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Noxious heat induces fMRI activation in two anatomically distinct clusters within the nucleus accumbens

I. Aharon [a,1], L. Becerraa [c,1], C.F. Chabris [b], D. Borsooka [c,*]

[a] *Athinoula Martinos NMR Center for Medical Imaging, Massachusetts General Hospital, MA, USA*
[b] *Department of Psychology, Harvard University, Cambridge, MA 02138, USA*
[c] *P.A.I.N. Group, Brain Imaging Center, McLean Hospital, Belmont, MA 02478-9106, USA*

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Abstract

Using functional magnetic resonance imaging (fMRI) we found that a noxious thermal stimulus (46 °C) to the hand activates the nucleus accumbens (NAc) in humans, while a non-noxious warm stimulus (41 °C) does not. Following the noxious stimulus, two distinct foci of decreased activation were observed showing distinct time course profiles. One focus was anterior, superior, and lateral and the second that was more posterior, inferior, and medial. The anatomical segregation may correlate with the functional components of the NAc, i.e., shell and core. The results support heterogeneity of function within the NAc and have implications for the understanding the contribution of NAc function to processing of pain and analgesia.

Keywords: Nucleus accumbens; Pain; Aversion; fMRI

Electrophysiological, pharmacological and imaging studies point to the nucleus accumbens (NAc) as one CNS site that may mediate functions involved in both reward and aversion [5,15]. One hypothesis to explain how the NAc could serve this putative dual role is that neurons within the structure can be separated into distinct functional groups, each playing a different role in hedonic responses or motivated behavior [10]. Based upon histological characteristics in animals and humans, the NAc is divided into at least two major sub-territories, the core and the shell, distinguished by unique input–output patterns [14,18]. Differential function within each component of the NAc, while reported in animal studies [13,27] has not been reported in humans.

Functional activity in the human accumbens has been reported using imaging studies following rewarding [1,19] and aversive stimuli [5,11]. Differential function within each component of the NAc, while reported in animal studies [13,17,27] has not been reported in humans. Some animal work suggests that the overall function is that the shell is involved in motivational valence and motivational value in the core [3].

Here, we report in healthy human subjects, a noxious thermal stimulus (46 °C) that produces a decrease signal in the NAc could be separated into two clusters. Each cluster may represent functional components of the core and shell.

Six healthy volunteers (male, right-handed, 31.0 ± 3.7 mean ± S.E.M.) participated in the study, which was approved by the Massachusetts General Hospital committee for experimentation on human subjects. Prior to scanning, subjects were instructed on how to rate their pain intensity during the scan using a standard 11-point Likerts visual analogue scale (VAS), where one end represents no pain and the other represents maximal pain imaginable.

A Peltier thermode system [6] was used to deliver two (a non-painful warm (41 °C) stimulus and a painful hot (46 °C) stimulus) square-wave stimuli (stimulus duration = 29 s; interstimulus interval = 36 s; ramp rate 4°/s; baseline temperature = 35 °C, Fig. 1A) to the back of the left hand (using an elastic strap) within the magnet. The 41 °C stimulus was administered prior to the 46 °C stimulus ash shown in Fig. 1A. Each thermal stimulus was presented four times in the run.

Scanning was performed in a 1.5 T scanner (GE Medical systems, Milwaukee, WI, USA). For the anatomical scan, a conventional 3D sagittal T1-weighted, SPGR sequence was acquired (60 slices 2.8 mm thick, 1.2 mm resolution in-plane)

* Corresponding author: Tel.: +1 617 855 31 95; fax: +1 617 855 3772.
E-mail address: dborsook@mclean.harvard.edu (D. Borsooka).
[1] Both the authors contributed equally.

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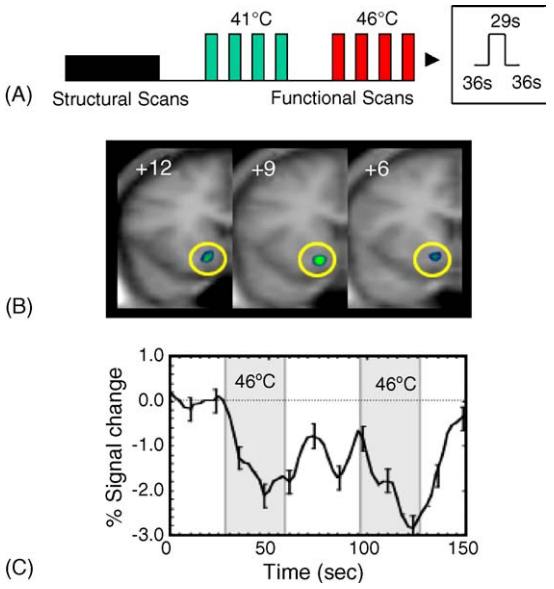

Fig. 1. Experimental paradigm and fMRI activation in the NAc following noxious heat: (A) the experimental paradigm indicating the four stimuli for 41 and 46 °C; (B) statistical maps of decrease activation from the 46 °C stimulus in three slices through the region of the brain containing the NAc; and (C) average decrease signal (±S.E.M.) change following 46 °C stimulus. A voxel-by-voxel analysis was done using a Bonferroni correction of 4×10^{-4} for statistical significance. Prior to generating statistical maps data was motion-corrected and inspected for gross motion larger than 2 mm. Statistical maps were generated for each individual using a *t*-statistics. Individual probability maps were multiplied and the resulting map used for inspection of activated areas. Percent signal changes were calculated using the baseline time points as 100% reference. Each time course was time adjusted before averaging. The gray bars indicate the time when the thermal stimulus was applied.

for atlas registration and for prescription of functional scans. Twenty contiguous slices (7 mm thick) were prescribed perpendicular to the AC–PC line extending from the anterior frontal pole through the cerebellum. A high-resolution T1-weighted echo planar sequence was acquired for preliminary analysis of statistical maps. For the functional studies, an asymmetric spin echo echo planar sequence was used (TR/TE = 2.5 s/70 ms) on the 20 prescribed slices, 100 images per slice were acquired for each functional scan.

Two analyses were performed on the data: (a) a standard analysis and (b) a cluster analysis. The standard analysis was used to obtain statistical maps of activation and average time courses for activated structures. Given that registration to an atlas does not always accurately map brain structures, a cluster approach was applied to the original data.

Data was preprocessed as described previously [5,6]. Briefly, functional data was motion corrected, globally normalized, and transformed into Talairach space. Functional data was averaged in time across subjects. Averaged data was smoothed using a Gaussian filter with an isotropic kernel of 5 mm. Voxel-by-voxel statistical analysis (*t*-test) was performed.

To probe for possible heterogeneity of activation time courses within the NAc we used an exploratory procedure based on cluster analysis. First, we manually selected all of the voxels comprising the NAc in each hemisphere of each subject (i.e., 12 clusters for the six subjects), using a segmentation method that has previously been reported [7] for obtaining the time course of the blood oxygen level dependent (BOLD) signal from each of these voxels. The signal time-course from each voxel was normalized to begin at a value of 1000, and any linear trend was removed. We used the SYSTAT software package (SPSS, Richmond, CA) to conduct separate hierarchical cluster analyses of the Pearson correlation matrices for the left and right NAc voxel sets for each individual subject (using the complete linkage method). This was necessary because each subject's NAc morphology is different, thus there is no simple way to average their voxel sets, and there are too few voxels involved to make an anatomical warping procedure worthwhile.

VAS scores of pain intensity reported by subjects during the experiment averaged 3.1 ± 1.1 for the 41 °C stimuli and increased significantly to 8.2 ± 1.6 for the 46 °C stimuli ($p < 0.01$; *t*-test).

Using voxel-by-voxel statistical analysis (*t*-test), we observed deactivation or a decrease in signal ($2.1 \pm 0.7\%$) bilaterally in the NAc following noxious (46 °C; $p < 1.5 \times 10^{-7}$) but not non-noxious (41 °C; $p > 0.05$) thermal stimuli (Fig. 1 B). When the thermal stimulus is terminated the signal begins to increase towards baseline, decreasing again at the onset of the second stimulus in the train (Fig. 1C). We do not show time courses for the third and fourth epochs since we have previously reported (in a similar paradigm) that there is attenuation of the signal at these points following a 46 °C stimulus [6]. To confirm this with respect to the NAc data collected in this experiment we calculated the standard deviation of the average BOLD signal time course from each subject's left and right NAc separately for the first two noxious thermal epochs and the last two noxious thermal epochs. For both the left and right NAc, the S.D. was higher during the first two epochs than the last two epochs (9.99 versus 9.21, t [5] = 2.12, $p = 0.044$, one-tailed). The remainder of the data presented here refers to the first two 46 °C stimuli. NAc activation has been previously detected in groups of six subjects using the same paradigm [6].

Using the method described above for cluster analysis, we analyzed data from 12 clusters (left and right from each subject). In 9 of 12 cases the algorithm produced two relatively large top-level clusters, which we used for further analysis (Fig. 2). In the remaining three cases, one very small and one very large top-level cluster were produced. In these cases, we discarded the very small cluster and used the two largest sub-clusters of the very large cluster for further analysis. Thus, in each case we were able to group all or nearly all of the voxels into two large clusters.

For each subject's left and right NAc, we computed the Pearson correlations between the time course of each voxel with the average time course of all the voxels in its cluster. Voxels that were "in between" the two clusters, defined as having correlations with the two cluster averages that differed by less than 0.10, were iteratively removed from the clusters by removing the voxel with the smallest difference at each iteration until all voxels in each cluster were at least 0.10 more correlated with their own cluster than with the other cluster. As a result, the correlation between the average time courses (across subjects) of

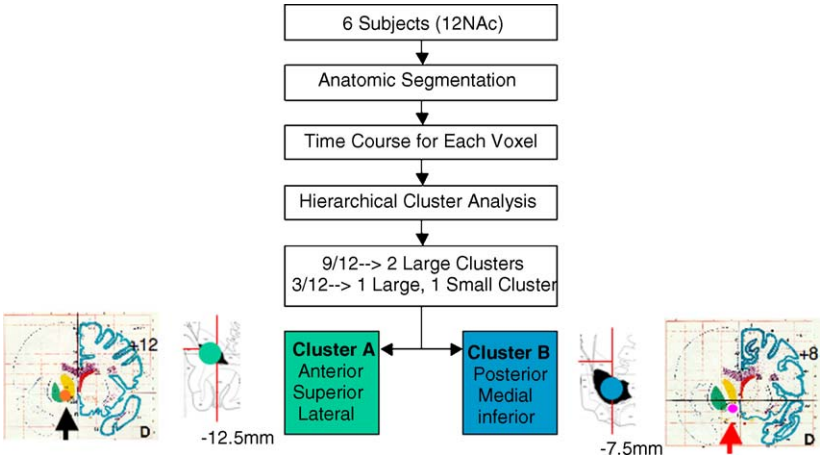

Fig. 2. Cluster activation superimposed anatomical maps. Cluster analysis algorithm is shown in the top part of the figure. Using data for the coordinates of maximal activation shown in Table 1, clusters A and B were plotted onto the human neuroanatomical maps [25] of the region (top) showing the NAc in black (center figures) or onto the normalized atlas of the human brain [16] left (+12) or right (+8) sides of the figure. Note that cluster A is found more anterior superior and lateral, while cluster B is more posterior medial and inferior (see text for details).

clusters A and B declined from 0.60 to 0.51 on the left and from 0.76 to 0.70 on the right. The resulting clusters were reduced in size (number of voxels) by an average of 17%, but maintained their relative locations. Further exploration indicated that other hierarchical clustering methods tended to produce similar results; thus, these clusters were retained for further analysis.

In all cases the time course-based distinction between the clusters was reinforced by spatial contiguity. Within each NAc, we designated the cluster that was more anterior, superior, and lateral as cluster A and the cluster that was more posterior, inferior, and medial, as cluster B. We compared the locations of the two main clusters using two measures: (a) the coordinates of the center of mass and (b) the coordinates of the voxel showing maximal activation (Table 1). For the left NAc, the average distance between the centers of mass for clusters A and B is 6.4 mm, while the average distance between the maximum voxel activated in the two clusters is 6.7 mm. The locations of the activations within each cluster are illustrated in Fig. 2. The figure shows serial coronal slices, taken from the atlas of Talairach and Tournoux [25]. Based on the left side NAc data shown in Table 1, the rostral, medial cluster (cluster A) and the more caudal, lateral cluster (cluster B) were mapped onto these two standard atlases [16,25] with the approximate 6–7 mm separation. This demonstrates that, anatomically, clusters A and B correspond roughly to the core and shell subdivisions of the NAc, respectively, as described in the animal literature [27], and may be differentiated anatomically in the humans [23].

The average time courses of activation in each cluster following noxious and non-noxious thermal stimuli are shown in Fig. 3. There were no significant activations in either cluster in response to non-noxious thermal stimuli (Fig. 3A). In cluster A (“core”) the signal decreases during the first noxious stimulus and returns to baseline during the inter stimulus interval, and then follows a similar course during administration of the second noxious stimulus. In the “shell” cluster B the signal increases prior to the onset of the first noxious stimulus and then decreases rapidly during the noxious stimulus. After increasing towards baseline during the intervening neutral stimulus, the signal increases above baseline during the second noxious stimulus (Fig. 3C). Thus, this region of the NAc is activated during both the first and second noxious stimuli, but in opposite directions.

To quantify the signal changes within each cluster during the neutral and noxious thermal stimuli, we calculated the slope

Table 1
Activation in NAc clusters A and B: right vs. left hemispheres

	Cluster A			Cluster B		
	R-L	S-I	A-P	R-L	S-I	A-P
Left side						
Coordinates of center of mass	−5	−9	12	−9	−5	9
Average distance between centers of mass (mm)	6.40 ± 1.42					
Coordinates of maximum *t*-test	−4	−10	12	−8	−8	7
Average distance between maximum *t*-test voxels (mm)	6.71 ± 1.23					
Right side						
Coordinates of center of mass	5	0	10	8	−1	8
Average distance between centers of mass (mm)	3.74 ± 1.56					
Coordinates of maximum *t*-test	10	−1	12	6	0	8
Average distance between maximum *t*-test voxels (mm)	5.74 ± 1.53					

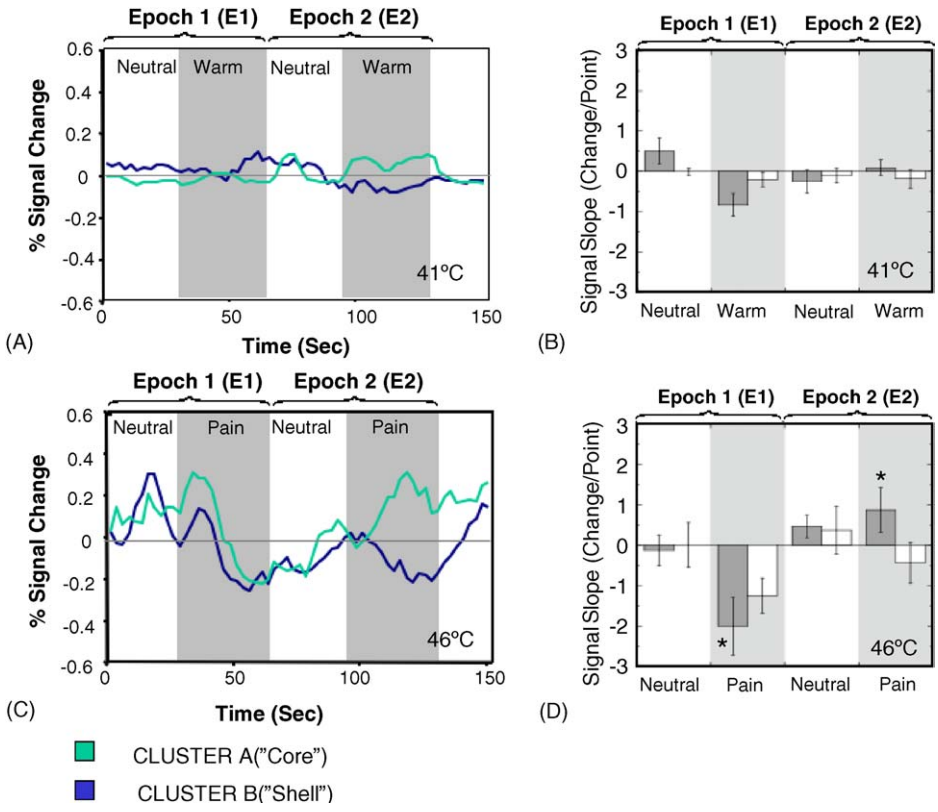

Fig. 3. Time course analysis for clusters A and B following a 41 or 46 °C stimulus: left panels (A and C) averaged, smoothed time-courses for clusters A (green) and B (blue) following a 41 or 46 °C stimulus. We temporally smoothed the time courses of all voxels with a five-position centered moving average operator, and then applied a procedure to "sharpen" the distinctions between clusters A (green) and B (blue) as described in the text; and right panels (B and D) slopes of best-fitting lines to the time courses of response in clusters A and B during each of the first two pain and neutral epochs are shown. Data is averaged from both hemispheres of all subjects. Note that no significant difference is observed between cluster A or B during either the first (E1) or second (E2) stimuli for the 41 °C paradigm during either the neutral (35 °C) or pain episodes. As indicated by the asterisks, significant differences are observed between cluster A and B for both the pain (46 °C) episodes, but not for the neutral episodes (35 °C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of the article.)

of the best-fitting line to the time courses during each of the first two thermal stimulus epochs as well as the first two neutral epochs for each subject, hemisphere, and cluster. The resulting set of slopes (measuring change in signal per time point) was explored with repeated-measures ANOVA, which indicated no significant effects or interactions involving the hemisphere variable. Accordingly, we averaged the left- and right-side slopes for each cluster for each subject. This analysis confirms a significant difference between the responses in the "core" and "shell" clusters (A and B) following noxious heat (Fig. 3D). Comparison of the slopes for both clusters during the first and second noxious epochs indicate significant differences ($p < 0.05$ student t-test). The amplitude of the signal changes for both clusters is greatest during the first pain epoch, while the largest difference between the clusters appears during the second pain epoch.

The results identify two distinct clusters of activation within the NAc with different responses to noxious thermal stimulation. These clusters may represent the anatomically defined core and shell regions, which have been shown to have distinct patterns of connectivity, as described above. The differences in the nature of the response in these two clusters may represent distinct functional roles for the core and shell of the NAc. However, any discussion of the core-shell organization should be accompanied by an acknowledgment that the true organization of the NAc involves a richly subtle mix of compartments, and that exclusive reference to core-shell organization may result in a gross under-appreciation of this mix in humans.

In terms of function, the initial increase in the BOLD signal prior to the first noxious stimulus may correlate with the subjects' expectation of the painful stimulus, which may include stress [22]. The decrease during the first stimulus itself might reflect relief of this "expectation stress." The pattern of the BOLD signal in cluster B (the "shell") during the second noxious epoch does not follow the pattern during the first epoch, and increases during the second noxious epoch. In contrast, in cluster A (the "core") there is no signal change prior to the first noxious stimulus, and the signal decreases to a similar level during the first and second painful stimuli, increasing towards baseline during the intervening neutral epoch (Fig. 3C). The changes in the "shell" may correlate more directly with a functional aversive response to the noxious stimulus.

The results may lend support to the notion that the NAc is involved in the dynamic interpretation of aversive vs. non-aversive or rewarding information [2]. Following noxious stimulation, the decrease in activation may reflect a response to

aversion in the NAc, with the return to baseline activation later reflecting a reward response since a decrease in pain is rewarding. In other studies, a small decrease in noxious pain stimuli results in a decrease in psychological ratings equal in magnitude to that when the stimulus actually returns to baseline [12].

In a recent functional magnetic resonance imaging (fMRI) study, both negative and positive NAc activation were observed in a group of subjects who were presented with stimuli perceived as rewarding or aversive [1]. One possible mechanism by which events with different motivational salience may produce positive and negative activation in the same anatomical region is through the involvement of different neuronal populations, such as dopaminergic and glutamatergic neurons [20]. In this manner, specific afferent systems may contribute to an "aversive" state, while others may contribute to a "rewarding" state. Indeed, different changes in neurochemical gradients in the shell have been observed following presentation of rewarding and aversive stimuli in animals [9].

Clusters A and B were deliberately constructed post hoc in such a way as to contain voxels that were dissimilar in time course profiles, so it is intriguing that they appear to correspond both anatomically and functionally to the core and shell of the NAc. Using higher field magnets and focusing on NAc during data acquisition, which we did not do in this experiment, should provide increased resolution in future studies. However, it is interesting to note that separate clusters within the human NAc appear to respond differently to the expectation, experience, and termination of pain, suggesting a functional mechanism by which the NAc might be involved in detecting changes in stimulus valence in humans and animals [5,19]. However, it should be pointed out that these accumbal sub-regions may also be related to other functions (e.g., sensory gating, novelty detection, modulation of stimulus salience, learning or escape motivation) [21,26]. For example, the shell could mediate antinociception by gating sensory information via pathways from medium spiny GABA projection neurons to ventral pallidal neurons [8]. In addition, the reversal of the response in the core from first to second trial may reflect a role in 'learning' which is not observed in the "shell", allowing for adaptation to the stimulus as previously observed in humans for the overall response [6].

The differences between the clusters occurred within a single stimulus epoch, or at least did not coincide precisely with changes in the stimulus. Thus, our analysis illustrates that exploring the time course data from fMRI experiments beyond simple correlation with stimuli [4] is valuable for the identification of activation occurring before or during stimuli, as well for distinguishing NAc subdivisions.

Animal studies have recently shown that the NAc may play important roles in pain modulation [24,28]. Given the reward circuitry's significant contribution to the processing of pain, these results demonstrate that detailed neuroimaging studies of NAc activity are feasible and may be a useful indicator or measure of the aversive nature of pain stimuli. This may have implications for the evaluation of analgesic effectiveness in surrogate models of pain or in chronic/pathological pain in which a 'reward deficit syndrome' may be present [10].

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