Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens

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The nucleus accumbens mediates both appetitive motivation for rewards and fearful motivation toward threats, which are generated in part by glutamate-related circuits organized in a keyboard fashion. At rostral sites of the medial shell, localized glutamate disruptions typically generate intense appetitive behaviors in rats, but the disruption incrementally generates fearful behaviors as microinjection sites move more caudally. We found that exposure to stressful environments caused caudal fear-generating zones to expand rostrally, filling ~90% of the shell. Conversely, a preferred home environment caused fear-generating zones to shrink and appetitive-generating zones to expand caudally, filling ~90% of the shell. Thus, the emotional environments retuned the generation of motivation in corticolimbic circuits.

The capacity of situations to retune limbic function has hardly been explored. Here we examined how environmental ambience shapes adaptive behaviors by reorganizing appetitive versus defensive function maps in nucleus accumbens.

Appetitive and defensive motivations are generated along an anatomical rostrocaudal gradient by glutamatergic circuits in the medial shell, analogous to a limbic 'affective keyboard'[1–4]. Each microinjection of the AMPA glutamate antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX, or related drug)[2,3] disrupts glutamate signals from the prefrontal cortex, amygdala and hippocampus in a spherical <0.75-mm radius of the shell. Just as a keyboard has many notes, microinjections of DNQX evoke many different combinations of appetitive and fearful behaviors, corresponding to their location along the rostrocaudal gradient[2,3]. In rostral regions of the medial shell, DNQX microinjections generate intense appetitive behaviors in rats: large increases in eating behavior and food intake, and the establishment of conditioned place preferences[1–4]. In contrast, identical DNQX microinjections in the caudal shell generate equally intense, but negatively valenced, fearful behaviors: distress vocalizations, escape attempts, conditioned place avoidance and 'defensive treading'[2,3]. Defensive treading in particular is an instinctive antipredator behavior of many rodents, which in the wild serves to throw dirt at an offending snake, scorpion or other threat, and in the lab serves to bury noxious objects and to construct defensive mounds[2,5–7]. Intermediate sites in the shell evoke various mixtures of appetitive and fearful behaviors corresponding to their relative position along the rostrocaudal gradient[2,3].

Here we tested a hypothesis describing how nucleus accumbens generates both reward and fear: namely, that the emotional ambience of external environments retunes the valence of functions generated at particular locations in the medial shell. Three different environments were used to manipulate ambience: a home-like environment that the rats preferred (familiar, dark and quiet), a conventional standard laboratory environment and a stressful or overstimulating environment that the rats avoided (bombardment with extra-bright light and unpredictably loud patterns of sound: punk-rock music by Iggy Pop; see **Supplementary Methods** and **Results** online).

First, we confirmed that the rats preferred the home environment and avoided the stressful environment (compared with the standard environment), by testing relative self-administration of selected pairs of environments (home versus standard and standard versus stressful environments; $n = 16$ rats) in a three-chamber apparatus. Here, rats could turn on the light and sound components of a specific environment by entering one end compartment (randomly assigned), and then turn those off and turn another environment on by leaving the first compartment and entering the opposite end. The rats preferred to turn on the dark and quiet home condition over the brighter standard condition by a 9:1 ratio in a 1-h test (cumulative duration, $F_{1,63} = 609.58$, $P < 0.001$; **Supplementary Fig. 1** online). Similarly, rats self-administered the sound and light levels of the standard environment more than they did the stressful environment by a 9:1 ratio ($F_{3,63} = 15.52$, $P < 0.001$).

Next, we tested how environments changed the valence of motivated behaviors generated by local DNQX in the medial shell when rats had to stay in that environment after a microinjection (without being able to choose or terminate the environment). Rats received bilateral microinjections of DNQX or vehicle (order counterbalanced; sites staggered across rats to fill the medial shell for each environment group) and were then placed in their assigned environment for 1 h ($n = 87$ rats total, 27–31 rats per environment; it required about 25 plumes to fill the medial shell). Motivated behavior was later quantified in video analysis: appetitive eating or drinking behavior directed toward food and water versus defensive-treading behavior, which generally shoved granular bedding toward the transparent chamber wall exposed to the experimenter[2,3].

We mapped localization of fear versus feeding functions that were generated by microinjections using a Fos plume tool. Mapping symbols were scaled in size to match Fos plumes (0.01-mm³ centers surrounded by middle and outer halos with volumes of 0.5–1.7 mm³) and color-coded to match the valence intensity of motivated behaviors evoked by DNQX microinjection at each site (relative to control levels shown by the same rat after vehicle microinjection; **Fig. 1** and **Supplementary Fig. 2** online).

In the standard condition, Fos plume maps revealed the typical rostrocaudal gradient described above (**Figs. 2** and **3**; see also

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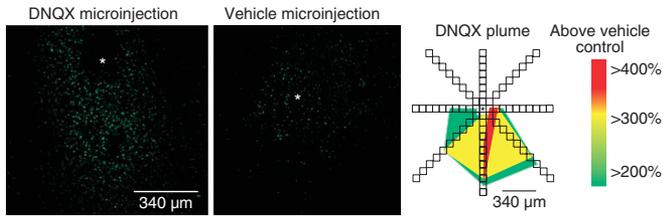
Figure 1 Examples of Fos plumes surrounding microinjections. A Fos plume surrounding a DNQX microinjection site in nucleus accumbens (left) is shown compared with vehicle control microinjection. Colored plume map shows average elevations of Fos caused by DNQX microinjection, relative to vehicle. The 2.5-mm rostrocaudal extent of the medial shell can contain up to eight adjacent plume centers or two to three halos without overlapping, perhaps accounting for keyboard effects.

Supplementary Figs. 3–7 online); DNQX in the rostral half of the shell stimulated a >200% increase in eating behavior (duration elevation from vehicle levels) at most sites anterior to the major islands of Calleja (stretching 0.8 mm between bregma coordinates +1.2 and +2.0 mm, ANOVA $F_{1,83} = 50.98$, $P < 0.001$). Approximately 75% of rostral sites were 'purely appetitive', producing only eating behavior and no defensive behavior. Conversely, in the caudal half of the shell, DNQX microinjections in the standard environment produced >400% increases in fearful defensive-treading behavior (posterior to the major island of Calleja: a 0.6-mm distance between +0.4 and +1.0 bregma, $F_{1,52} = 15.95$, $P < 0.001$) and most caudal sites were 'purely fearful' in the standard environment. Approximately 26% of sites evoked 'mixed valence' motivations, simultaneously increasing both appetitive-eating and defensive-treading behaviors in the same rat (mostly mid-rostrocaudal sites), and 19% of DNQX sites were 'silent' under the standard condition, producing no more motivated behaviors than vehicle (randomly distributed anatomically).
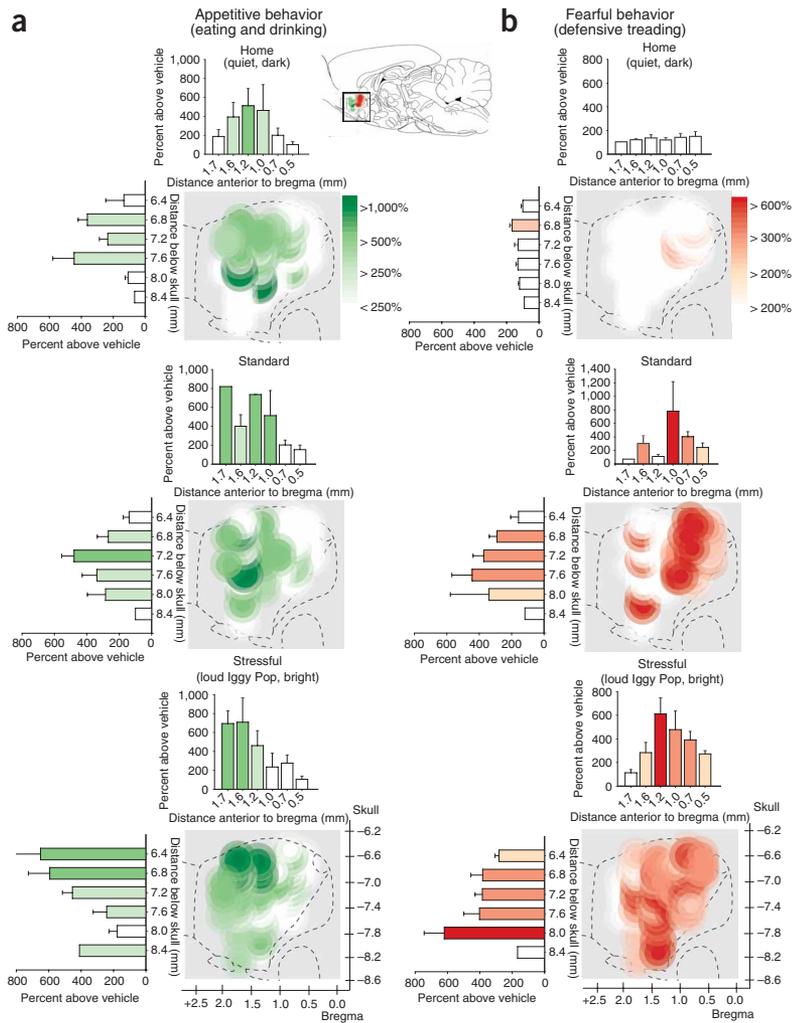
Figure 2 Fos plume maps of appetitive versus fearful behaviors generated by DNQX microinjections. Sagittal maps of ingestive behaviors (eating and drinking, left) and fearful behaviors (defensive treading, right) elicited at each microinjection site are shown. Bars show behaviors at anatomical levels (keyboard effects are visible as incremental step-wise changes across successive bars). (**a**) Appetitive-ingestive behavior was primarily elicited from the rostral medial shell. The dark and quiet home environment that the rats preferred expanded the positive appetitive-generation zone in a caudal direction. The stressful environment containing loud Iggy Pop music and bright lights (which rats avoided when able) expanded the fearful generation zone in a rostral direction and promoted valence mixtures. (**b**) Defensive-treading behavior was produced by DNQX microinjections in the caudal half of medial shell. The preferred home environment shrunk the defensive-treading zone to the caudal tip. Conversely, the stressful environment expanded the defensive treading zone in the rostral direction. These procedures were approved by the Committee on Use and Care of Animals at the University of Michigan.
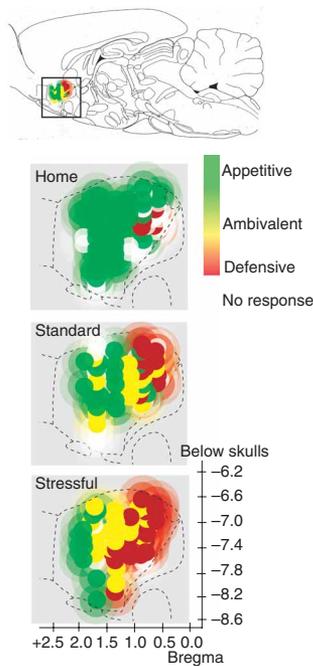
In the preferred home condition (familiar, dark and quiet), the appetitive zone for DNQX-stimulated eating or drinking behavior expanded to fill >90% of the entire shell, invading most of the caudal half (1.5-mm rostrocaudal distance between +0.5 and +2.0 mm, $F_{5,55} = 2.69$, $P = 0.03$; **Figs. 2** and **3**). Conversely, the defensive or fearful zone shrank to about one-third of its original size, becoming restricted to the far caudal tip (even there DNQX generated only very mild levels of defensive behavior, $F_{5,55} = 2.95$, $P = 0.02$). Two-thirds of all microinjection sites became purely ingestion-generating in the home condition, eliciting no defensive behavior at all ($\chi^2 = 31.49$, $P < 0.001$). Approximately 33% of shell sites were silent, predominantly located in caudal shell, and there were no mixed valence sites in the home environment.

In the stressful condition containing bright light and raucous Iggy Pop sound, the fearful zone expanded to fill >80% of the medial shell, heavily invading the rostral half. Defensive treading was elicited from a >1.0-mm rostrocaudal length and a >2-mm^3 volume of shell, or roughly twice the size of the fearful zone in the standard condition ($F_{1,27} = 43.37$, $P < 0.001$; **Figs. 2** and **3**). Nearly 90% of sites generated fearful defensive-treading behavior in the loud and bright environment ($\chi^2 = 35.00$, $P < 0.001$).

For sites in the caudal half of the shell, the stressful condition additionally increased the intensity of DNQX-generated fearful behavior by >50% above standard ($F_{2,86} = 17.75$, $P < 0.001$).

Figure 3 Summary map of glutamatergic valence generation in the medial shell. Purely appetitive sites are shown in green (where DNQX elicited only eating or drinking behaviors). Purely fearful sites are shown in red (where DNQX elicited only defensive-treading behavior). Ambivalent or mixed valence sites are shown in yellow (where DNQX microinjection elicited both appetitive-eating behavior and defensive-treading behavior from the same rat). The home environment expanded the purely appetitive zone compared with the standard environment, whereas the stressful environment expanded the fearful and ambivalent zones.

Only in the far rostral tip of the shell did any site remain purely ingestive in the loud and bright condition.

However, we note that, despite the expansion of the fear zone size and intensity in the stressful condition, appetitive-eating behavior was still robustly stimulated by DNQX, at least at some rostral sites (even if robust defensive behavior was stimulated at the same site). Therefore, most rostral sites became motivationally mixed or ambivalent in the stressful condition. Finally, behaviorally silent sites virtually disappeared in the stressful condition ($\chi^2 = 10.70$, $P = 0.005$).

Remapping by environmental ambience was powerful enough to completely switch the appetitive versus fearful valence of DNQX-motivated behaviors generated at many intermediate locations between the rostral and caudal tips (locations were defined as 0.2-mm^2 squares containing sites). Valence switches occurred for over 40% of the medial shell locations, even though DNQX microinjections at those locations were nearly always potent enough affectively to cause highly valenced behavior of at least one type (either appetitive, fearful or both). Environmental retuning completely reversed the valence generated at 14% of the locations in the shell (from purely positive to purely negative, or vice versa), and caused an expansion from single pure valence into a mixture of both appetitive and fearful behaviors or a retraction from mixed to pure at a further 28% of the locations. Only the extreme rostral tip of the shell remained consistently appetitive, whereas the extreme caudal tip always generated fear.

Future research may clarify the neurobiological mechanisms that allow environments to switch valence generation at sites. Speculatively, one general possibility is that environments modulate DNQX effects on neurons in local microinjection spheres (for example, via local

neuromodulators). This would imply that a Fos plume contains multiple valence circuits or circuit modes, able to be triggered differentially by DNQX depending on other local factors[8]. Alternatively, environmental modulation might alter processing in distant brain structures, rather than change the local impact of DNQX in the medial shell. In that case, the same local disruption signal would serve as an affective building block that could be flexibly constructed into either appetitive motivation or fearful motivation by larger limbic circuits, depending on the current states of target structures. These general possibilities are not mutually exclusive.

In terms of corresponding psychological mechanisms, a possibility consistent with the above neurobiological alternatives is that motivational salience may be generated by mesocorticolimbic circuits with flexible valence, either as positive incentive salience or negative fearful salience[9–12]. Positive incentive salience makes reward-related stimuli such as food become perceived as more 'wanted' and attractive[9,10], and when sensitized to high levels is thought to contribute to compulsive pursuits of drugs or other addictive rewards[13,14]. By contrast, overlapping mesocorticolimbic circuits have been suggested to generate a negative form of fearful salience that makes threat-related stimuli become perceived as frightening, and at excessive levels, fearful salience may contribute to pathological defensive motivations such as paranoia in schizophrenia[11].

In summary, our data suggest that the incentive or fearful valence of motivation generated by a particular local glutamate disruption in nucleus accumbens depends at least on two factors: the location along a rostrocaudal affective keyboard, which assigns an anatomically determined bias of appetitive or fearful valence, and current signals about environmental ambience that can flip the valence generated by disruption of moderately biased local circuits in the medial shell. Corticolimbic circuits involving nucleus accumbens may therefore flexibly remap affective-generating functions from moment to moment as external situations change. Such remapping has implications for understanding how limbic circuits normally tune adaptive motivations and for understanding pathological motivation disorders, including addiction and schizophrenia.

Note: Supplementary information is available on the Nature Neuroscience website.

1. Maldonado-Irizarry, C.S., Swanson, C.J. & Kelley, A.E. *J. Neurosci.* **15**, 6779–6788 (1995).
2. Reynolds, S.M. & Berridge, K.C. *J. Neurosci.* **22**, 7308–7320 (2002).
3. Reynolds, S.M. & Berridge, K.C. *Eur. J. Neurosci.* **17**, 2187–2200 (2003).
4. Stratford, T.R. & Kelley, A.E. *J. Neurosci.* **17**, 4434–4440 (1997).
5. Owings, D.H. & Morton, E.S. *Animal Vocal Communication: a New Approach* (Cambridge University Press, Cambridge, 1998).
6. Reynolds, S.M. & Berridge, K.C. *J. Neurosci.* **21**, 3261–3270 (2001).
7. Wilkie, D.M., MacLennan, A.J. & Pinel, J.P. *J. Exp. Anal. Behav.* **31**, 299–306 (1979).
8. Valenstein, E.S., Cox, V.C. & Kakolewski, J.W. *Science* **163**, 1084 (1969).
9. Berridge, K.C. *Psychopharmacology (Berl.)* **191**, 391–431 (2007).
10. Berridge, K.C. & Robinson, T.E. *Brain Res. Brain Res. Rev.* **28**, 309–369 (1998).
11. Kapur, S. *Am. J. Psychiatry* **160**, 13–23 (2003).
12. Kringelbach, M.L. & Rolls, E.T. *Prog. Neurobiol.* **72**, 341–372 (2004).
13. Robinson, T.E. & Berridge, K.C. *Annu. Rev. Psychol.* **54**, 25–53 (2003).
14. Everitt, B.J. & Robbins, T.W. *Nat. Neurosci.* **8**, 1481–1489 (2005).