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Fear and Feeding in the Nucleus Accumbens Shell: Rostrocaudal Segregation of GABA-Elicited Defensive Behavior Versus Eating Behavior

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This study examined localization of positive versus negative motivational functions mediated by GABA circuits within the accumbens shell. Microinjections of a $GABA_A$ agonist (0, 25, 75, and 225 ng/0.5 μl muscimol) in rostral shell sites elicited appetitive increases in eating behavior. In contrast, microinjections in caudal shell sites elicited defensive burying or paw-treading behavior. Rats whose microinjections landed bilaterally outside of the accumbens shell did not display either behavior. Defensive treading elicited by caudal shell muscimol microinjection appeared to be a negative motivated response to threat (similar in parameters and orientation to normal defensive burying of a threatening electrified shock prod). The nucleus accumbens shell thus appears functionally heterogeneous in coding motivational valence. The demonstration that muscimol elicits positive eating behavior from rostral shell versus negative defensive behavior from caudal shell suggests in particular that GABAergic substrates of positive and negative types of motivated behavior in the nucleus accumbens shell are segregated along a rostrocaudal gradient.

Key words: accumbens shell; GABA; food intake; reward; appetite; motivation; glutamate; dopamine; fear; defense; aggression; mesolimbic; microinjection

GABAergic medium spiny neurons in the nucleus accumbens shell are implicated in the control of appetitive behavior and reward. Regarding eating behavior specifically, robust increases in food intake by rats are elicited by microinjection in the medial accumbens shell of either $GABA_A$ or $GABA_B$ receptor agonists (or non-NMDA glutamate antagonists) (Maldonado-Irizarry et al., 1995; Stratford and Kelley, 1997; Basso and Kelley, 1999).

Aside from its role in positive appetitive behavior, the accumbens shell has also been implicated in negative motivational states, such as stress, fear, and defensive behavioral responses elicited by noxious or threatening stimuli (Inoue et al., 1994; Beck and Fibiger, 1995; Gray, 1995; Salamone et al., 1997; Berridge et al., 1999; Liberzon et al., 1999). Footshock increases extracellular dopamine in the accumbens shell but not core (Kalivas and Duffy, 1995), and increased accumbens dopamine or DOPAC have also been reported after other noxious stimuli, such as tail pinch, anxiogenic drugs, bright novel environments, or immobilization stress (Thierry et al., 1976; D'Angio et al., 1987; Bertolucci-D'Angio et al., 1990; McCullough and Salamone, 1992; Berridge et al., 1999). Even conditioned stimuli for fear, such as auditory or context cues that have been paired with shock, may produce increases in accumbens dopamine and accumbens Fos expression (Beck and Fibiger, 1995; Young et al., 1998).

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Regarding the relationship of accumbens GABA neurotransmission to stress, presentation of a conditioned signal for shock increases GABA levels in the medial accumbens shell (Saul'skaya and Marsden, 1998). Thus, GABA neurotransmission in the accumbens shell may play a role in defensive or fear-related behavior, as well as in positively motivated behavior.

Rodents have evolved a natural defensive reaction, in the form of defensive burying behavior, as a species-specific response to a variety of threatening stimuli (e.g., electric shocks, scorpions, rattlesnakes, etc.) (Owings and Coss, 1977; Wilkie et al., 1979; Bolles and Fanselow, 1980; Pinel et al., 1992; Treit et al., 1993; Rodgers et al., 1997; Londei et al., 1998; Treit et al., 1998). Defensive burying consists of vigorous treading-like movements of the forepaws that splash wood shavings, sand, or similar substrate toward the threatening object, sometimes burying it entirely. Rats emit defensive treading toward an electrified "shock prod" (Fig. 1*A*) (Treit et al., 1981) and toward the food that was paired with LiCl-induced illness (Parker, 1988). Mice emit defensive treading to bury a live scorpion (Londei et al., 1998), and ground squirrels defend their burrow by emitting similar defensive treading and sand kicking toward a rattlesnake during anti-predator mobbing (Fig. 1*B*) (Owings and Coss, 1977). Anxiolytic drugs reduce defensive treading behavior of rats toward a shock prod (Treit, 1985), as do lesions of the central nucleus of the amygdala (Kopchia et al., 1992). Defensive treading therefore appears to constitute a negative motivated reaction to a variety of noxious stimuli that pose a near and immediate threat (Rodgers et al., 1997).

In this study, we compared eating versus defensive treading behavior elicited by $GABA_A$ receptor activation after muscimol microinjection in the accumbens shell. Our goal was to examine the GABAergic localization of appetitive and defensive motivational function within accumbens shell.

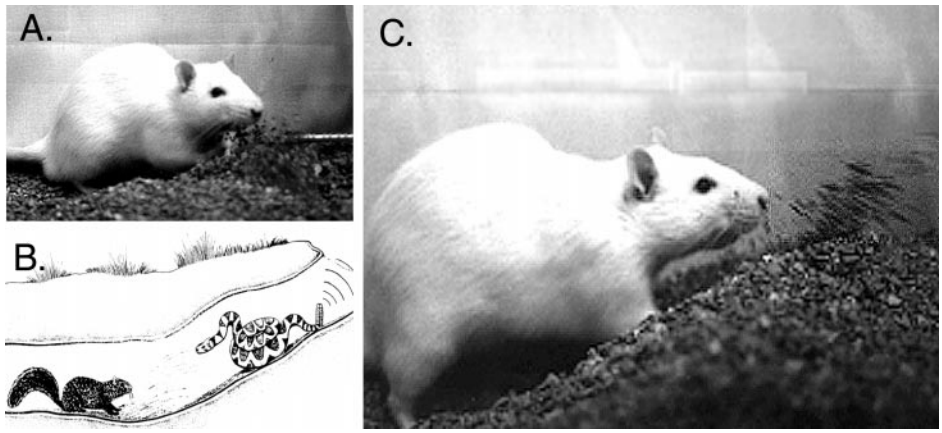

Figure 1. Examples of defensive treading or burying behavior elicited from rodent species by threatening stimuli or by accumbens GABA activation. *A*, Normal rat defensively burying electrified shock prod that shocked it. *B*, Belding's ground squirrel defending maternal burrow against a predatory rattlesnake in the wild (reprinted from Owings and Morton, 1998 with permission). *C*, Rat emitting defensive treading of the type elicited by muscimol microinjection at caudal sites in accumbens shell. Note the midair substrate in front of rat, thrown up by defensive treading movements in *A* and *C*.

Table 1. Sterotaxic surgical coordinates for experiment 1

n	AP+	ML±	DV−
9	3.6	1.0	5.8
6	3.4	0.8	5.8
15	2.9	0.8	5.8
7	2.7	1.2	5.6
11	2.5	0.8	5.5
12	2.1	1.2	5.6

MATERIALS AND METHODS

Experiment 1: segregation of accumbens shell sites for elicitation of eating behavior versus defensive treading behavior

Subjects

A total of 60 male and female Sprague Dawley rats (280–320 gm at the time of surgery) were group-housed (~21°C; 12 hr light/dark cycle) with *ad libitum* food (Purina Rat Chow) and water (tap water) available.

Surgery

Rats were pretreated with 0.1 ml of atropine sulfate and anesthetized with a mixture of ketamine HCl and xylazine (80 and 5 mg/kg, respectively) and placed in a David Kopf Instruments (Tujunga, CA) sterotaxic apparatus with the incisor bar set at 5.0 mm above interaural zero to avoid the lateral ventricles. Chronic microinjection guide cannulas (23 gauge) were implanted bilaterally 2.0 mm above the intended target. Accumbens shell targets differed mainly in anteroposterior (AP) values (between +2.1 and +3.6 mm anterior to bregma), which progressed through the shell in 0.3 mm increments, with only minor alterations in mediolateral (ML) coordinates (±0.8 to ±1.2) and dorsoventral (DV) coordinates (−5.5 to −5.8 below surface) to accommodate the changing contours of the accumbens shell (Table 1). Microinjection guide cannulas were anchored to the skull with screws and acrylic cement. A stainless steel obturator was inserted into each guide cannula to help prevent occlusions. Each rat received prophylactic penicillin (aquacillin; 45,000 U, i.m.) after surgery. At least 7 d were allowed for recovery before the beginning of behavioral testing.

Drugs and intracerebral injections

Muscimol (Sigma, St. Louis, MO) was dissolved in sterile 0.15 M saline, which was also used for vehicle control microinjections (0.5 μl). For the first experiment, a dose of 75 ng/side muscimol (resulting in a total dose of 150 ng/animal) was chosen because it was intermediate between the two most effective doses (50 and 100 ng) for eliciting eating as reported by Stratford and Kelley (1997). Microinjections were made with a stainless steel injector cannula (29 gauge), extending 2.0 mm beyond the ventral tip of the guide, and attached to a syringe pump via PE-20 tubing. The animals were gently hand-held and bilaterally infused with a volume of 0.5 μl at a rate of 0.30 μl/min. Animals received muscimol and vehicle microinjections in a counterbalanced order with 48 hr rest between injections. After infusion, the injectors remained in place for an additional 60 sec to allow for drug diffusion before the obturators were replaced, and the animal was placed immediately in the test chamber. For this and subsequent experiments, animals were habituated to the test chambers for 4 consecutive days before the beginning of behavioral testing and received a vehicle microinjection on the final day of habituation.

Behavioral testing

The transparent test chambers (23 × 20 × 45 cm) contained wood shavings spread 2.0 cm in depth evenly across the chamber floor. A preweighed amount of food (Purina Rat Chow pellets) was placed on the chamber floor, and a tap water spout was available during each 60 min test session.

Food and water intake were recorded by both weight and duration of eating or drinking behavior. The behavior of each rat was videotaped for detailed off-line behavioral analysis. The videotapes were subsequently scored by an experimenter blind to drug treatment and analyzed for (1) time spent eating (defined as the amount of time the animal's mouth was either touching a food pellet or chewing), (2) drinking (amount of time a rat's tongue was in contact with the water spout), (3) paw treading (defined as rapidly repeated forward-and-backward movements of either a single forepaw or rapidly alternating bilateral forward-and-backward movements of both forepaws, which shoved or sprayed the wood shavings forward, (4) head covering (burying the head underneath the wood shavings covering the snout and eyes), (5) grooming (defined as paw strokes over the face or licking of the paws or body), (6) general locomotion (front-to-back cage crossings), and (7) resting (tucking head against chest without movement for >5 sec). Duration of each behavior was scored in terms of seconds spent engaged in it by each rat.

Environmental influence

We noticed in pilot experiments that our magnitude of food intake elicited after muscimol injections was slightly less than that reported by Kelley and colleagues after equivalent doses (Stratford and Kelley, 1997; Basso and Kelley, 1999). We surmised that extraneous environmental stimuli, such as wood shavings, might exert an inhibitory influence on elicited eating because our animals had always been tested for food intake in chambers containing both wood shavings and food, whereas previous studies by Kelley and colleagues had tested intake with only food present (no shavings). To test this possibility, the effects of muscimol infusions on food intake were compared in two environmental stimulus conditions: food and wood shavings both present versus food only present (shavings absent). A waterspout was always available during testing.

Histology

After behavioral testing, rats were deeply anesthetized with sodium pentobarbital, microinjected with ink for anatomical localization of injection sites, and perfused transcardially with buffered saline, followed by a buffered 4% paraformaldehyde solution. The brains were removed, post-fixed, sectioned (40 μm), mounted on slides, and stained with cresyl violet. Cannula placements were mapped onto the corresponding atlas drawings of Paxinos and Watson (1986). The data from animals with

cannula placements falling outside the accumbens shell were considered separately in the statistical analysis.

Statistical analysis

Each behavior was initially examined with a two-way repeated measures ANOVA (drug × anatomical level) with one factor (drug) repetition. When significant main effects were found, additional analysis was performed with one-way repeated measures ANOVA with *post hoc* comparisons conducted by Bonferroni test.

Experiment 2: dose–response effects for elicited eating versus defensive treading behavior

Surgery

Twenty-five female Sprague Dawley rats were implanted bilaterally with chronic indwelling cannulas in the medial accumbens shell as in experiment 1. Fifteen rats received cannulas aimed at the rostral shell (AP, +3.1; ML, ±0.8; DV, −5.8), and 10 rats received cannulas aimed at the caudal shell (AP, +2.1; ML, ±1.2; DV, −5.6).

Experimental design

Each animal received bilateral injections of 0, 25, 75, or 225 ng of muscimol (dissolved in 0.5 μl of saline) before testing. The order of doses was administered across rats in a counterbalanced order. Testing of eating behavior and of defensive treading behavior and all analysis and histology procedures were as described above.

Statistical analysis

Effects of muscimol doses on each behavior at each anatomical level were examined by one-way repeated measures ANOVA, followed by multiple comparisons conducted with Bonferroni tests.

Experiment 3: comparison of muscimol-elicited treading with "real defensive treading" elicited by electric shock prod

To determine whether the paw-treading behavior elicited by GABA receptor activation in the caudal shell reflects a motivated response to threat similar to natural defensive treading, we compared motor and orientation parameters of muscimol-elicited treading with those of normal defensive treading behavior elicited from undrugged rats by encounter with an actual threatening object, namely, an electrified shock prod (Treit et al., 1981).

Experimental design

Eight female Sprague Dawley rats were habituated to the 29 × 39 × 39 cm Plexiglas testing chamber containing 5.0 cm of wood shavings on the floor, for 4 consecutive days. On the fifth day, undrugged animals were placed into the chambers, and an electrified metal shock prod (9 cm, 1 mA) was inserted into the front of the chamber at a height of 2 cm (Treit et al., 1981). Rats were allowed to explore the chamber and to touch or avoid the prod as they chose for 30 min while behavior was videotaped for later analysis. A separate group of eight female rats received bilateral muscimol microinjections into caudal accumbens shell (75 ng/0.5 μl). These rats were selected from experiment 1 on the basis of having shown vigorous defensive treading elicited by shell GABA receptor activation as described above. The rats that received muscimol microinjections were tested in similar chambers with wood shavings on the floor (but without the shock prod). The videotaped behavior of both groups was subjected to identical video analysis.

Videotape analysis

Orientation toward chamber and external stimuli. The orientation of defensive treading behavior was scored in terms of whether the spray of shavings was directed against the front, sides, or back of the cage using a 360° circle in which 0° represented a radial line from the midline of the cage front (the shock prod was always inserted in the front; the video camera, experimenter, and light source were also visible beyond the transparent front wall).

Mound construction. The number, size (height and length), shape, and location of mounds constructed during treading behavior were measured by video analysis. A mound was defined as a pile of shavings >1 cm in height constructed as a consequence of the rat's treading movements. The physical parameters of the mound were calculated by comparing the measured video image size with measures of standard mounds of known size, and mound parameters were plotted to show their position in a map diagram of the test chamber.

Movement parameters. Six motor parameters of treading movements were analyzed frame-by-frame (30 frames per second) or in slow motion (1/10-1/2 actual speed): (1) cycle duration was the interval between forepaw extension and retraction to the point of origin (in milliseconds); (2) bout duration was duration of a series of paw-treading strokes without >1 sec pause; (3) number of cycles per bout was the number of forelimb extension–retraction cycles performed within each bout; (4) limb extension was length of maximal extension of a paw from the point of origin (determined from the video screen by first measuring the video image length from the rat's nose tip to its ear and using that to calculate the movement extension length); (5) unilateral versus bilateral paw use was percentage of treading bouts performed with one forelimb only compared with percentage performed with both forelimbs; and (6) direction of forelimb strokes was direction of forelimb strokes relative to the rat's midline and classified as midline movement (0°) or lateral movement (>45°).

Statistical analysis

Direction of each type of paw-treading behavior was examined with one-way repeated measures ANOVA. Size of mounds constructed by treading behavior was assessed with Mann–Whitney Rank Sum test. Differences in movement parameters between shock prod and muscimol-induced treading were examined with Mann–Whitney Rank Sum test.

RESULTS

Experiment 1: segregation of accumbens shell sites for elicitation of eating behavior versus defensive treading behavior

Eating behavior elicited by GABA agonist: rostral shell localization

For the purpose of initial analysis, the shell was divided into three rostrocaudal levels: a rostral level (2.2–1.6 mm anterior to bregma; ~40% of the shell; $n = 19$), a middle level (1.4–1.2 mm anterior to bregma; ~20%; $n = 6$), and a caudal level (1.0–0.4 mm anterior to bregma; ~40%; $n = 23$). Drinking, grooming, locomotion, and resting behaviors were not altered by muscimol microinjection at any level. Robust eating behavior was elicited by muscimol microinjection (75 ng) into the medial accumbens shell but only at rostral and middle sites. A two-way ANOVA that compared rostrocaudal site (three levels) against drug condition (muscimol versus vehicle) found a main effect of rostrocaudal site ($F_{(2,101)} = 8.75$; $p < 0.001$), a main effect of drug versus vehicle (ANOVA; $F_{(1,101)} = 10.32$; $p < 0.002$), and a significant drug by site interaction (ANOVA; drug × level, $F_{(2,101)} = 29.73$; $p < 0.001$) (Fig. 2). A one-way ANOVA comparing rostrocaudal levels indicated that muscimol elicited greater food intake when infused into either the rostral or middle shell levels compared with the caudal shell level (for muscimol rostral vs caudal, $p < 0.001$; middle vs caudal, $p < 0.002$).

Muscimol microinjection at sites in the rostral one-third of the shell (2.2–1.6 mm anterior to bregma) most dramatically increased eating over vehicle-elicited baseline intake (one-way repeated measures ANOVA; drug, $F_{(1,39)} = 49.82$; $p < 0.001$) (Fig. 3). Food intake was approximately doubled after muscimol compared with vehicle injections at every site within the rostral level: 2.20 mm anterior to bregma (ANOVA; drug, $F_{(1,11)} = 18.62$; $p < 0.01$), 1.70 mm (ANOVA; drug, $F_{(1,13)} = 11.70$; $p < 0.02$), and 1.60 mm (ANOVA; drug, $F_{(1,15)} = 28.05$; $p < 0.002$) (Fig. 3). At sites within the middle AP level (1.4–1.2 mm anterior to bregma), muscimol produced only a marginally significant increase in food intake over vehicle (ANOVA; drug, $F_{(1,11)} = 5.60$; $p = 0.06$). In contrast, muscimol administration into the caudal level of the accumbens shell (1.0–0.4 mm anterior to bregma) actually caused a significant decrease of food intake to ~50% of vehicle control

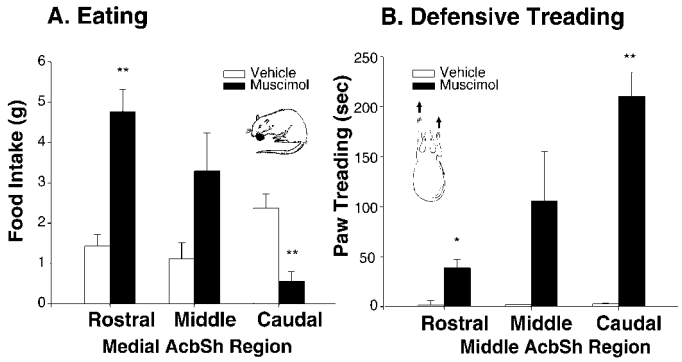

Figure 2. Mean ± SEM food intake and time spent paw treading after vehicle or 75 ng muscimol injections into different regions of the medial accumbens shell. *A*, Muscimol increased eating behavior compared with vehicle when microinjected into the rostral shell but decreased food intake below vehicle when injected into the caudal shell (cumulative total over 60 min trial). *B*, Paw-treading behavior was markedly increased by muscimol injections compared with vehicle injections in the caudal accumbens shell and only slightly increased by rostral muscimol infusions. $^{*}p < 0.002$; $^{**}p < 0.001$, significant muscimol compared with vehicle effect.

amounts (one-way repeated measures ANOVA; drug, $F_{(1,51)} = 15.60$; $p < 0.001$). Food intake was suppressed by muscimol compared with vehicle at two caudal sites, corresponding to 1.00 mm anterior to bregma (ANOVA; drug, $F_{(1,21)} = 18.47$; $p < 0.002$) and 0.48 mm (ANOVA; drug, $F_{(1,5)} = 24.75$; $p < 0.04$) (Fig. 3).

Environmental modulation of eating

Comparison of food intake after rostral muscimol injection in animals tested with both food and wood shavings present versus with food only (wood shavings absent) revealed that food intake was greater when wood shavings were absent (paired *t* test; $p < 0.02$). This suggests that extraneous stimuli such as wood shavings may inhibit muscimol-elicited food intake. Suppression by extraneous stimuli may account for why we observed slightly lower amounts of food intake after muscimol microinjection (even in rostral shell) than found previously by Kelley and colleagues (Stratford and Kelley, 1997; Basso and Kelley, 1999).

Defensive treading behavior elicited by GABA agonist: caudal shell localization

The rostrocaudal organization of defensive treading behavior elicited by GABA receptor activation within the accumbens was the reverse of eating behavior. Muscimol injections evoked strong defensive treading behavior from caudal and middle sites in the medial accumbens shell but very little treading from rostral sites. A two-way ANOVA comparing rostrocaudal site (three levels) and drug conditions (muscimol versus vehicle) found a significant main effect of site ($F_{(2,101)} = 17.66$; $p < 0.001$), a significant main effect of drug ($F_{(1,101)} = 48.49$; $p < 0.001$), and a significant interaction between site and drug ($F_{(2,101)} = 18.58$; $p < 0.001$) (Fig. 2).

A subsequent one-way ANOVA of sites showed that muscimol at sites within the caudal one-third of the shell (1.0–0.4 mm anterior to bregma) produced the most vigorous defensive treading, which was 10 times more intense than at more rostral sites ($F_{(2,49)} = 18.05$; $p < 0.001$) (Fig. 2). Muscimol microinjection at caudal sites robustly increased paw-treading behavior over vehicle-elicited levels, eliciting 2–6 min of cumulative defensive treading after muscimol compared with only a few seconds at most after vehicle (one-way ANOVA; drug, $F_{(1,51)} = 79.00$; $p < 0.001$) (Fig. 3). Within this caudal shell zone, muscimol increased defensive treading behavior over vehicle levels at sites corresponding to 1.00 mm anterior to bregma (ANOVA; drug, $F_{(1,21)} = 46.97$; $p < 0.001$) and 0.70 mm (ANOVA; drug, $F_{(1,23)} = 94.78$; $p < 0.001$).

Defensive treading movements consisted of rhythmic cycles of vigorous and repetitive forelimb paw thrusts forward-and-backward (1.6–3.2 cm extension length), which served to shove or spray wood shavings 1–3 inches in front of the rat (usually 0–60° in front, but on occasion deviating in angle as far as 90°) in bouts of between 0.5 and 6 sec, usually with pauses of several seconds between successive bouts. Each bout contained 2–21 individual forelimb extension–retraction cycles (3.7–6.0 Hz). Most defensive treading bouts consisted of several unilateral paw pushes (69% of bouts), followed by several pushes by the other paw, although some bouts consisted of bilateral paw pushes emitted in an alternating right–left–right–left pattern (31% of bouts).

Defensive treading bouts typically resulted in the construction of elevated mounds of wood shavings placed in front of the rat (5–10 cm in height–width and up to 20 cm in length). Between the rat and the mound, a low trough or depression in the surface was also created by the displacement of shavings. Rats did not emit treading movements randomly, but instead coordinated their defensive treading bouts toward their mound locations so that the mounds tended to increase in size over successive treading bouts. In addition, the mounds themselves were not placed randomly but instead were constructed in strategic positions within the cage, usually placed to block the rat's exposure to the transparent front of the chamber and less commonly placed in back corners (as though the corners were also perceived as minor sources of threat). These features gave observers the impression of a coordinated defensive reaction toward the location of the mound rather than a simple series of stereotyped movements.

Only marginal defensive treading was elicited by muscimol at sites within the middle AP level (1.4–1.2 mm anterior to bregma) (one-way repeated measures ANOVA; drug, $F_{(1,11)} = 4.59$; $p = 0.08$) Sporadic defensive treading was still elicited by muscimol at sites in the rostral one-third of the shell (2.2–1.6 mm anterior to bregma) (ANOVA; drug, $F_{(1,37)} = 13.20$; $p < 0.002$), specifically at two AP levels: 1.70 mm anterior to bregma (ANOVA; drug, $F_{(1,13)} = 7.84$; $p < 0.05$) and 1.60 mm (ANOVA; drug, $F_{(1,15)} = 6.29$; $p < 0.05$). However, in the rostral shell, muscimol elicited only 10% the amount of defensive treading elicited at caudal sites, and many rats showed no defensive treading at all after rostral muscimol microinjections.

Rats that received muscimol infusions in caudal shell (but only caudal shell) often emitted distress vocalizations upon being handled at the end of the test session and attempted to bite the experimenter and to escape. The heightened defensiveness of rats that received caudal muscimol microinjections was sometimes so strong that the animals could not be retrieved for several hours after the test session.

Anatomical map: comparison of eating versus defensive function localization within accumbens shell

To construct an anatomical map of functional localization, functional criteria were set for the elicitation of food intake and paw treading, and microinjection sites that met them were plotted on a digitized stereotaxic atlas. An "eating site" was considered to be any site at which muscimol microinjection increased food intake at least 150% over vehicle baseline (or elicited at least 1 gm food

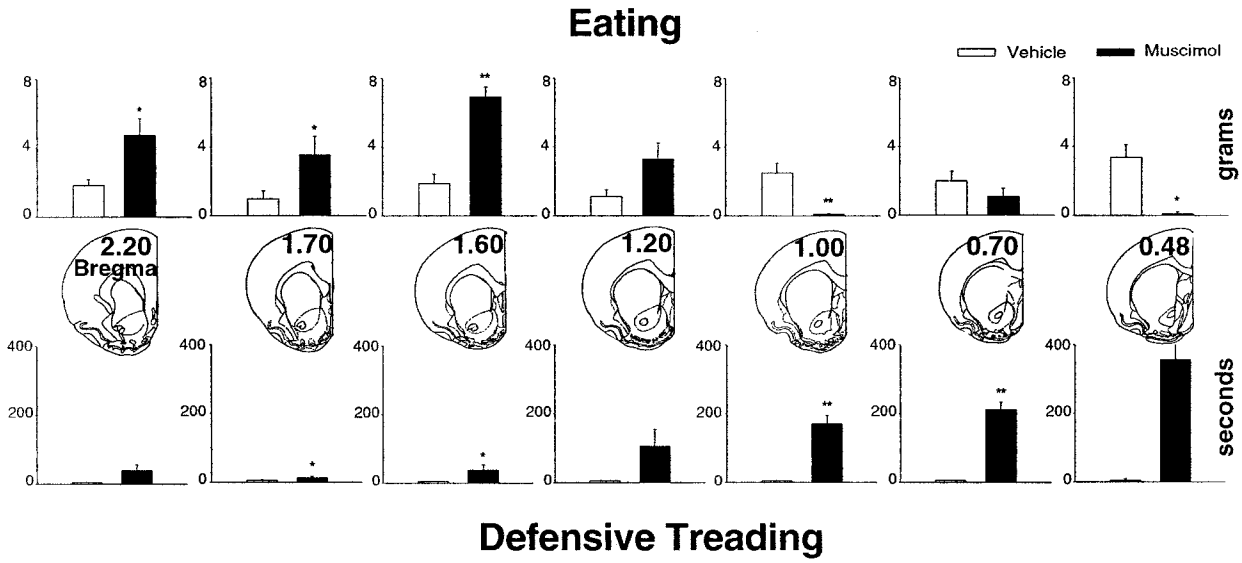

Figure 3. Mean ± SEM food intake (*top row*) and time spent paw treading (*bottom row*) after vehicle or muscimol injections at each rostrocaudal level of the accumbens shell. $*p < 0.05$; $**p < 0.001$ muscimol compared with vehicle.

intake in cases in which food intake was zero after vehicle microinjection). A "defensive treading site" was considered to be any site at which muscimol elicited at least 100 sec of total cumulative paw-treading behavior (which was orders of magnitude greater than vehicle levels because treading was generally zero after vehicle microinjection). A site could be classified as both an eating site and a defensive treading site if it met both criteria. Sites that met neither criteria were considered to be functionally negative.

Eating sites were clearly concentrated in the rostral one-half of the accumbens shell (Figs. 4, 5), from the rostral tip of the shell to a point at which the anterior commissure merges and the nucleus of the vertical limb diagonal band begins. Defensive treading sites were clustered in the caudal one-third of the accumbens shell, beginning at the level of the optic nerve, just behind the tenia tecta. The most rostral defensive treading sites overlapped slightly with the most caudal eating sites, and a number of sites in this zone of overlap supported both types of muscimol-elicited behavior. "Negative sites," in which GABA activation produced neither behavior, were chiefly located bilaterally outside of the accumbens shell, usually placed laterally in or near the core of the accumbens, ventral to the shell in the islands of Calleja, or medial to the shell in the vertical limb diagonal band near the medial septal nucleus. Animals whose microinjections landed bilaterally outside of the accumbens shell showed neither eating nor defensive behavior (e.g., in accumbens core, at the core–shell border but not penetrating into the shell, or in other structures outside the shell). Thus, it appeared that these behaviors were attributable to activation of receptors within the shell.

In summary, accumbens muscimol administration into different sites elicited two different types of behavior with opposite motivational valence organized along a rostrocaudal gradient within the medial accumbens shell. Eating was elicited by $GABA_A$ receptor activation at rostral sites within medial shell, whereas defensive treading was elicited at caudal sites. The functional regions appeared to overlap slightly, and some intermediate shell sites supported both behaviors.

Experiment 2: dose–response effects for elicited eating versus defensive treading behavior

When the effects of 0, 25, 75, and 225 ng of muscimol doses were compared at rostral eating sites and caudal defensive treading sites, different dose–response behavior was seen (Fig. 6). Paw treading increased with dose in an approximately linear manner until reaching an asymptote, whereas the greatest food intake was produced by the lowest muscimol dose.

Dose–response effects for rostral shell eating behavior

Muscimol microinjection at rostral eating sites significantly increased food intake over vehicle baseline levels (one-way repeated measures ANOVA; dose, $F_{(3,43)} = 7.92$; $p < 0.001$) at the two lowest doses (specific dose compared with vehicle; 25 ng, $p < 0.001$; 75 ng, $p < 0.04$; 225 ng, $p = 0.11$). However, the 25 ng dose of muscimol appeared to be the most effective for eliciting eating behavior, more than doubling food intake and causing a larger increase than higher doses (25 ng dose compared with 225 ng dose, $p < 0.05$; paired t test). In contrast, muscimol infusions into the caudal shell suppressed eating to 80–10% of vehicle levels in a linear dose–response manner (one-way repeated measures ANOVA; dose, $F_{(3,46)} = 11.70$; $p < 0.001$) (specific dose compared with vehicle; 25 ng, $p = 0.31$; 75 ng, $p < 0.001$; 225 ng, $p < 0.001$).

Dose–response effects for caudal shell defensive treading behavior

Muscimol microinjections into the caudal shell elicited robust paw treading (one-way repeated measures ANOVA; dose, $F_{(3,46)} = 9.66$; $p < 0.001$) (specific dose compared with vehicle; 25 ng, $p = 0.55$; 75 ng, $p < 0.001$; 225 ng, $p < 0.001$). The highest two caudal doses produced the greatest amount of treading, which averaged over 3 min of cumulative treading, although a ceiling effect appeared at ~75 ng. The highest 225 ng caudal dose caused two rats to become immobile for several hours, beginning ~40 min after microinjection, during which time they lay spread-eagle and were unresponsive when touched. Rostral microinjections of muscimol also elicited

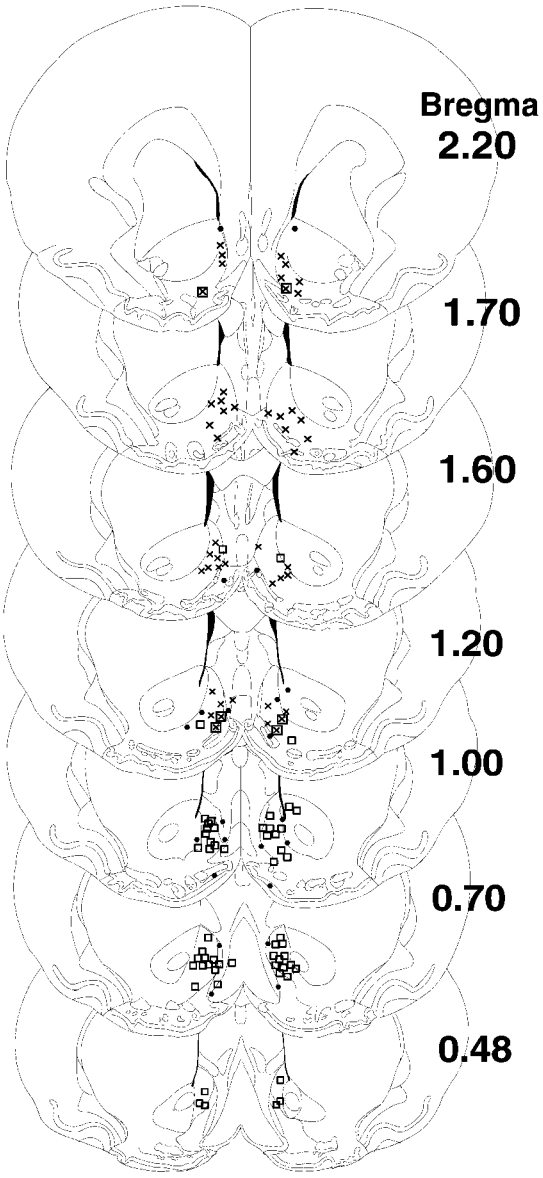

Figure 4. Coronal map of microinjection sites for appetitive behavior versus defensive behavior elicited within the accumbens shell. Eating sites are denoted by *crosses* and are restricted to the rostral accumbens shell. Defensive treading sites are denoted by *open squares* and are concentrated in the caudal one-third of the shell. Mixed eating and defensive treading sites, in which both behaviors were elicited by muscimol, are denoted by *cross-filled squares* and appear in intermediate levels. Negative sites, in which neither behavior was elicited, are denoted by *filled circles*, typically placed near the outside of the shell. Atlas based on Paxinos and Watson, 1986.

small but significant amounts of defensive treading at the highest two doses (one-way repeated measures ANOVA; dose, $F_{(3,42)} = 6.69$; $p < 0.001$) (specific dose compared with vehicle; 25 ng, $p = 0.63$; 75 ng, $p < 0.02$; 225 ng, $p < 0.001$).

Experiment 3: comparison of muscimol-elicited treading with real defensive treading elicited by electric shock prod

Orientation toward external stimuli

Normal defensive burying behavior elicited from undrugged rats by a shock prod was compared with defensive burying elicited by muscimol microinjection (75 ng) in the caudal accumbens. The two types of burying behavior were similar in both movement pattern and mound construction. All rats in the undrugged shock-prod condition explored the chamber and touched the electrified prod one or two times (mean, 1.4 ± 0.2 touches) with their paw or snout, withdrawing immediately and vigorously upon receiving a shock. Defensive burying behavior was subsequently emitted by those rats (109.5 ± 24.3 sec/30 min) toward the shock prod positioned at the front of the cage (compared with sides or back of the chamber; one-way repeated measures ANOVA comparing direction of treading orientation; $F_{(3,23)} = 222.72$; $p < 0.001$).

In comparison, rats that received muscimol microinjections in the caudal accumbens shell (but in the absence of the shock prod) similarly tended to orient their defensive burying behavior (268.3 ± 62.9 sec/60 min) toward the transparent front of the chamber (which faced the camera, experimenter, and light source) than toward the more sheltered sides or back of the cage (which faced opaque surfaces; one-way repeated measures ANOVA; direction, $F_{(2,23)} = 19.12$; $p < 0.001$) (Fig. 7).

Mound construction

Defensive burying behavior elicited by shock prod and caudal muscimol microinjection both resulted in the construction of similar bedding mounds that were of similar height and size (shock prod mound height, 5.4 ± 0.7 cm; length, 21.4 ± 1.5 cm; width, 14.0 ± 0.4 cm; muscimol mound height, 4.4 ± 0.5; length, 10.3 ± 2.1 cm; width, 18.6 ± 1.1 cm). Seven of eight rats encountering a shock prod buried the prod entirely under a mound of wood shavings, whereas seven of eight muscimol-treated rats constructed long mounds (over 15-cm-long) that extended across the entire front wall of their chambers. One difference in mound construction was that rats encountering a shock prod constructed only one mound (burying the shock prod), whereas three of eight rats after muscimol microinjections constructed more than one mound: a major mound at the front of the chamber as described above, and one or two smaller mounds (2.2 ± 0.2 cm in height) in the back corners.

Movement parameters

There were no significant differences in movement parameters between defensive burying elicited by a shock prod compared with defensive burying elicited by caudal shell muscimol regarding either cycle duration, number of cycles per bout, percentage of unilateral versus bilateral paw cycles, or direction of forelimb stroke (Table 2). The only significant differences in motor parameters between the two forms of treading behavior was that bout duration for muscimol-induced paw treading was very slightly longer than for shock-induced paw treading ($p < 0.05$), and conversely, the length of forelimb extension was longer during shock-induced burying than during muscimol-induced burying ($p < 0.01$). These differences were very small (<25%), and in general the movements appeared highly similar in defensive treading behavior elicited by shell muscimol microinjection and in normal defensive burying behavior elicited by an electric shock prod.

DISCUSSION

Our results showed that $GABA_A$ receptor activation in nucleus accumbens shell can produce either appetitive eating behavior or defensive treading behavior (in which paw treading movements spray wood shavings into protective mounds), depending on whether the muscimol microinjection site is in rostral versus caudal shell. Microinjection in rostral shell elicited positive eating behavior, especially at low doses (e.g., 25 ng). That confirms previous reports of food intake after accumbens muscimol (Strat-

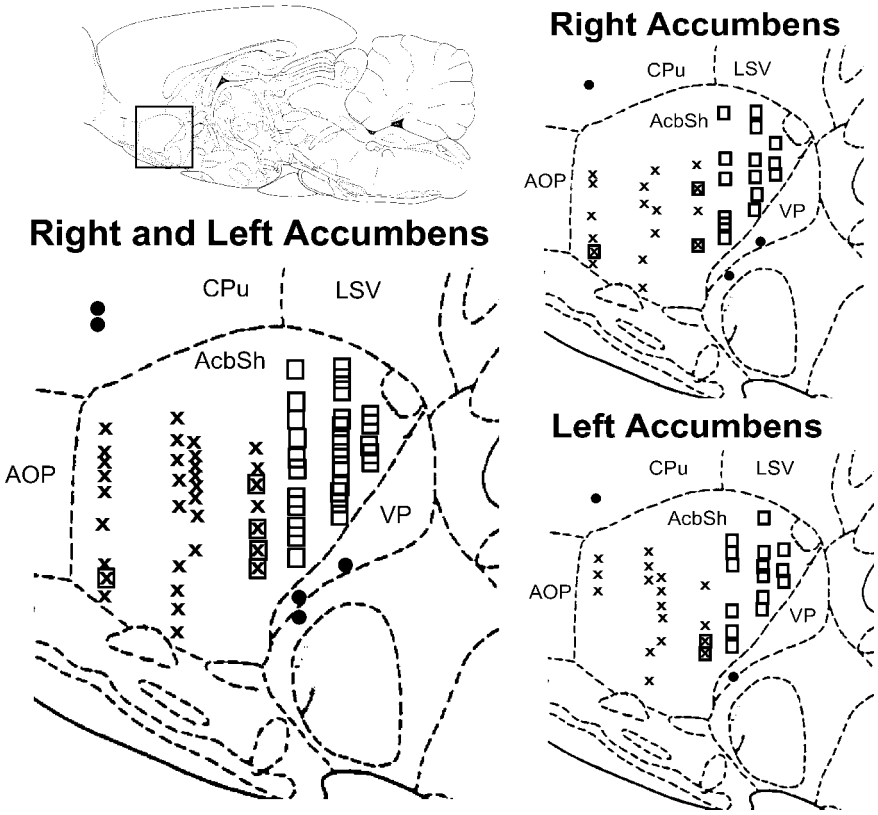

Figure 5. Sagittal map of microinjection sites for appetitive versus defensive behavior. Eating sites are denoted by *crosses* and are restricted to the rostral accumbens shell. Defensive treading sites are denoted by *open squares* and are clustered in the caudal shell. Mixed eating and defensive treading sites that caused both behaviors are denoted by *cross-filled squares* and appear at midshell levels. Negative sites are denoted by *filled circles* and are located outside the accumbens shell. Only microinjection sites are shown that were located in the sagittal plane ~0.9 mm lateral from midline (~85% of total sites; atlas based on Paxinos and Watson, 1986).

ford and Kelley, 1997; Basso and Kelley, 1999). In contrast, muscimol microinjection in caudal shell produced negative defensive burying behavior, especially after high doses (e.g., 225 ng). Defensive treading elicited by accumbens microinjection has not to our knowledge been reported previously. GABAergic inhibition of medium spiny neurons and their projections may be the cellular mechanism for both positive and negative types of muscimol-elicited behavior (Carlezon and Wise, 1996; Stratford and Kelley, 1997, 1999; Zahm, 2000).

Defensive treading behavior: motor stereotypy or fearful response to threat?

Normal rodents display defensive treading behavior toward threatening stimuli (electric shock, scorpions, predators, noxious foods, etc). However, is paw-treading behavior caused by caudal shell muscimol a motivated defensive response to a perceived threat? Or is it instead a simple motor pattern triggered in the absence of motivational valence?

Three lines of evidence indicate that burying behavior after caudal shell muscimol was motivated rather than motor. First, movement parameters used in muscimol-elicited treading were similar to those of normal motivated defensive treading elicited by a shock prod. Second, muscimol-elicited treading was coordinated as though to defend against major features of the environment. Mounds constructed after caudal shell muscimol were placed strategically in the chamber. For example, the major mound was always placed as a barrier between the rat and the transparent front of the chamber, beyond which were other objects in the room, such as the experimenter, camera, and light source. Third, muscimol-elicited treading behavior was often followed by other defensive behaviors when touched, such as distress vocalization, biting, and escape attempts. In other words, caudal shell muscimol appeared to cause rats to respond as

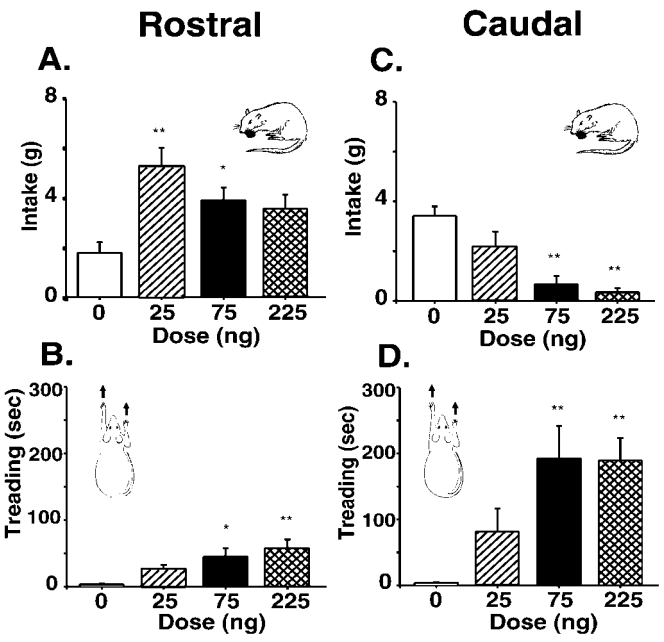

Figure 6. Dose–response functions for mean ± SEM food intake and defensive paw-treading behavior after muscimol microinjection into the rostral and caudal shell. *A*, Food intake was significantly increased by the two lowest doses of muscimol compared with vehicle infused into the rostral shell, especially by the 25 ng dose. *B*, Defensive treading was slightly increased by rostral shell muscimol at the higher 75 and 225 ng doses compared with vehicle. *C*, Food intake was significantly decreased below vehicle levels by muscimol microinjection within the caudal shell. *D*, Defensive treading was significantly increased by muscimol microinjection compared with vehicle microinjection into the caudal shell, especially at the 75 and 225 ng doses. $^{*}p < 0.05$; $^{**}p < 0.001$ muscimol doses compared with vehicle.

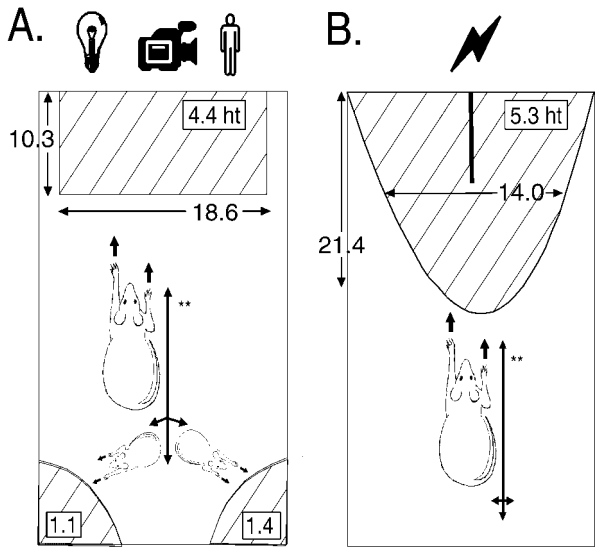

Figure 7. Comparison of defensive treading direction and bedding mound placement within the test chamber of rats exposed to either caudal shell muscimol (without shock prod present) or a shock prod (without microinjection). *Striped areas* represent distribution of shaving mounds, dimensions are indicated by mound *arrows* (in centimeters), and *numbers in squares* indicate height (in centimeters) of mounds. The orientation or direction of actual treading behavior is indicated by *arrows* next to the rat. Each *arrow length* indicates the relative proportion of treading duration directed toward each of the four cage sides. *A*, Treading induced by muscimol microinjection in caudal shell was directed predominantly toward the front of the cage, which faced the light source, camera, and experimenter, and resulted in mounds of wood shavings spread along the cage front and occasionally in smaller mounds located in back corners. *B*, Shock prod-elicited treading from undrugged rats was also directed primarily toward the cage front, which contained the electric prod, and resulted in a large bedding mound that covered the shock prod. $^{**}p < 0.001$, significant difference in direction of treading for each type of treading.

though to a threat and as though defensive burying was one component of their defensive response.

A conclusion that $GABA_A$ receptor activation in caudal shell causes a motivated defensive or fearful reaction does not imply that it is identical to conventional fear-inducing procedures, such as classical conditioning of freeze or startle responses to a signal for shock (LeDoux, 1998; Fanselow and LeDoux, 1999; Maren, 1999), or even identical to natural defensive burying reactions elicited by a shock prod or other threatening stimulus (Treit et al., 1981). Fear may not be a single reaction caused identically by all of these. Several investigators have suggested that there may exist different types of fearful reaction, which serve different purposes (Bolles and Fanselow, 1980; Marks and Nesse, 1994; Kagan and Schulkin, 1995), and are mediated by different neural systems (Treit et al., 1993; Gray and McNaughton, 1996; Killcross et al., 1997; Davis and Lee, 1998; Rosen and Schulkin, 1998; Lehmann et al., 2000). For example, Gray and McNaughton (1996) suggested that different neural systems may mediate active panic defense reactions versus passive inhibitory withdrawal reactions. If so, defensive burying seems likely to correspond most closely to active defense. Alternatively, the defensive state triggered by caudal shell muscimol might be different from all types of naturally triggered fearful reactions, being instead one isolated fragment of normal fear processes. For example, Berridge and Robinson (1998) suggested that, just as positive incentive salience may be contributed by mesolimbic systems to appetitive motivation and reward, so also a related but negative mesolimbic form of motivational salience might contribute attention-grabbing properties to frightening stimuli. A $GABA_A$ agonist in caudal accumbens shell could conceivably cause aversive motivational salience to be attributed to the neural representation of stimuli, such as the experimenter and other objects in the room, causing the rat to perceive them as frighteningly salient. In contrast, the positive form of the same process triggered in rostral shell could cause attribution of positive incentive salience to food stimuli, leading to appetitive eating behavior. Finally, it is possible that the eating behavior evoked by rostral shell muscimol is related to stress-induced eating caused by stimuli, such as tail pinch (Antelman et al., 1975), and that defensive treading represents a related response to stress coded by rostral versus caudal shell GABAergic substrates. All such conjectures need to be evaluated by future research, but it seems clear at least that the treading behavior elicited by muscimol in caudal accumbens shell was an active defensive response (rather than a motor reflex or a passive withdrawal response) emitted to repel, diminish, and sometimes bury a fearful stimulus.

Localization of function within accumbens shell

Our results revealed rostrocaudal segregation of behavioral valence coded by $GABA_A$ substrates in the accumbens shell. Eating is typically viewed as a positive or appetitive behavior and was elicited only by $GABA_A$ agonist microinjections in the rostral shell (1.2–2.7 mm anterior to bregma). This region corresponds to the same coordinates at which Kelly and colleagues found muscimol-elicited eating (Stratford and Kelley, 1997; Basso and Kelley, 1999). In contrast, we also found that microinjections in caudal shell, behind the feeding site, not only failed to increase food intake but actually decreased eating behavior.

Highest amounts of negative defensive burying behavior are elicited predominately by $GABA_A$ receptor stimulation of caudal shell regions (AP, +1.2 to +0.48 mm). Slight defensive treading was also elicited by high doses at rostral sites but at much lower intensity than at caudal sites. It is as yet unclear whether the slight treading behavior after rostral microinjection results directly

Table 2. Forelimb motor parameters in paw treading elicited by a shock prod versus muscimol microinjections into caudal accumbens shell

Parameter	Electric probe	Muscimol
Cycle duration	199.06 ± 3.16 msec	206.13 ± 2.99 msec
Bout duration	1.096 ± 0.102 sec*	1.380 ± 0.149 sec*
Number of cycles per bout	5.27 ± 0.45 c/b	5.79 ± 0.50 c/b
Limb extension length	2.66 ± 0.07 cm*	2.35 ± 0.06 cm*
Percentage of unilateral versus bilateral paw cycles	72.89 ± 8.30% unilateral	66.64 ± 8.34% unilateral
Directions of forelimb strokes	79.14 ± 6.10% midline	74.98 ± 7.04% midline

$^{*}p < 0.05$ muscimol compared with shock prod-elicited treading.

from action on rostral receptors there or instead from drug diffusion to more caudal receptors. Thus, GABA substrates in rostral regions of the medial shell trigger an apparently positive motivated behavior, whereas GABA substrates in caudal shell trigger an apparently negative motivated behavior. Microinjection sites in the accumbens core or other structures outside the shell failed to elicit either behavior.

Functional interaction between neuroanatomical and neurochemical coding

Our rostral shell region for GABAergic eating behavior overlaps considerably with an earlier map by our laboratory of an opioid eating site in the shell, in which morphine microinjection caused increased food intake (Peciña and Berridge, 2000). However, the appetitive opioid eating site of Peciña and Berridge (+1.0 to +2.2 mm anterior to bregma) also extended posteriorly into our caudal shell region in which GABAergic muscimol elicited negative defensive burying. An overlap between positive and negative motivational systems in caudal shell is also consistent with our previous finding using a pure conditioned incentive paradigm that amphetamine microinjection in this same caudal shell site increases appetitive cue-triggered bar pressing for a sucrose reward (Wyvell and Berridge, 2000) and with findings of greatest reward effects in place preference and brain self-stimulation paradigms after histamine receptor blockade in the caudal shell (Zimmermann et al., 1999). These comparisons indicate that positive–negative function is determined interactively by neurochemical receptor activation, as well as by neuroanatomical localization of function within the nucleus accumbens shell.

Neurobiological bases of rostrocaudal shell segregation of appetitive–defensive function

Rostral versus caudal portions of the accumbens shell differ in cell morphology, connectivity, and neurochemical organization (Herkenham et al., 1984; Phillipson and Griffiths, 1985; Oades and Halliday, 1987; Zahm and Brog, 1992; Brog et al., 1993; Groenewegen et al., 1993; Zahm and Heimer, 1993; Voorn et al., 1994; Gorelova and Yang, 1997; Usuda et al., 1998). Although the entire shell receives afferent projections from the prefrontal cortex, subiculum, amygdala, ventral pallidum, lateral hypothalamus, ventral tegmental area, etc., the rostral shell receives denser innervation from the subiculum and basolateral amygdala, whereas the caudal shell receives sparser projections from those structures (Phillipson and Griffiths, 1985; Brog et al., 1993). Furthermore, segregated projections from different regions within afferent structures that project differentially to rostral and caudal shell may be another source of functional variance (Oades and Halliday, 1987; Groenewegen et al., 1993; Gorelova and Yang, 1997). Regarding efferent projections, both rostral and caudal shell regions project to the ventral pallidum, lateral hypothalamus, ventral tegmental area, substantia nigra compacta, pedunculopontine nuclei, and periaqueductal gray area. However, the rostral shell may send denser efferents to the lateral preoptic area, globus pallidus, and substantia nigra pars reticulata, whereas the caudal shell sends denser projections to anterior regions of the extended amygdala and bed nucleus of the stria terminalis and the locus ceruleus (Heimer et al., 1991; Zahm and Heimer, 1993; Usuda et al., 1998).

Neurochemically, the rostral shell has higher levels of D1 and D2 mRNA and opioid enkephalin mRNA (Bardo and Hammer, 1991; Voorn and Docter, 1992). In contrast, the caudal shell has higher levels of cholecystokinin, acetylcholinesterase, vasopressin–oxytocin receptors, and greater norepinephrine and serotonin innervation (Zaborszky et al., 1985; Meredith et al., 1989; Zhou et al., 1991; Tribollet et al., 1992; Berridge et al., 1997; Veinante and Freund-Mercier, 1997; Delfs et al., 1998). These various neurochemical and neuroanatomical differences between rostral versus caudal shell may contribute to the functional differences we have reported here.

Conclusion

Motivational functions are segregated within the nucleus accumbens shell. $GABA_A$ receptor activation in the rostral accumbens shell elicits food intake (especially at relatively low doses), whereas $GABA_A$ receptor activation in the caudal shell elicits defensive treading behavior (especially at high doses). The rostrocaudal segregation of positive eating versus negative defensive behavior by GABAergic systems indicates that the nucleus accumbens shell may heterogeneously code behavioral function and motivational valence.

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