Unsupervised Fuzzy Clustering Analysis Supports Behavioral Cutoff Criteria in an Animal Model of Posttraumatic Stress Disorder

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Background: *Unsupervised fuzzy clustering (UFC) analysis is a mathematical technique that groups together objects in the multidimensional feature space according to a specified similarity measurement, thereby yielding clusters of similar data points that can be represented by a set of prototypes or centroids.*

Methods: *Since clinical studies of mental disorders distinguish between affected and unaffected individuals, we designed an inclusion/exclusion criteria (cutoff behavioral criteria [CBC]) approach for animal behavioral studies. The effect of classifying the study population into clearly affected versus clearly unaffected individuals according to behaviors on two behavioral paradigms was statistically significant.*

Results: *Here the raw data from previous studies were subjected to UFC algorithms as a means of objectively testing the validity of the concept of the CBC for our experimental model. The first UFC algorithm yielded two clearly discrete clusters, found to consist almost exclusively of the exposed animals in the one and unexposed animals in the other. The second algorithm yielded three clusters corresponding to animals designated as clearly affected, partially affected, and clearly unaffected. The algorithm for physiological data in addition to behavioral data failed to elicit discrete clusters.*

Conclusions: *The UFC analysis yielded data that support the conceptual contention of the CBC and lends additional validity to our previous behavioral studies.*

Key Words: Posttraumatic stress disorder, animal model, unsupervised fuzzy clustering, anxiety, stress, behavioral criteria

Posttraumatic stress disorder (PTSD) is an incapacitating chronic disorder ensuing from exposure to traumatic events involving a perceived threat to life or physical integrity. The diagnosis of PTSD depends on an index traumatic event occurring more than 1 month prior to onset. Symptoms must be present over a period of at least 1 month, including intrusive re-experiencing of the traumatic event, persistent avoidance and emotional numbing, and persistent symptoms of physiological arousal (American Psychiatric Association 1994).

Epidemiological studies of PTSD indicate that only a proportion of those exposed to an event will go on to develop the chronic syndrome (Breslau et al 1998; Helzer et al 1987; Resnick et al 1993). The discrepancy between the proportion of the general population exposed to potentially traumatic events and those who eventually fulfill criteria for the disorder suggests qualitative differences in vulnerability and/or resilience.

In light of the difficulty associated with prospective studies of the etiology of PTSD in humans and because PTSD explicitly requires presence of a precipitating environmental event, animal models are valuable.

The rationale for the use of animal models of human conditions is to enable experimentation and sample sizes that are impossible in humans for ethical/moral or practical/technical reasons. For the animal model to be useful, it must approximate the human disorder as validly as possible, always keeping in mind the risks inherent in "over-humanizing" animal behaviors.

Numerous animal models have been developed where intense stressful experiences have been shown to result in long-term alterations in psychological and physiological functioning reflected in biobehavioral tests, which mimic many of those seen in PTSD. Irrespective of the study design, the exposed animals displayed a diverse range of responses, and yet, the results were conceptualized and discussed as involving the entire exposed population. The clinical syndrome, however, clearly affects only a proportion of the exposed (Yehuda and McFarlane 1995) and thus reflects the fact that different subjects respond differently. The sequelae of exposure to extreme events range from normal adaptive responses to a maladaptive fixation on the event. It could thus be said that on a conceptual level, individual variations in response are integral to PTSD, probably reflecting individual vulnerability/resilience factors. Since animals display a range of responses, the exposed population cannot be homogeneous, practically or conceptually, and some animals appear to be more vulnerable than others.

In a previous study, the authors examined the effect of classifying exposed rats into clearly behaviorally affected (maladapted [MA]) and clearly unaffected (well-adapted [WA]) individuals according to the distribution of behaviors on the elevated plus maze (EPM) paradigm, by applying cutoff behavioral criteria (CBC) in imitation of the guiding principle for the inclusion and exclusion criteria applied to human conditions in diagnostic systems, such as DSM or ICD, and in most clinical trails (Cohen et al 2003). Only about 25% of the rats developed significant behavioral disruptions (MA) as the result of exposure to the stressor, as compared with 1.3% in unexposed control rats. The prevalence of WA rats was only 24.7% in the exposed groups, as compared with 80% in the control group. Maladapted rats also exhibited significantly higher plasma corticosterone and adrenocorticotropic hormone (ACTH) concentrations, increased sympathetic activity, diminished vagal tone, and increased sympatho-

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vagal balance. The authors therefore suggested that application of CBCs is both feasible and valid.

Unsupervised fuzzy clustering (UFC) analysis is a mathematical technique that groups together objects in the multidimensional feature space according to a specified similarity measurement, thereby yielding clusters of similar data points which can be represented by a set of prototypes or centroids (see Appendix). Each data point is attached to each cluster with different degrees of membership. This tool identifies meaningful structures among multidirectional data, exposing new information and models in the global data (Gath and Geva 1989; Geva and Pratt 1994).

The rationale for electing to use UFC rather than deterministic clustering methods, such as K-means, is based on a number of considerations:

1. Since the data are not well separated, they may be best modeled by probabilistically belonging to more than one cluster.
2. The UFC algorithm uses exponential distance, since the distribution of the different clusters is variable in terms of the means and variance. Methods which assume Euclidean distances and not exponential (compatible with Gaussian distribution mixture) will not provide accurate answers.
3. The number of clusters and their centroids are unknown ahead of time. The UFC method resolves this issue (see steps 10 and 11 in the Appendix) by beginning with one group at the center of data and with each iteration adding another group at a location in which membership in the previous groups is small, while examining the partition validity criteria until reaching the maximal number of groups (Gath and Geva 1989; Geva 1998, 1999; Geva and Pratt 1994). The number of clusters providing maximal validity criteria measurements is the one chosen for optimal partition. The idea is to find the partition that models the data with minimum number of Gaussian clusters.

The authors thus set out to process the raw data of behavioral studies in which the CBCs were applied to segregate the animals according to observed behaviors to examine whether the UFC analysis would group the animals in a similar manner to the behavioral grouping.

The aim of the present study, then, was to further examine the feasibility and validity of applying the CBC concept in animal studies, using unsupervised fuzzy clustering.

Methods and Materials

Experimental Procedures

Animals. Three hundred male Sprague-Dawley rats weighing 150 to 200 gm were employed. Animals were habituated to the housing conditions for at least 10 days and handled once daily. The animals were housed four per cage at stable temperature and reversed 12-hour light/dark cycle with ad libitum food and water. All testing was performed during the dark phase using a dim light.

Exposure to Stressor. The stress paradigm consisted of placing the test animals on well-soiled cat litter for 10 minutes (in use by the cat for 2 days, sifted for stools). The control animals were exposed to fresh, unused litter for the same amount of time.

Behavioral Paradigms

Behavioral tests were done 7 days after exposure to stress. The assessment of rats after 7 days has previously been established by Adamec et al (1997) and is accepted as reflecting PTSD and not acute stress reaction. Our results (Cohen et al 2004) have shown repeatedly that as of day 7, the prevalence rate remains constant at about 25% until day 30, i.e., reflect long-standing changes.

The Elevated Plus Maze. The maze employed is a four-armed black opaque Perspex platform (Columbus Instrument, Columbus, Ohio), elevated 50 cm above the ground, as described by File (1993). Each 5-minute session was recorded using an overhead video camera connected to a monitor/recorder in an adjacent observation room. All test sessions were analyzed by ETHO-VISION program (Noldus Information Technology, Wageningen, The Netherlands). Five measures of behavior were assessed: time spent in the open arms, time spent in the closed arms, number of entries into open arms, number of entries into closed arms, and total entries into all arms (total activity).

Acoustic Startle Response. Startle response was measured using two ventilated startle chambers (SR-LAB system, San Diego Instruments, San Diego, California).

Full description of the data and procedures is given in Cohen et al (2003).

Behavioral Assessments for Determination of Cutoff Behavioral Criteria

The assessments were designed in two steps.

Step I: Assessment of Overall Behavioral Effect. Prior to attempting to distinguish the differently affected subgroups, we routinely performed a preliminary assessment of the overall response of the exposed population. This was intended to demonstrate that exposure to the stressor did, in fact, have significant overall behavioral effects on the exposed rats as a group compared with control animals. The data were also examined to determine if there was a sufficient range of varying degrees of behavioral changes.

Behavioral changes, such as extremely compromised exploratory behavior on the plus maze and markedly increased startle reaction that does not undergo any adaptation, reflect anxiety like behaviors, fearfulness, and hypervigilance. In keeping with the work of Blanchard et al (1990, 1993), Adamec et al (1999), Adamec and Shallow (1993), and our own previous studies (Cohen et al 1996, 1999, 2000, 2003, 2004), the observed behaviors at this time point were considered to reflect relatively long-term and persistent changes.

Step II: Application of the CBC to the Data. Having established that the stressor had an effect on the rats and that not all animals responded to it in the same manner, we focused only on animals that demonstrated extremes of behavioral change, on the one hand, maladapted, or virtually no change on the other, well-adapted.

The cutoff behavioral criteria are a means of distinguishing individuals with extreme behavioral disturbances from those virtually unaffected by the same stress conditions, using two simple, well-documented models for behavioral assessment of stress-related behaviors. The models were selected since they are widely applied and familiar, while also being related to familiar groups of PTSD symptoms, namely avoidance behaviors and startle response to sudden stimuli. The criteria were arbitrarily determined by defining the most extreme degrees of behavioral disruption in each model (Cohen et al 2003, 2004; Cohen and Zohar 2004).

To maximize the resolution and by doing so to minimize the chance of including false-positives, we defined the behavioral cutoff criteria to represent the most extreme degree of behavioral