

## BEHAVIORAL NEUROSCIENCE

# Metabotropic glutamate receptor blockade in nucleus accumbens shell shifts affective valence towards fear and disgust

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*Keywords:* defensive treading, eating, motivation, rat, taste reactivity

## Abstract

Glutamatergic inputs to the nucleus accumbens (NAc) modulate both appetitive and fearful motivation. It has been suggested that pathological disturbances of glutamate signaling in NAc contribute to motivation disorders, ranging from excessive desire in drug addiction to paranoia in schizophrenia. Metabotropic glutamate receptors are of special interest, as metabotropic Group II receptor (mglu2/3) agonists have been proposed as potential treatments for both addiction and schizophrenia. Here we tested whether local mglu2/3 receptor blockade in the medial shell of the rat NAc can generate intense distortions of motivation or affect, which might model clinical dysfunction. We found that microinjection of the mglu2/3 antagonist LY341495 at sites throughout medial shell suppressed appetitive motivation to eat and drink. Simultaneously, LY341495 microinjections generated fearful motivation in the form of defensive treading or burying. To assess whether the valence shift extended into a parallel hedonic shift from affective 'liking' to 'disliking' we employed the taste reactivity test, which measures affective orofacial reactions to the sensory pleasure or displeasure of tastes. We found that LY341495 microinjections reduced positive 'liking' reactions to sucrose and enhanced 'disliking' reactions. Overall, mglu2/3 antagonism at most shell sites produced a similar valence shift from positive to negative. This pattern comprised (i) generation of fearful behaviors, and (ii) induction of aversive affective reactions, together with (iii) loss of appetitive ingestion and (iv) loss of 'liking' for rewards. These results are discussed in terms of implications for clinical disorders and the influence of corticolimbic glutamate inputs to NAc in the generation of motivation and affect.

## Introduction

The nucleus accumbens (NAc) is best known for its role in appetitive motivated behaviors such as reward seeking (Ikemoto & Panksepp, 1999; Cardinal & Everitt, 2004; Kalivas & Volkow, 2005; Kelley *et al.*, 2005; Nicola, 2007; Sesack & Grace, 2010), but it also plays a role in negatively valenced motivation and affect, such as fear, pain, stress and disgust (Blackburn *et al.*, 1992; Salamone, 1994; Horvitz, 2000; Levita *et al.*, 2002, 2008; Reynolds & Berridge, 2002; Scott *et al.*, 2006; Kerfoot *et al.*, 2007; Delgado *et al.*, 2008; Carlezon & Thomas, 2009). Glutamate inputs to NAc medial shell participate in the bivalent generation of motivations (Maldonado-Irizarry *et al.*, 1995; Kelley & Swanson, 1997; Reynolds & Berridge, 2003), and anatomically arise predominantly from prefrontal cortex and cortex-related structures such as basolateral amygdala, hippocampus and thalamus (Beckstead, 1979; Christie *et al.*, 1987; Fuller *et al.*, 1987; Sesack *et al.*, 1989; McDonald, 1991; Swanson, 2005; Wolf *et al.*, 2005; Zahm, 2006; Belujon & Grace, 2008). Disruptions of glutamate signaling are implicated in a variety of motivation disorders including addiction, depression and schizophrenia (Moghaddam, 2004; Lapish

*et al.*, 2006; Uys & LaLumiere, 2008; Floresco *et al.*, 2009; Guo *et al.*, 2009; Kalivas, 2009).

Metabotropic glutamate receptors may be especially implicated in psychopathology: specifically, the metabotropic Group II glutamate receptors (mglu2/3) subtypes have been suggested as targets for the pharmacological treatment of depression, anxiety, schizophrenia and addiction (Kenny & Markou, 2004; Moghaddam, 2004; Swanson *et al.*, 2005; Patil *et al.*, 2007). Group II mglu receptors are prevalent in NAc as well as other limbic structures (Kenny & Markou, 2004; Gu *et al.*, 2008) and there is evidence that agonists for NAc mglu2/3 receptors may suppress reinstatement of drug seeking and conditioned place preference (Markou *et al.*, 2004; Bossert *et al.*, 2006; Gerdjikov & Beninger, 2006; Liechti *et al.*, 2007). Given the increasing interest in agonists for mglu2/3 receptors as potential medications for disorders of motivation, we wanted to understand whether blocking mglu2/3 receptors could conversely induce any pathological motivational states.

Here, we investigated whether localized blockade of mglu2/3 receptors in NAc medial shell modulates the generation of motivated behavior or affective reactions to tastes. Following microinjections of the mglu2/3 antagonist LY341495 into the NAc shell, rats were tested for spontaneous appetitive eating and drinking and for fearful behavior in the form of defensive treading, an antipredator behavior which

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Received 30 June 2010, revised 12 October 2010, accepted 8 November 2010

rodents emit in the wild to rattlesnakes and in the laboratory to shock prods and other noxious objects (Coss & Owings, 1978; Treit *et al.*, 1981). We also tested for modulation of affective reactions elicited by sweet or bitter tastes infused into the rat's mouth. Disruptions of mglu2/3 transmission were found to suppress feeding and drinking behavior while promoting defensive treading behavior, suggesting a fearful or anxious state. Independently, in the taste reactivity paradigm we found that mglu2/3 disruptions suppressed 'liking' reactions to sweetness and conversely increased 'disliking' reactions of disgust that are normally reserved for bitter tastes.

## Materials and methods

### Synopsis

In order to independently test the effect of mglu2/3 blockade on motivated behaviors and affective reactions to taste, rats were randomly separated into food intake and taste reactivity groups. Independent groups of rats were tested for the effects of mglu2/3 blockade on generation of spontaneous appetitive and fearful motivated behavior, or modulation of 'liking' reactions to the taste of sweet sucrose solution and for 'disliking' reactions to bitter quinine solution in the taste reactivity test. Additionally, we analyzed local Fos expression in order to assess the functional impact and spread of drug microinjections on surrounding tissue.

### Subjects

Sprague–Dawley rats (male,  $n = 44$ : food intake testing,  $n = 16$ ; taste reactivity testing,  $n = 14$ ; Fos plume analysis,  $n = 14$ ; 300–500 g at the time of surgery) were housed on a 12 : 12-h reverse light:dark cycle at  $\sim 21$  °C with *ad lib* access to food (Purina Rat Chow) and water (tap water). All experimental procedures were approved by the University Committee on the Use and Care of Animals at the University of Michigan.

### Surgery

All rats were implanted with permanent bilateral cranial cannulae aimed at points throughout the anatomical extent of the medial shell of NAc or at anatomical control sites. The coordinates used for each rat were bilaterally symmetrical, but placements were staggered across rats in anteroposterior and dorsoventral planes so that the group's placements filled the medial shell. Rats were placed under surgical anesthesia using ketamine (80 mg/kg) and xylazine (5 mg/kg) and pretreated with atropine (0.04 mg/kg) to prevent respiratory distress, after which they were placed in a stereotaxic apparatus (David Kopf Instruments). All rats received bilateral implantation of 14-mm 23-gauge stainless steel guide cannulae aimed 2 mm above target sites. The incisor bar was set at 5.0 mm above intra-aural zero so that cannulae were angled to avoid the lateral ventricles. For medial shell rats ( $n = 41$ ) placements were adjusted in order to achieve microinjection sites spaced throughout the shell, and were aimed at coordinates anteroposterior (AP) +2.4 to +3.4, mediolateral (ML)  $\pm 0.8$  to 1.0 mm and dorsoventral (DV)  $-5.5$  to  $-5.7$  mm in comparison to bregma. Anatomical control rats ( $n = 3$ ) received cannulae aimed at sites dorsal and medial to NAc shell, including the intermediate and ventral areas of the lateral septum. Cannulae were anchored to the skull using four surgical screws and dental acrylic, and stainless steel obturators were inserted to avoid cannulae occlusion. An independent group ( $n = 14$ ), designated for taste reactivity testing, received implantation of permanent oral cannulae for the infusion of

taste solutions in addition to the microinjection cannulae in medial shell. Oral cannulae were made of ethyl vinyl acetate microbore tubing (0.04 inch inner diameter; Cole-Parmer, Vernon Hills, IL, USA), and each was inserted into the mouth just lateral to the first maxillary molar and run subcutaneously along the zygomatic arch to the top of the skull, where the cannula exited through an incision. There, the tubing was secured to a 15-mm 19-gauge stainless steel cannula with wiring and dental acrylic. Post-surgery, each rat received chloramphenicol sodium succinate (60 mg/kg) to prevent infection, and buprenorphine hydrochloride (0.3 mg/kg) for pain relief. Rats recovered for at least 7 days before testing began.

### Drugs and intracerebral microinjections

We dissolved the selective and highly potent antagonist for mglu2/3 receptors, (2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xanth-9-yl) propanoic acid (LY341495; Tocris) in  $\sim 10$   $\mu$ L of 0.1 M NaOH and brought to dose volume using 0.9% sterile saline solution. The pH was normalized to 7.4 using HCl for both drug and vehicle microinjections. Rats received the following microinjections, counterbalanced for order and spaced 48 h apart: either 5  $\mu$ g LY341495 in 0.5  $\mu$ L vehicle per side, or vehicle alone 0.5  $\mu$ L per side as within-subject control. At a speed of 0.3  $\mu$ L/m, drugs were bilaterally infused through stainless steel injectors (29-gauge) which extended 2 mm beyond the guide cannula into the NAc shell and were attached to the syringe pump via PE-20 tubing. Following infusion, injectors were left in place for 1 min to allow drug to diffuse away from the microinjector tip, after which obturators were replaced and rats were placed in the testing chamber.

### Behavioral tests of fear and feeding

To assess spontaneous motivated behaviors, following 3 days of preexposure in the home cage to palatable chocolates (M&Ms) in order to counter any neophobia, and 3 days of handling, rats implanted with intracranial cannulae ( $n = 16$ ) were preliminarily habituated to the food intake procedure and apparatus for 4 days. On the fourth day of habituation rats also received a mock microinjection of vehicle prior to entering the testing chamber, in order to habituate them to the microinjection procedure. On the test day, rats received either LY341495 or vehicle and were placed immediately in the transparent testing chamber, which contained  $\sim 3$  cm deep of bedding, *ad lib* water and  $\sim 20$  g of preweighed M&Ms. These stimuli allowed the expression of eating and drinking behavior as well as of defensive treading behavior (using the granular bedding). Behavior was taped for 60 min post-microinjection, and scored later offline for analysis.

### Behavioral taste reactivity test

Rats ( $n = 14$ ) implanted with oral cannulae and intracranial cannulae were habituated to the taste reactivity chambers for 4 days prior to testing. On the fourth day of habituation rats received mock microinjection of vehicle, and mock infusions of distilled water. On the test days rats received either LY341495 or vehicle, and PE-50 tubing was attached to an oral cannula. Rats were then placed immediately in the taste reactivity chamber, which had a transparent floor under which was angled a mirror from which reactions were taped. After a 10-min delay a 1 mL volume of either sucrose (100 mM) or quinine (30  $\mu$ M) was infused into the rat's mouth over a 1-min period. Each rat was retested again at 35 min after microin-

jection with a second 1-mL infusion of the same taste solution to examine the time course of modulation. Sucrose solution and quinine solution were presented on separate days, for a total of four test days (once after LY341495 microinjection, once after vehicle microinjection) in an alternating order that was counterbalanced across rats. Orofacial reactions to taste were videorecorded and analyzed later offline by an observer who was blind to the drug condition.

### Behavioral video scoring

#### Spontaneous fear and feeding behaviors

An experimenter blind to treatment scored each tape for the total time (seconds) rats spent eating (mouth in contact with food pellet or continual chewing movements), drinking (tongue in contact with spout), defensive treading (rapid, alternating thrusts of the forepaws, which spray or push bedding material to the front of the rat) and grooming. In addition to total time spent in each behavior, the experimenter recorded the number of bouts, or episodes, of appetitive behavior as well. For appetitive motivated behaviors (eating and drinking) the end of a bout was marked by a pause in behavior of at least 5 s (which typically extended into a much longer break in behavior). A total number for each behavior was scored for more discrete events including food carrying (grasping and transportation of food pellets in the mouth), food sniffs, rears and cage crosses.

#### Hedonic taste reactivity scoring

A trained observer, blind to experimental and taste solution condition, scored the hedonic, aversive and neutral reactions to tastes in slow motion (1/25 to 1/10 speed) on Observer software (Noldus, Netherlands). Totals for each type of reaction were tallied using a bin system which was designed to allow comparison of hedonic and aversively valenced reactions, and is described elsewhere (Berridge & Grill, 1984; Berridge, 2000). Hedonic reactions included rhythmic tongue protrusions along the midline, lateral tongue protrusions and paw-licks, whereas aversive reactions consisted of face washing, gapes, chin rubs, forelimb flails and headshakes. Neutral reactions to tastes are less consistently linked to hedonic valence, and consist of neutral mouth movements, passive drip and grooming behavior. Behaviors which typically occur as discrete events were counted each time they occurred; they included lateral tongue protrusions, gapes, chin rubs, forelimb flails and headshakes. Behaviors which occur generally in succession were binned based on the typical length and frequency of these reactions; tongue protrusions were scored in 2-s bins and paw-licks, face washing, mouth movements and grooming consisted of 5-s bins.

### Histology

Following all testing, rats used for behavioral tests were deeply anesthetized with an overdose of sodium pentobarbital and decapitated. Brains were removed and fixed in 10% paraformaldehyde overnight, and then cryoprotected in 25% sucrose solution for at least 2 days. Brains were then sliced at 60  $\mu$ m on a freezing microtome, and stained with Cresyl violet for verification of microinjection sites. Bilateral microinjection sites were placed on coronal slices from a rat brain atlas (Paxinos & Watson, 2007). Later these placements were used to position bilateral injection sites for each rat onto sagittal and horizontal slices, which were then color-coded for behavioral changes in the figures.

In order to quantify localization of function for mglu2/3 receptors within medial shell, we also measured functional spread of drug via

changes in local Fos expression in a separate group of rats ( $n = 14$ : vehicle, 6; LY341495, 8). Local plumes of modulated Fos expression are produced surrounding the microinjection site of drugs such as DNQX, which blocks AMPA–kainate receptors, as well for drugs that modulate neurotransmission at opioid, cannabinoid, dopaminergic or GABAergic synapses (Peciña & Berridge, 2000, 2005; Smith & Berridge, 2005; Pecina *et al.*, 2006; Mahler *et al.*, 2007; Faure *et al.*, 2008, 2010). Here, following a single microinjection of either LY341495 or vehicle, brain slices were processed for Fos expression and analyzed for local Fos plumes as described previously (Peciña & Berridge, 2005; Smith & Berridge, 2005, 2007; Mahler *et al.*, 2007; Faure *et al.*, 2008; Reynolds & Berridge, 2008).

### Statistical analysis

The effect of drug microinjection on behaviors of interest was tested using both one-factor (drug) repeated-measures ANOVA and/or a two-factor mixed within- and between-subjects ANOVA (drug  $\times$  anatomical level: rostral, middle and caudal; or dorsal and ventral). Taste reactivity data was also analyzed using a two-factor within-subject ANOVA (drug  $\times$  time). For statistical analysis rostral or caudal classification was determined by placing rats with AP coordinates  $> +1.8$  mm ahead of bregma in the rostral group, and rats with AP coordinates  $< +1.6$  mm ahead of bregma in the caudal group. Dorsal versus ventral classification was determined by placing rats with DV coordinates  $> 7.6$  mm below the skull in the ventral group, and rats with DV coordinates  $< 7.4$  mm below the skull in the dorsal group. Rats with placements between these cut-offs, or with asymmetrical placements in both halves, were designated as middle. For the purposes of visualizing changes in behavior produced at particular sites, the anatomical placements of rats were divided further into six rostrocaudal and six dorsoventral bins and data from each bin was represented in a bar graph superimposed next to maps of behavioral effects produced at individual sites in each figure.

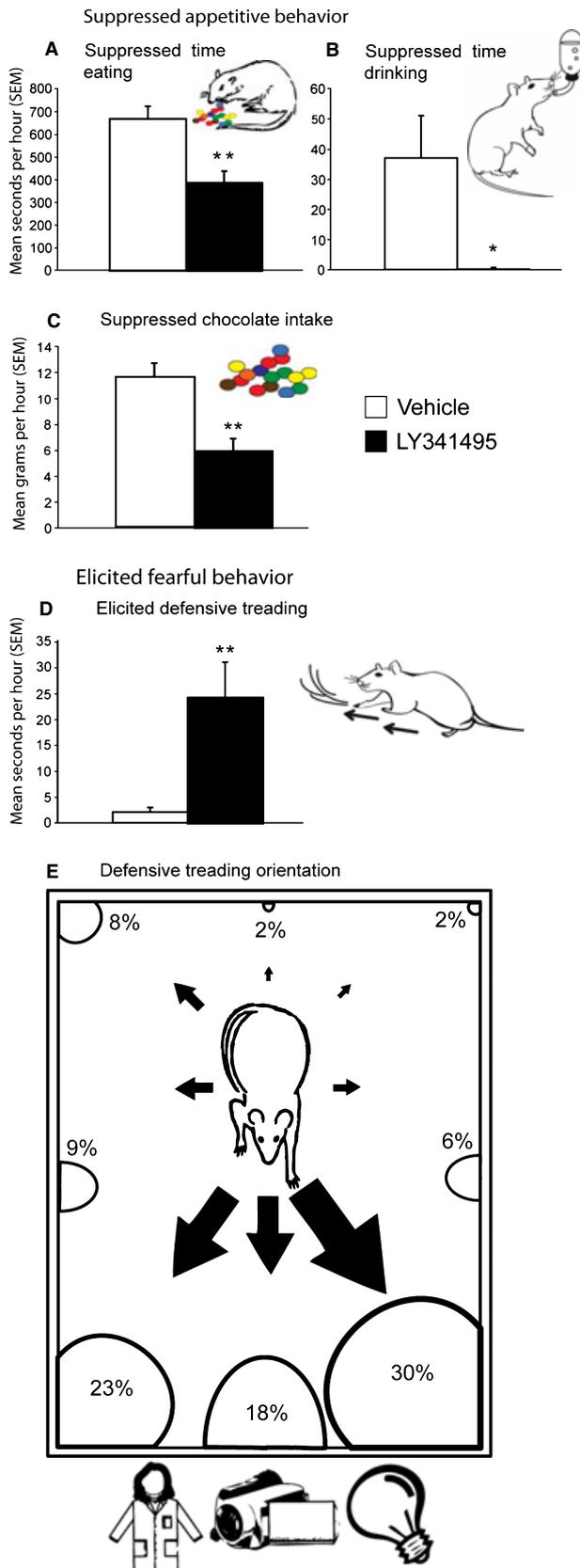
## Results

### LY341495 suppressed appetitively motivated behaviors throughout medial shell

Microinjections of the mglu2/3 antagonist LY341495 in the medial shell suppressed eating behavior and intake of palatable chocolate to below half of vehicle control levels (main effect of drug: M&M intake grams,  $F_{1,15} = 14.837$ ,  $P = 0.002$ ; eating duration,  $F_{1,15} = 10.522$ ,  $P = 0.006$ ; Figs 1A and C, and 2). The reduction in eating behavior reflected primarily a decrease in the number of ingestive bouts initiated ( $F_{1,15} = 8.770$ ,  $P = 0.011$ ) more than a decrease in the duration spent eating after initiation (main effect of drug,  $F_{1,15} = 1.672$ ,  $P = 0.111$ ), suggesting that LY341495 disrupted the initiation of eating more than its maintenance. Similarly, LY341495 microinjections also suppressed drinking behavior and water intake to nearly zero (main effect of drug,  $F_{1,15} = 7.835$ ,  $P = 0.015$ ; Fig. 1B). Nearly all sites within the medial shell were equally effective for LY341495 suppression of eating and drinking behaviors, suggesting the entire medial shell is relatively homogeneous for mglu2/3 appetitive suppression. Specifically, there were no differences between the intensity of suppression induced at rostral sites versus caudal sites (drug  $\times$  rostrocaudal level interaction: M&M intake grams,  $F_{2,14} = 0.900$ ,  $P = 0.449$ ; eating duration,  $F_{2,14} = 2.153$ ,  $P = 0.187$ ; drinking time,  $F_{2,14} = 1.597$ ,  $P = 0.268$ ) or dorsal versus ventral sites (drug  $\times$  dorsoventral level interaction: M&M intake grams,  $F_{2,14} = 0.812$ ,  $P = 0.482$ ; eating duration,  $F_{2,14} = 1.700$ ,  $P = 0.250$ ;

drinking time,  $F_{2,14} = 2.366$ ,  $P = 0.164$ ; Fig. 2A). The suppression of behavior by mglu2/3 blockade was relatively specific to consumption of food and water, as LY341495 did not significantly affect other food-

related behaviors such as food carrying or food sniffs (main effect of drug, food carrying,  $F_{1,15} = 0.147$ ,  $P = 0.482$ ; mean  $\pm$  SEM,  $7.94 \pm 1.42$  vs.  $8.75 \pm 1.70$  on vehicle; food sniffs,  $F_{1,15} = 0.066$ ,  $P = 0.909$ ; mean  $\pm$  SEM,  $17.1 \pm 2.40$  vs.  $17.5 \pm 1.56$  on vehicle).



### LY341495 generated fearful antipredator behavior throughout medial shell

Microinjections of the mglu2/3 antagonist LY341495 also generated defensive treading behavior at an intensity almost 10 times above vehicle levels (which are generally near zero; main effect of drug,  $F_{1,15} = 6.310$ ,  $P = 0.026$ ; mean  $\pm$  SEM,  $24.1 \pm 6.90$  vs.  $2.59 \pm 1.21$  s on vehicle; Fig. 1D). Supporting the idea that defensive treading was a motivated fearful behavior emitted towards particular stimuli perceived as threatening by the rat, the treading behavior after LY341495 was not randomly directed throughout the cage (i.e., as a motor stereotypy), but was typically 3–10 times more likely to be focused and directed toward a small set of specific stimulus targets: either the transparent front of the cage which revealed objects in the room and lights ( $18 \pm 3\%$  SEM to the revealed front versus  $2 \pm 1.53\%$  SEM to the back) or its brightly lit, reflective corners ( $23$  and  $31 \pm 7\%$  SEM to the front corners versus  $6$  and  $9\%$  to the sides of the chamber; main effect of direction,  $F_{1,13} = 4.590$ ,  $P < 0.001$ ; Fig. 1E). The strong directional focus of the defensive burying and treading movements stimulated by LY341495 supports the interpretation of this behavior as a coordinated defensive reaction, reflecting a fearful motivated state. Like the feeding and drinking effects, defensive treading was elicited by LY341495 at nearly every site throughout the medial shell, and not limited to a particular rostral or caudal zone (drug  $\times$  rostrocaudal level,  $F_{2,14} = 0.243$ ,  $P = 0.790$ ) or dorsal or ventral zone (drug  $\times$  dorsoventral level,  $F_{2,14} = 1.076$ ,  $P = 0.436$ ; Fig. 2B).

### Other behaviors

General changes in activity level or capacity do not appear to account for the changes in motivated behavior described above, as LY341495 did not alter normal levels of locomotor behavior (main effect of drug: rears,  $F_{1,15} = 0.299$ ,  $P = 0.723$ , mean  $\pm$  SEM,  $97.1 \pm 13.1$  vs.  $101.4 \pm 8.62$  on vehicle; cage crosses,  $F_{1,15} = 1.302$ ,  $P = 0.307$ , mean  $\pm$  SEM,  $91.5 \pm 13.5$  vs.  $77.4 \pm 8.24$  on vehicle). Burrowing and burrow-treading were generally not observed and were not elevated by LY341495 microinjections. The drug did moderately suppress the time spent in grooming behavior (main effect of drug,  $F_{1,15} = 5.320$ ,  $P = 0.038$ ) to about two-thirds of vehicle levels (mean  $\pm$  SEM,  $196.7 \pm 49.2$  vs.  $291.1 \pm 34.4$  s on vehicle), but that may have been due partly to the reduction in number of eating bouts, which would in turn reduce the incidence of post-prandial grooming. In general, LY341495 microinjections in medial shell appeared to most specifically suppress appetitive behavior and to stimulate defensive behavior.

FIG. 1. Motivated behavior summary. Changes in (A) eating duration, (B) drinking duration, (C) food intake and (D) defensive treading behavior elicited by vehicle or LY341495 microinjections into NAc shell. (E) Directional orientation of defensive treading elicited by LY341495 is shown by the size of arrows pointing from rat and size of semicircles in each direction (numbers are mean percentage of treading in each direction). Defensive treading was typically pointed at the transparent front of the chamber (which revealed sights of the experimenter and objects in the room beyond), or at corners of the plastic tub chambers (which may have reflected back bright patterns of light). We interpret the directedness of treading as indicating that the behavior was fearfully motivated and targeted toward specific stimuli that the rat perceived as most threatening, rather than merely a motor stereotypy. Data shown are combined for rats with rostral and caudal microinjection sites. Error bars indicate SEM; \* $P < 0.05$ , \*\* $P < 0.01$ .

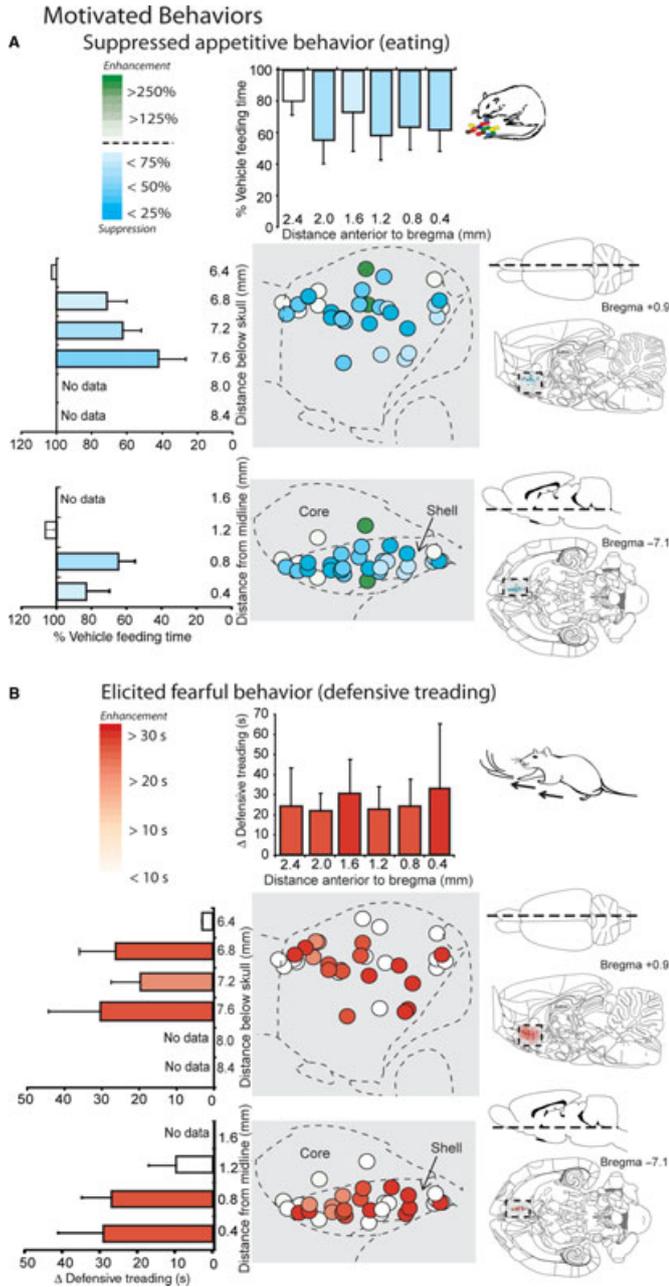


FIG. 2. Motivation shifts: LY341495 in NAc shell suppressed appetitive eating and drinking but stimulated fearful motivated behavior. Sagittal and horizontal maps of (A) appetitive eating and (B) defensive treading behavior generated by LY341495 mglu2/3 antagonism. Changes in the cumulative duration of eating behavior are shown as percentages of vehicle, while defensive treading behavior is shown as unit change from vehicle (given near-zero levels of treading baseline). LY341495 microinjections in medial shell suppressed appetitive eating and enhanced defensive behavior throughout medial NAc shell. Histogram bars below and to the sides of the maps show mean values (error bars are SEM) for each behavior at rostrocaudal, dorsoventral and mediolateral levels.

*LY341495 suppressed hedonic reactions and increased aversive reactions to sucrose*

Because LY341495 strongly suppressed motivation ‘wanting’ to eat, at least as reflected in intake of palatable chocolates, we further tested for any corresponding change in ‘liking’ reactions to the hedonic impact of food reward. In the taste reactivity test, liquid sucrose infusions into

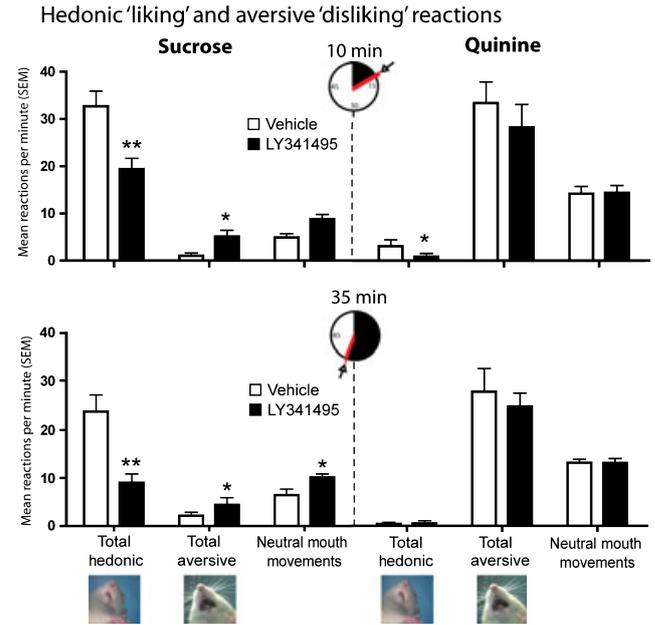


FIG. 3. Affective reactions summary. Changes in hedonic, aversive and neutral mouth movements elicited by sucrose (left) at 10 min (top) and 35 min (bottom), or by quinine (right) at 10 min (top) and 35 min (bottom) after microinjection of vehicle or LY341495 into accumbens shell. Data shown are combined for rats with rostral and caudal microinjection sites. Error bars indicate SEM; \* $P < 0.05$ , \*\* $P < 0.01$ .

the mouth ordinarily elicit primarily positive hedonic reactions (rhythmic midline tongue protrusions, lateral tongue protrusions or lip licking, and paw licking behaviors) after control vehicle microinjections, and all these hedonic components were suppressed by LY341495 (Figs 3 and 4). The number of positive hedonic reactions to sucrose taste was reduced by blockade of mglu2/3 receptors with LY341495 in medial shell to less than half of vehicle control levels (main effect of drug,  $F_{1,10} = 20.171$ ,  $P = 0.001$ ; mean  $\pm$  SEM,  $9.07 \pm 1.79$  vs.  $23.9 \pm 3.46$  on vehicle; Fig. 3). Blockade of mglu2/3 receptors suppressed hedonic reactions homogeneously at all sites throughout medial shell (drug  $\times$  rostrocaudal level interaction,  $F_{1,9} = 1.018$ ,  $P = 0.426$ ; drug  $\times$  dorsoventral level interaction,  $F_{1,9} = 1.091$ ,  $P = 0.344$ ; Fig. 4). Suppression of positive hedonic reactions by LY341495 was consistent at both 10 and 35 min after microinjection (time  $\times$  drug interaction,  $F_{1,10} = 0.413$ ,  $P = 0.535$ ), though there was an overall reduction in hedonic reactions between the 10- and 35-min infusions, regardless of drug condition (main effect of time,  $F_{1,10} = 11.419$ ,  $P = 0.007$ ; Fig. 3). Conversely, mglu2/3 receptor blockade with LY341495 increased overall aversive reactions to sucrose (e.g., gapes), which normally are elicited only by bitter quinine or other avoided tastes (main effect of drug,  $F_{1,10} = 12.653$ ,  $P = 0.005$ ; drug  $\times$  rostrocaudal level interaction,  $F_{1,9} = 0.916$ ,  $P = 0.458$ ; drug  $\times$  dorsoventral interaction,  $F_{1,9} = 0.163$ ,  $P = 0.703$ ; Figs 3 and 5). Aversive reactions were almost quadrupled over near-zero control levels when sucrose was tested at 10 min after LY341495 microinjection (mean  $\pm$  SEM,  $3.93 \pm 1.29$  vs.  $1.07 \pm 0.578$  on vehicle), and still more than doubled later at 35 min (mean  $\pm$  SEM,  $4.43 \pm 1.49$  vs.  $2.14 \pm 0.725$ ; Figs 3 and 5). Finally, the relatively non-valenced neutral mouth movements were moderately enhanced, by  $\sim 50$ – $75\%$ , which may also be consistent with a shift in the perception of sweet taste from highly positive to more mixed in valence (main effect of drug,  $F_{1,10} = 6.223$ ,  $P = 0.032$ ; mean  $\pm$  SEM,  $6.82 \pm 0.98$  at 10 min and  $8.18 \pm 1.36$  at 35 min vs.  $5.00 \pm 1.01$  at

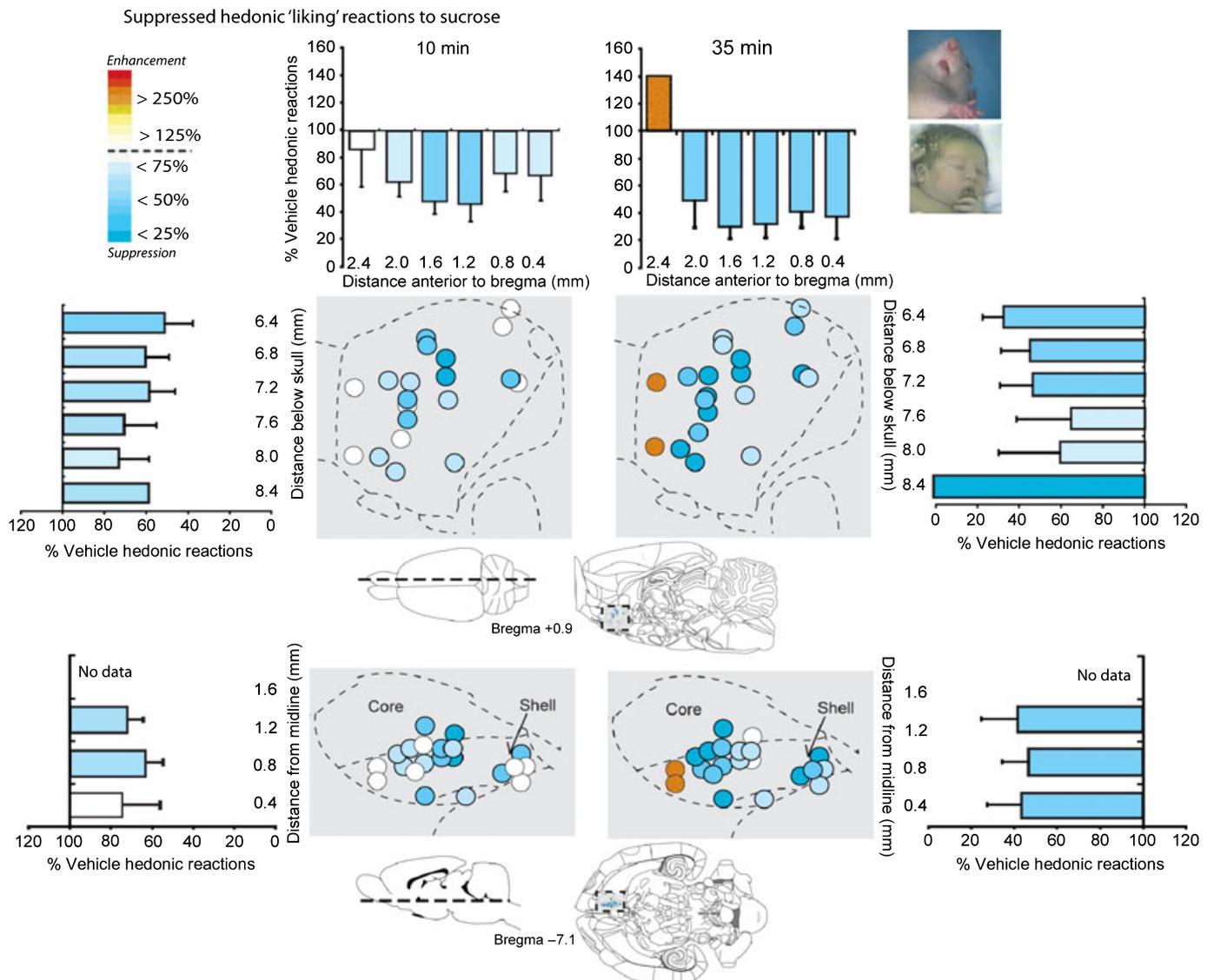


FIG. 4. Hedonic 'liking' suppression: LY341495 in NAc shell suppressed sucrose hedonic reactions. Sagittal and horizontal maps of sites where LY341495 suppressed positive hedonic 'liking' reactions to sucrose taste at 10 min after the microinjection (left) and at 35 min after the microinjection (right; both 1-min tests). The entire medial shell supported LY341495 suppression of positive hedonic impact, with the possible anatomical exception of a moderately far rostral strip (0.1 mm AP width directly beneath the genu of the corpus callosum). Histogram bars show mean change from vehicle (error bars are SEM) for each reaction pattern at rostrocaudal, dorsoventral and mediolateral levels throughout medial shell.

10 min and  $4.63 \pm 1.03$  at 35 min on vehicle; Fig. 3). The combination of strong suppression of highly hedonic reactions, moderate increase in neutral reactions, and strong increase in negative aversive reactions to sucrose indicates a shift in palatability from positive to negative rather than an overall motor effect on orofacial reactions as a whole (Fig. 3). Hedonic suppression and aversive magnification was produced at similar intensities by most sites throughout the 90% of medial shell that was posterior to the far-rostral strip (i.e. at or caudal to the genu of corpus callosum), without evidence of localization or of a rostrocaudal gradient (Figs 4 and 5).

However, a possible anatomical exception to the pattern of hedonic suppression and aversive magnification may have occurred in an anterior 10% strip of medial shell located just ahead of the genu of the corpus callosum and immediately adjacent to the rostral pole (AP +2.4 mm). This was suggested by data from the rat with the furthest rostral placements, where LY341495 actually elevated hedonic reactions to sucrose to 140% of vehicle control levels (Fig. 4). While

more data would be needed to draw firm conclusions, we note that this anterior strip in medial shell has been found to generate anomalous patterns of taste reactivity in previous studies (e.g., unique positive hedonic enhancement by GABAergic agonist muscimol microinjections that contrasts to a gradient of hedonic suppression and aversive enhancement in the rest of medial shell; Reynolds & Berridge, 2002; Faure *et al.*, 2010).

#### LY341495 did not affect aversive reactivity to quinine

Aversive reactions were ordinarily elicited robustly by quinine, and remained high and unchanged after LY341495 injection at either 10 or 35 min (main effect of drug,  $F_{1,10} = 4.141$ ,  $P = 0.072$ ; time  $\times$  drug interaction,  $F_{1,10} = 2.280$ ,  $P = 0.165$ ; Fig. 3). Only modest numbers of positive hedonic reactions were ever elicited by quinine in control condition, but hedonic reactions were suppressed even further by the mglu2/3 blockade to < 40% of vehicle control levels (main effect of

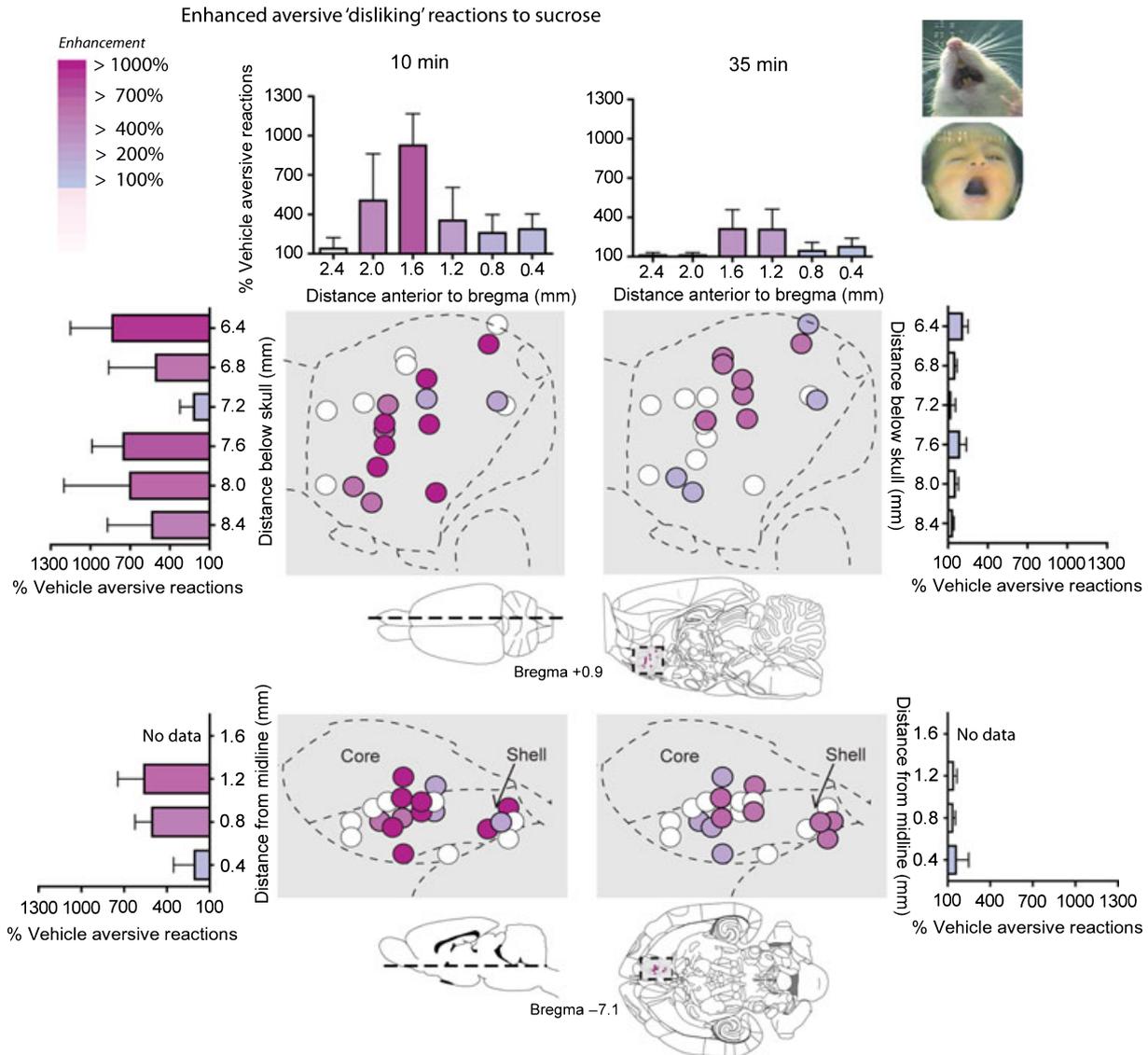


FIG. 5. Aversive 'disliking' enhancement: LY341495 in NAc shell enhanced sucrose aversive reactions. Sagittal and horizontal maps of sites where LY341495 enhanced aversive 'disliking' reactions to sucrose taste at 10 min after the microinjection (left) and at 35 min after the microinjection (right; both 1-min tests). Histograms bars show mean change from vehicle (error bars are SEM) for each reaction pattern at rostrocaudal, dorsoventral and mediolateral levels throughout medial shell.

drug,  $F_{1,10} = 5.918$ ,  $P = 0.038$ ; time  $\times$  drug interaction,  $F_{1,10} = 2.562$ ,  $P = 0.111$ ; Fig. 3). LY341495 blockade did not alter neutral mouth movements elicited by quinine, which remained at relatively low levels (main effect of drug,  $F_{1,10} = 5.053$ ,  $P = 0.051$ ; Fig. 3).

*Anatomical control sites*

LY341495 microinjection at control placements dorsal to medial shell, largely contained in lateral septum, had no significant effect on taste reactivity behaviors: mglu2/3 receptor blockade had no significant effect on hedonic reactions (sucrose, main effect of drug,  $F_{1,2} = 16.333$ ,  $P = 0.056$ ; quinine, main effect of drug, ( $F_{1,2} = 0.730$ ,  $P = 0.483$ ), aversive reactions (sucrose, main effect of drug,  $F_{1,2} = 3.000$ ,  $P = 0.225$ ; quinine, main effect of drug,  $F_{1,2} = 3.510$ ,  $P = 0.202$ ), or neutral mouth movements (sucrose, main effect of drug,  $F_{1,2} = 7.737$ ,  $P = 0.109$ ; quinine, main effect of drug,  $F_{1,2} = 0.964$ ,  $P = 0.430$ ).

Although our microinjection placements in NAc specifically targeted the medial shell, it is worth addressing whether there was any contribution from diffusion into the core. In our study, two rats in the food intake test had at least a unilateral placement that impinged into the core (with the contralateral placement in medial shell). However, these unilateral-core rats did not show the same suppression of intake seen in those rats with bilateral medial shell hits, and one even showed an increase in eating time (Fig. 2A). Additionally, neither rat met the lowest criteria for defensive treading behavior (10 s; Fig. 2B). Those observations suggest that the medial shell makes much more of a contribution than the core to mglu2/3 influences in NAc over fear induction and appetitive suppression, just as for ionotropic AMPA glutamate modulation of the same behaviors (Reynolds & Berridge, 2003). Even so, while microinjection of LY341495 that are centered in medial shell of NAc produce the behavioral effects described here, future studies would be needed to unequivocally state whether the core contributes anything to mglu2/3 effects.

### Local Fos plumes not induced by LY341495

Microinjections of LY341495 produced no discernable change in Fos expression immediately surrounding the microinjection site when compared to vehicle microinjections (main effect of drug,  $F_{1,11} = 0.464$ ,  $P = 0.504$ ). Blockade of mglu2/3 receptors failed to cause either excitatory local Fos plumes of elevated protein translation or inhibitory anti-plumes of suppressed translation compared to vehicle, as drug and vehicle microinjections induced identical Fos expression levels surrounding the center of injection (both slightly elevated over normal tissue baselines). The lack of any local change in Fos expression after a LY341495 microinjection is unusual and contrasts with the excitatory plumes produced by the ionotropic glutamate AMPA antagonist (DNQX), and by agonists for mu opioid (DAMGO), CRF (CRF) or endocannabinoid (anandamide) receptors, as well as amphetamine and GABA blockade (bicuculline) in NAc medial shell; lack of any local Fos change at the mglu2/3 blockade site also contrasts to the inhibitory antiplumes produced in medial shell at sites of a GABA agonist (muscimol) or an opioid antagonist (naloxone) (Peciña & Berridge, 2000, 2005; Smith & Berridge, 2005; Pecina *et al.*, 2006; Mahler *et al.*, 2007; Faure *et al.*, 2008, 2010). One possible explanation for absence of any Fos change for LY341495 might be that mglu2/3 receptors are located predominantly on presynaptic terminals rather than on the postsynaptic medium spiny neurons, which are more directly impacted by the other drugs mentioned above. However, assessment of the spread of microinjection impact was relatively unimportant for interpretation of this experiment given that the behavioral effects of LY341495 microinjections described earlier were homogeneous throughout the entire medial shell. Therefore all maps of behavioral effects constructed for figures were based only on the pinpoint locations of the center for each microinjection placement. For any future experiment that finds LY341495 to involve functional localization, other markers of neuronal activation that are more sensitive to terminal mglu2/3 blockade would be required.

### Discussion

Here, we found that local blockade of mglu2/3 receptors by LY341495 microinjections in the NAc medial shell suppressed appetitive eating of palatable chocolates and suppressed positive hedonic ('liking') reactions normally induced by the sensory pleasure of sweetness. The effects on motivation and hedonic impact could be related, as a reduction in 'liking' for sweet taste could contribute to reduced 'wanting', though it is also possible that appetitive motivation is directly suppressed by NAc mglu2/3 blockade. Concomitantly the same LY341495 microinjections generated increases in spontaneous fearful motivation as reflected by 10-fold increases in a species-specific antipredator behavior, defensive treading, and in parallel increased the number of aversive disgust reactions such as bitter-typical gapes to the normally pleasant taste of sucrose. These shifts of motivation and affect from positive valence to negative valence occurred after LY341495 microinjections homogeneously throughout the entire medial shell, and were not anatomically segregated or localized to any subregion of shell. Overall, NAc mglu2/3 blockade appears to push the animals from appetitive motivation and positive hedonic reactions into a more affectively negative, but still highly motivated, state of fear and disgust.

### Metabotropic versus ionotropic glutamate disruptions

Our finding of a negative shift in motivation and affective valence produced throughout the entire medial shell by metabotropic gluta-

mate antagonism contrasts strongly with the anatomically-segregated 'keyboard pattern' of desire and dread generation produced by disruptions of ionotropic AMPA glutamate signals in the same structure. Local microinjections of the AMPA antagonist DNQX in the rostral half of medial shell are known to produce intense appetitive behaviors such as hyperphagia (Maldonado-Irizarry *et al.*, 1995; Stratford *et al.*, 1998; Reynolds & Berridge, 2003, 2008; Faure *et al.*, 2008), whereas the equivalent DNQX microinjections in the caudal half of medial shell instead produce fearful anti-predator responses similar to those observed here such as defensive treading and burying in a standard laboratory setting, distress vocalizations, escape attempts, etc. (Reynolds & Berridge, 2003, 2008; Faure *et al.*, 2008). A similar rostrocaudal gradient of bivalent motivation is also produced by local GABA<sub>A</sub> stimulation caused by muscimol microinjections in the medial shell: appetitive stimulation at rostral sites, and fearful stimulation (with appetitive suppression) at caudal sites (Stratford & Kelley, 1997; Reynolds & Berridge, 2001, 2002). However, while both local GABA receptor activation and local ionotropic glutamate disruption generate similar keyboard gradients of appetitive versus fearful motivated behaviors, there are also differences between GABA and glutamate gradients in the medial shell. Only GABA muscimol microinjections additionally cause a corresponding affective gradient of positive 'liking' versus negative 'disliking' in hedonic reactions similar to the hedonic shift from 'liking' to disgust seen here after LY341495, whereas local blockade of ionotropic glutamate AMPA receptors by DNQX anywhere in medial NAc shell fails to alter 'liking' or 'disliking' (Reynolds & Berridge, 2002; Faure *et al.*, 2010).

Thus, two features of our current results of metabotropic glutamate signal blockade stand in stark contrast to that gradient pattern of effects produced by AMPA blockade of ionotropic glutamate receptors in the NAc medial shell. First, mglu2/3 receptor blockade by LY341495 penetrated as successfully into sensory pleasure reactions as into motivated behavior, suppressing positive 'liking' and amplifying negative 'disliking' reactions to sweetness, while simultaneously suppressing appetitive eating and generating motivated fearful behaviors. Second, mglu2/3 blockade homogeneously generated fear and shifted affective valence toward negative at all locations in the medial shell, rostral as well as caudal, without the bivalent keyboard pattern (rostral = appetitive and caudal = fearful) of ionotropic AMPA blockade (or of GABAergic inhibition).

### Limits to top-down penetration of sensory pleasure: not the case for mglu2/3?

We previously proposed that fast corticolimbic glutamate signals mediated by ionotropic receptors on NAc neurons were unable to control hedonic emotional processes generated in NAc, based on observations that DNQX local AMPA blockade in NAc shell sites failed to alter 'liking' reactions to tastes (Faure *et al.*, 2010). The ability of metabotropic mglu2/3 blockade here to suppress hedonic 'liking' and stimulate 'disliking' may possibly be reconciled with our previous conclusion, that ionotropic AMPA manipulations of motivation in shell do not penetrate into hedonic 'liking' or 'disliking', by distinguishing between fast ionotropic (AMPA) and slower metabotropic (mglu2/3) functions of glutamate in NAc. For example, metabotropic receptors more widely regulate the release of other neurotransmitters besides glutamate, including GABA, which more effectively influence hedonic impact in NAc (Fig. 6). Therefore, a hypothetical explanation for the effects of LY341495 on hedonic 'disliking' shifts might be that mglu2/3 microinjections recruit local

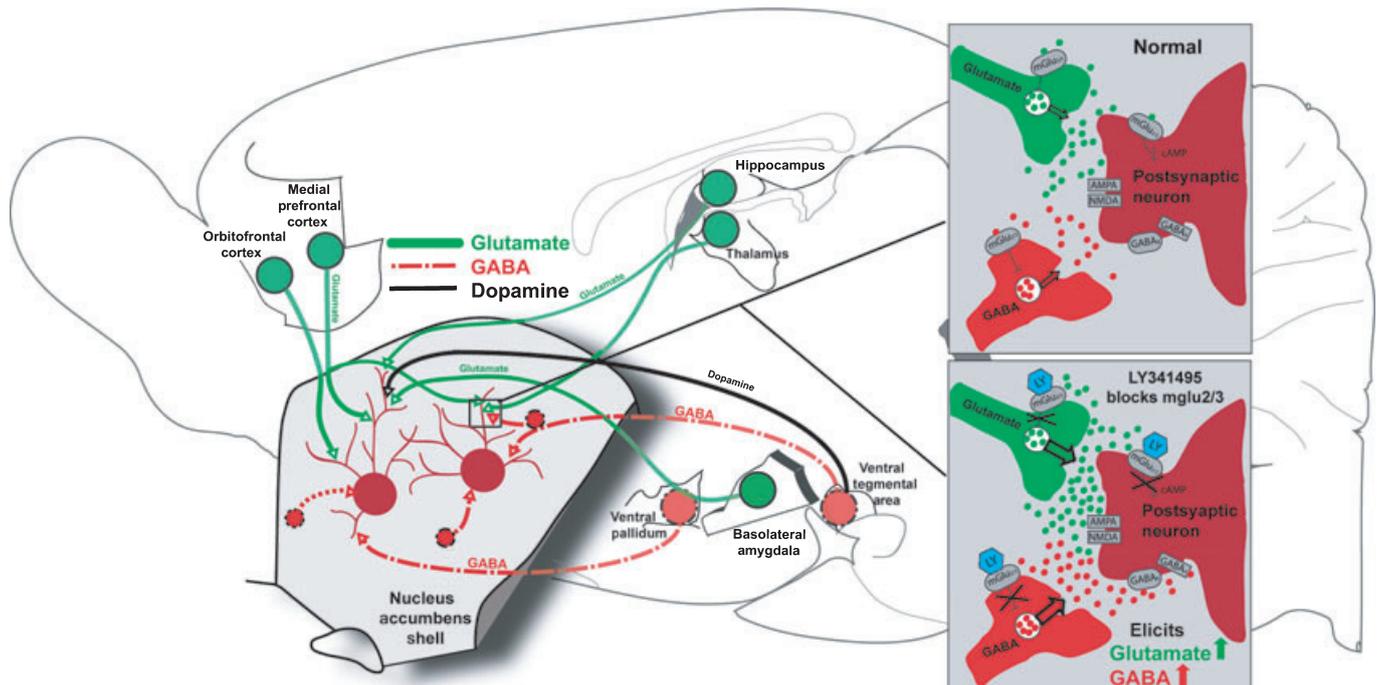


FIG. 6. Corticolimbic circuits impacted by metabotropic glutamate receptor blockade induced by LY341495 microinjection in NAc shell. Close-up of glutamatergic (green) and GABAergic (red) neurotransmission under normal conditions and in the presence of LY341495 (LY, blue), which blocks presynaptic mglu2/3 receptors and, consequently enhances release of both glutamate and GABA from their respective neurons (insets). Glutamatergic inputs (green) are shown from medial prefrontal cortex, orbitofrontal cortex, hippocampus, thalamus and basolateral amygdala. GABAergic medium spiny neurons (deep red) and GABAergic inputs (red) to NAc shell are shown from ventral pallidum and ventral tegmental area. Dopaminergic inputs are shown from the ventral tegmental area (black). Circuit diagram is modified from Faure *et al.*, 2010; with inset based on Swanson *et al.*, 2005. For interpretation of references to color in the figure legend, please refer to the Web version of this article.

GABA release by acting on presynaptic axons of GABA-releasing neurons. This hypothesis is based on reports that stimulation of mglu2/3 receptors can suppress GABA release (Cartmell & Schoepp, 2000; Schoepp, 2001) and our findings that GABA agonist and LY341495 microinjections can both suppress 'liking' and enhance 'disliking' in much of the NAc shell.

Alternatively, mglu2/3 blockade may access hedonic impact via direct glutamatergic actions, but via metabotropic receptor functions that recruit intracellular cascades absent from ionotropic receptor function (Spooren *et al.*, 2003). Generally, the responses of metabotropic glutamate receptors are slower in onset and longer-lasting than the fast excitatory synaptic transmission of ionotropic receptors (Conn & Pin, 1997; Simeone *et al.*, 2004). Additionally, Group II metabotropic glutamate receptors are located primarily presynaptically, as well as on glial cells and on the periphery of synaptic zones (Schoepp, 2001; Swanson *et al.*, 2005; Imre, 2007), while AMPA receptors are found almost entirely on the spines of postsynaptic membranes of medium spiny neurons in NAc (Meredith *et al.*, 1990; Sesack & Pickel, 1992; Johnson *et al.*, 1994; Chen *et al.*, 1998; Tarazi *et al.*, 1998a,b). Given that blockade of mglu2/3 receptors triggers intracellular processes not impacted by AMPA receptors, conceivably metabotropic receptors might alter hedonic processing through differential modulation of NAc neurons.

#### Expansion of fear-generating zone to rostral shell

We note two points relevant to understanding why LY341495-induced negative valence shifts homogeneously throughout the entire medial shell. First, there is at least a similarity to ionotropic effects in caudal shell, where both LY341495 and DNQX microinjections generate

fearful behaviors. Second, an anatomical expansion of fear generation into the rostral shell also has one known parallel in the gradients observed after ionotropic AMPA disruptions: DNQX microinjections at many rostral sites in the medial shell can generate fearful defensive treading (nearly as well as at caudal sites) when rats are tested in a stressful, highly noisy and brightly lit environment (Reynolds & Berridge, 2008). We hypothesize that mglu2/3 blockade may act similarly to just such a stressful environmental ambience, at least in terms of modulating the function of local sites in medial shell, and consequently expand the negative fear generation from caudal into rostral shell. The mechanism of such expansion, by either mglu2/3 blockade or stressful environment combined with AMPA blockade, will of course require further explanation.

#### Role of mglu2/3 receptors in fearful salience: relevance to schizophrenia

It has been suggested that aberrant and intense fearful salience, generated in part by NAc circuits, is involved in the paranoia of human schizophrenia (Kapur, 2003; Barch, 2005; Taylor *et al.*, 2005; Kienast & Heinz, 2006; Schmidt & Beninger, 2006; Jensen *et al.*, 2008; Walter *et al.*, 2010). Proposed potential future treatment options for schizophrenic paranoia include mglu2/3 agonists, which would have the opposite receptor effects from those of the antagonist LY341495 tested here, which generated fear. Group II agonists have anxiolytic and antipsychotic-like effects in animal models of schizophrenia (Swanson *et al.*, 2005; Schlumberger *et al.*, 2009). For example, systemic administration of mglu2/3 agonists blocks the expression of fear-potentiated startle (Helton *et al.*, 1998; Tizzano *et al.*, 2002; Grillon *et al.*, 2003; Rorick-Kehn *et al.*, 2007), increases

the time spent on the open arms of an elevated plus maze (Monn *et al.*, 1997; Helton *et al.*, 1998; Ferris *et al.*, 2001) and inhibits conditioned-avoidance responding (Rorick-Kehn *et al.*, 2007). Similarly, in phase II clinical trials LY2140023, an oral prodrug of LY404039, reduced both positive and negative symptoms of human schizophrenia (Patil *et al.*, 2007). The effectiveness of mglu2/3 agonists as potential antipsychotics might have more to do with effects on affective dysfunction involving motivation and emotion than on cognitive dysfunction, as the drugs do not appear effective in animal models of the cognitive deficits found in schizophrenia (Schlumberger *et al.*, 2009). Given these considerations and our results, we hypothesize that the effectiveness of mglu2/3 agonists may be linked to the dampening of fearful motivational salience, extending beyond the cognitive dysfunction these drugs were first considered to treat (Moghaddam, 2004).

Similar dampening of fearful salience has been proposed by Kapur and others to be the mechanism of action of typical and atypical antipsychotic medications, which block D2 dopamine receptors to prevent the enhancement of salience of negative stimuli (Kapur & Mamo, 2003; Kapur, 2004; Howes & Kapur, 2009; Heinz & Schlagenhauf, 2010). Presynaptic alterations in dopamine release may contribute to the fearful motivational state produced by mglu2/3 antagonism. Agonism at mglu2/3 receptors inhibits KCl-stimulated dopamine release (Chaki *et al.*, 2006), and blockade of these receptors increases extracellular dopamine levels in NAc shell (Karasawa *et al.*, 2006). Additionally, we have previously reported that endogenous dopamine is necessary for the generation of fearful motivation by local disruptions of ionotropic glutamate in the caudal shell as well as for the generation of appetitive motivation in the rostral shell (Faure *et al.*, 2008). All this suggests that interactions between glutamate and dopamine signals in NAc modulate negative fearful salience as well as positive incentive salience. In contrast, we suggest that dopaminergic transmission in NAc shell is unlikely to contribute to LY341495's suppression of hedonic 'liking' or amplification of 'disliking' reactions to sucrose. Neither suppression of dopamine function (using either systemic dopamine blockade or 6-OHDA lesions of dopamine projections) nor dopamine stimulation in NAc (by systemic administration or local microinjection of amphetamine, or by genetic mutation) have been found to alter hedonic reactions to tastes, and the bulk of the available evidence indicates that dopamine does not actually mediate 'liking' or the hedonic impact of sensory pleasure (Berridge *et al.*, 1989; Peciña *et al.*, 1997, 2003; Berridge & Robinson, 1998; Wyvell & Berridge, 2000, 2001; Tindell *et al.*, 2005; Berridge, 2007).

## Conclusions

Local blockade of mglu2/3 receptors by LY341495 microinjections in NAc shell shifts both motivation and hedonic affective reactions from positive valence to negative valence. The ability of LY341495 to negatively shift motivation from appetitive to fearful, and hedonic impact of a sensory reward from pleasant to disgusting, demonstrates the potential importance of normal mglu2/3 neurotransmission to appetitive motivation and positive affective reactions. This role of metabotropic Group II glutamate signals also may be related to why drugs which act as agonists at these receptors might help to restore normality in some cases of aberrant motivation, such as that found in schizophrenia and drug addiction.

## Acknowledgements

This research was supported by National Institutes of Health Grants DA015188 and MH63649. J.M.R. was supported by NIMH NRSA MH090602. We thank Ryan Selleck for assistance with Fos histology.

## Abbreviations

AP, anteroposterior; DV, dorsoventral; LY341495, (2S)-2-Amino-2-[(1S,2S)-2-carboxycycloprop-1-yl]-3-(xan th-9-yl) propanoic acid; mglu2/3, metabotropic Group II glutamate receptors; ML, mediodorsal; NAc, nucleus accumbens.

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