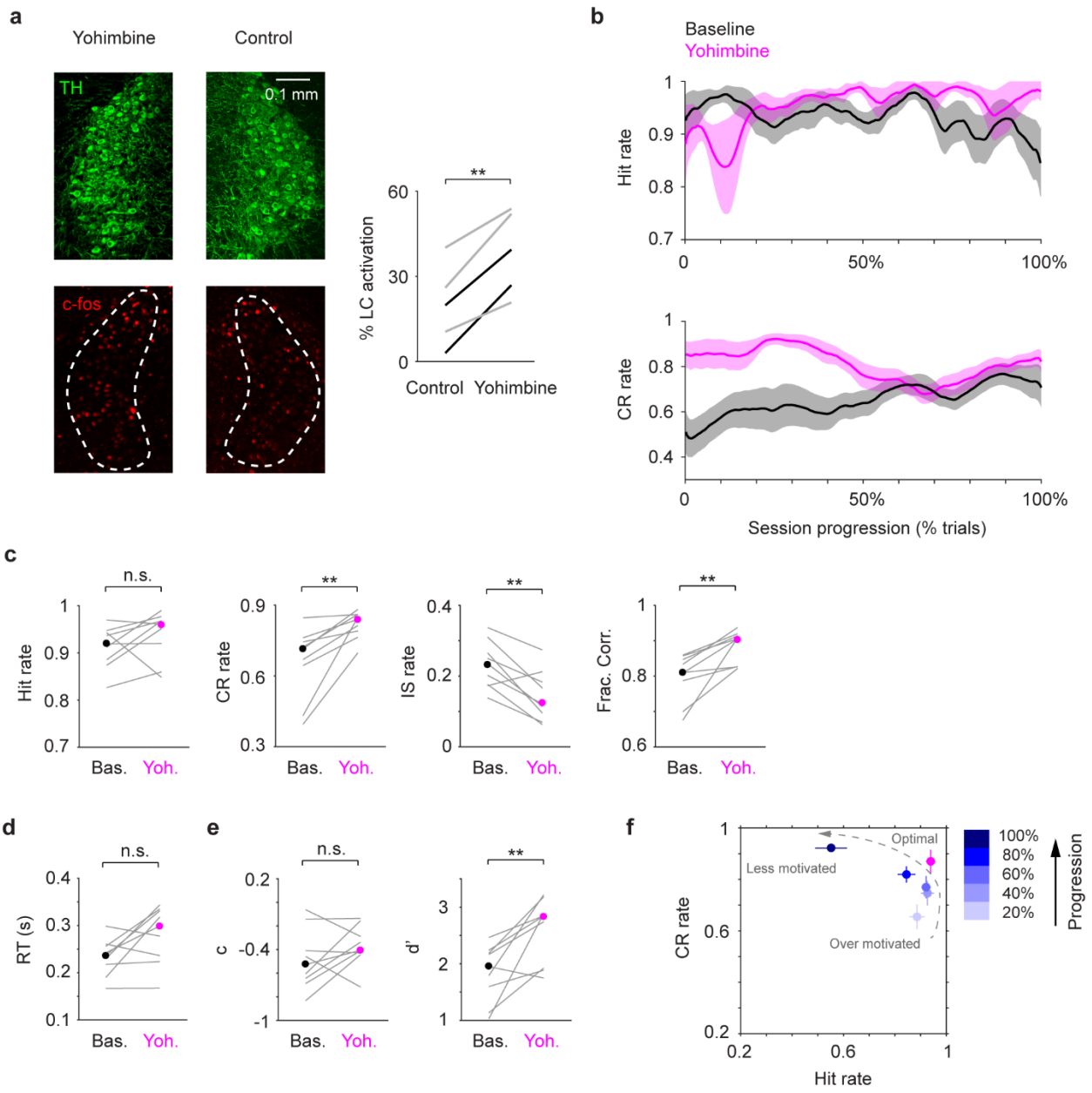

Figure 4. Localized yohimbine infusion improves tactile detection. **a.** Left: Example c-fos expression (red) in the LC (green) after localized yohimbine infusion. The contralateral LC serves as a basal level control. Right: c-fos expression was enhanced upon yohimbine infusion in 5 mice (P = 0.0033, two-tailed paired t-test. 2 under anesthesia, black lines; 3 during wakefulness, gray lines. Cell counts for individual mice are shown in Table 2). % LC activation was defined as the fraction of TH/c-fos double positive cells among TH positive cells. **b.** Mean single-session trajectories for Hit (top) and CR (bottom) rates during baseline and yohimbine sessions (± s.e.m.). Baseline sessions were recorded one day before infusion. **c-e.** Hit rate, CR

719 rate, IS rate, Fraction Correct, RT, decision bias (c) and detection sensitivity (d') for baseline (black dot,
720 median) and clonidine (magenta dot, median) sessions. Gray lines indicate individual consecutive two-day,
721 baseline-yohimbine pairs. Hit rate, P = 0.20, Signed rank = 11; CR rate, P = 0.0039, Signed rank = 0; IS
722 rate, P = 0.0078, Signed rank = 44; Frac. Corr., P = 0.0039, Signed rank = 0; RT, P = 0.074, Signed rank
723 = 7; c, P = 0.30, Signed rank = 11; d', P = 0.0078, Signed rank = 1. n = 9. **f.** CR rate vs. Hit rate trajectory
724 showing yohimbine transitioned mouse behavior to a near-optimal regime (high Hit rate and high CR rate),
725 similar to mouse behavior around the middle of normal baseline sessions. n.s., P > 0.05; ** P < 0.01.
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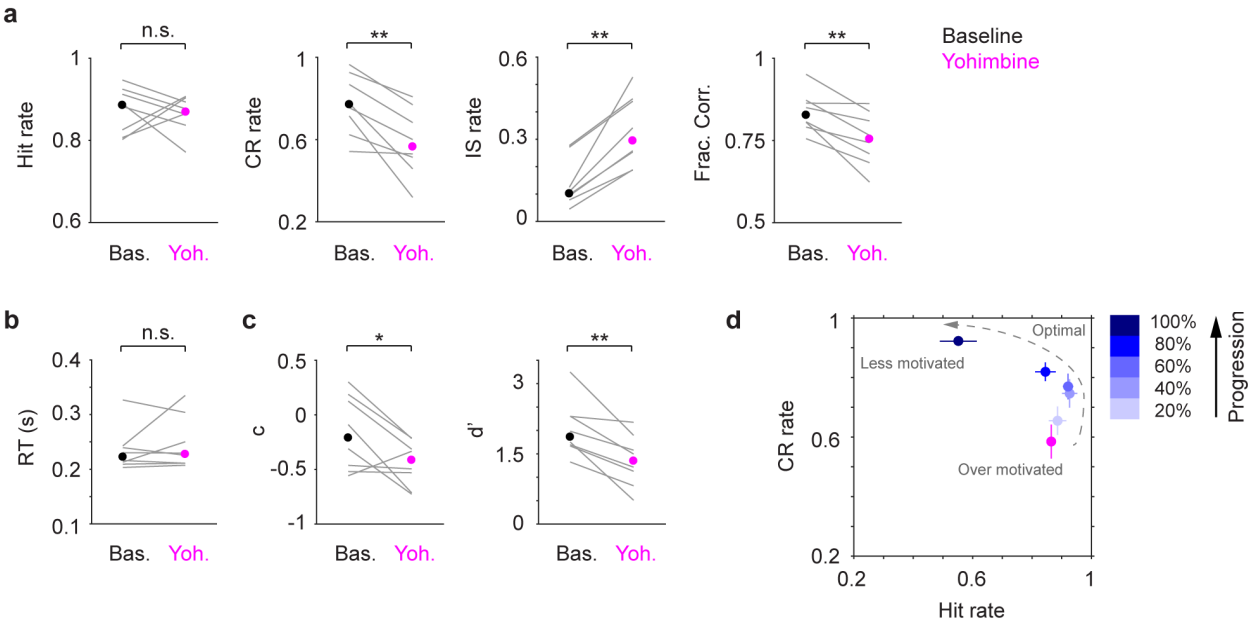

Figure 5. Systemic yohimbine treatment impairs tactile detection. **a-c.** Hit rate, CR rate, IS rate, Fraction Correct, RT, decision bias (c) and detection sensitivity (d') for baseline (black dot, mean) and yohimbine (magenta dot, mean) sessions. Gray lines indicate individual consecutive two-day, baseline-yohimbine pairs. Hit rate, P = 1, Signed rank = 18; CR rate, P = 0.0078, Signed rank = 36; IS rate, P = 0.0078, Signed rank = 0; Frac. Corr., P = 0.0078, Signed rank = 36; RT, P = 0.84, Signed rank = 16; c, P = 0.039, Signed rank = 33; d', P = 0.0078, Signed rank = 36. n = 8. **d.** CR rate vs. Hit rate trajectory showing yohimbine promotes impulsivity (high Hit rate and low CR rate), which coincides with mouse behavior at the beginning of normal baseline sessions. n.s., P > 0.05; * P < 0.05; ** P < 0.01.

Clonidine								
Mouse number	1		2		3		4	
	Left	Right	Left	Right	Left	Right	Left	Right
TH positive cells	424	406	389	252	299	325	169	178
TH/c-fos double positive cells	16	56	15	19	106	130	13	26
c-fos expression level (%)	3.8	13.8	3.9	7.5	35.5	40.0	7.7	14.6
	P < 1e-5		P = 0.0021		P = 0.060		P = 0.0049	

Table 1. Quantification of c-fos expression to examine the effect of localized clonidine infusion on LC activity in 4 awake mice. Clonidine was infused in the left LC. The right LC serves as a basal level control. Permutation test was performed (10^5 iterations) to compare c-fos expression levels between the left and right LC in individual mice.

Yohimbine										
Mouse number	1		2		3		4		5	
	Left	Right	Left	Right	Left	Right	Left	Right	Left	Right
TH positive cells	531	536	416	402	100	196	355	382	501	458
TH/c-fos double positive cells	209	106	112	12	52	51	191	153	104	48
c-fos expression level (%)	39.4	19.8	26.9	3.0	52.0	26.0	53.8	40.1	20.8	10.5
	P < 1e-5		P < 1e-5		P < 1e-5		P < 1e-5		P < 1e-5	

Table 2. Quantification of c-fos expression to examine the effect of localized yohimbine infusion on LC activity in 5 mice. Yohimbine was infused in the left LC (Mouse 1 and 2: anesthesia; Mouse 3-5: awake). The right LC serves as a basal level control. Permutation test was performed (10^5 iterations) to compare c-fos expression levels between the left and right LC in individual mice.