A Principled Method to Identify Individual Differences and Behavioral Shifts in Signaled Active
Avoidance

Angelos-Miltiadis Kryptos¹, Justin M. Moscarello², Robert M. Sears^{3,4,5}, Joseph E. LeDoux^{3,4,6},
& Isaac Galatzer-Levy⁷

¹ Department of Clinical Psychology, Utrecht University, Utrecht, the Netherlands ² Department of Psychological & Brain Sciences, Texas A & M University, College Station, TX, USA ³ Center for Neural Science, New York University, New York, NY, USA, ⁴ Emotional Brain Institute, Nathan Kline Institute for Psychiatric Research, Orangeburg, NY, USA ⁵ Department of Child and Adolescent Psychiatry, New York University School of Medicine, NY, USA ⁶ Department of Psychology, New York University, New York, NY, USA ⁷ Department of Psychiatry, New York University Langone Medical Center, NY, USA

Abstract

Signaled active avoidance (SigAA) is the key experimental procedure for studying the acquisition of instrumental responses towards conditioned threat cues. Traditional analytic approaches (e.g. general linear model) often obfuscate important individual differences. However, individual differences models (e.g. latent growth curve modeling) typically require large samples and onerous computational methods. Here, we present an analytic methodology that enables the detection of individual differences in SigAA performance at a high accuracy based at the $n=1$ level. We further show an online software that enables the easy application of our method to any SigAA data set.

Keywords: anxiety disorders, psychopathology, fear, defensive behaviors

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Signaled active avoidance (SigAA) is the dominant experimental paradigm for studying instrumental defensive responses. During SigAA, an animal is typically placed in a shuttle box where it is exposed to pairings of a neutral stimulus (CS; e.g. a sound) with an aversive unconditioned stimulus (US; electric stimulation), with the animal being able to cancel the US by shuttling to the adjacent chamber during CS presentation (Mowrer & Lamoreaux, 1942; Solomon & Wynne, 1953). This simple procedure has been widely used across the fields of psychology and neuroscience for uncovering the theoretical principles and the neural underpinnings of (maladaptive) avoidance (LeDoux, Moscarello, Sears, & Campese, 2017).

Statistical inferences of SigAA data are usually based on the analysis of average response rates, where a general linear model is fitted on the animals' mean responses (i.e. the traditional method). Despite the popularity of this approach, there are at least two limitations with it.

First, by averaging responses across the whole sample, the traditional method fails to account for individual differences in avoidance acquisition, with any deviations from the mean being essentially treated as noise (Lonsdorf & Merz, 2017). However, there is good evidence that there is individual heterogeneity in the ability to acquire and extinguish instrumental responses to aversive stimuli (Galatzer-Levy et al., 2013; Galatzer-Levy, 2014). Importantly, such heterogeneity has long been hypothesized to underlie fluctuations in psychological response to trauma (Bonanno, Kennedy, Galatzer-Levy, Lude, & Elfström, 2012).

Second, the traditional method fails to account for the phase-shift from a reactive threat detection state, where the animal acquires the CS-US associations (i.e. *Pavlovian learning*) and freezes in presence of the CS, to an active state, where the animal avoids the US presentation when the CS is presented (i.e. *instrumental learning*). Empirically, trajectories of individual differences characterized by these phase-shifts have been shown to better reflect avoidance acquisition than a linear pattern (Galatzer-Levy et al., 2014). This phase-shift is also predicted by the dominant theoretical accounts of avoidance learning (see Krypotos, Effting, Kindt, & Beckers,

2015 and LeDoux et al., 2017 for reviews).

Galatzer-Levy et al. (2014) showed that these shortcomings can be surpassed by using *Latent Class Growth Analysis* (LCGA), a cluster analytic method for identifying subgroups, within the sample data, defined by their rate of response change over time. Using LCGA, Galatzer-Levy et al. (2014) showed that the sample data could be reliably grouped into different subgroup, with each subgroup exhibiting a different rate of avoidance learning pattern (e.g. avoidance after one, two days etc.) or failing to learn to avoid within the allotted time. Importantly, the pattern of responses within the subgroups did not resemble the average group pattern.

The results above are important for at least two reasons. First, the LCGA results provided strong evidence of observable heterogeneity within the population as animals either did not learn to avoid at all, or acquired the instrumental response at different learning rates. Second, the subgroups that learned to avoid exhibited the predicted step-like pattern where, after acquiring the avoidance response, animals reached asymptotic levels of avoidance. As such, the theoretical prediction of a transition point from freezing to avoidance responses was supported.

Despite the advantages of LCGA over the traditional method for analyzing SigAA data, LCGA comes with onerous sample size requirements (Hertzog, Lindenberger, Ghisletta, & Oertzen, 2006), diminishing their relevance to animal research which typically uses small samples to examine fine-grained mechanisms. In order to overcome these shortcomings, we have developed a principled method for categorizing animals in different trajectory learning clusters that is based on LCGA but has no high sample requirements. Our method is based on the definition of an inflection point after which animals exhibit asymptotic avoidance responses.

For determining this inflection point, we first reanalyzed the second study of Galatzer-Levy et al. (2014). Full details about the experimental setup and statistical analyses of this data set are described in the original study. In short, 186 naive male Sprague-Dawley rats performed a signaled active avoidance task as explained above, for 5 days. The final LCGA solution suggested that the animals could be classified into 4 groups: Modal Avoiders (37% of the sample), Rapid

Avoiders (22% of the sample), Slow Avoiders (22% of the sample), and Non-Avoiders (20% of the sample) (see Figure 1).

We followed up the results of Galatzer-Levy et al. (2014) by computing Receiver operating characteristic (ROC) curves (Metz, 1978). ROC curves are routinely used in, among others, self-report development for testing how accurately a scale can distinguish individuals with and without a disorder (e.g. depression). The most common measure for describing a ROC curve is by computing the area under it. Values between 0 and .60 indicate failure of the test to discriminate between a random case to a noncase whereas values closer to 1 indicate excellent discrimination. For these analyses, we compared each class defined by the LCGA, with the rest of the classes. The results suggest that the group of animals were accurately placed into categories that can be adequately separated from each other (all ROC values $> .80$; detailed values at Table 1 of the supplemental material). Based on these outcomes, we determined that the inflection point after which freezing behavior turns to avoidance is when 55% of the trials are avoided on any single day.

Then, we tested the generalizability of our method by applying it to a new independent data set. This data set was derived from a study with parameters similar to the experiments that produced the data set described above, with the exception that animals performed only 4 daily sessions and that neither the CS nor the US were inactivated by shuttling. Instead, both the CS and the US continued to be presented for the full duration regardless of whether or not the animal shuttled during these stimuli.

In Figure 2 we plot the results of each group for each day. The groups are defined based on when, if ever, was the first time they crossed the defined inflection point. In Figure 1 of the supplemental material we plot, for each day, the mean results of the animals who had crossed the inflection point, compared to the animals that had not. For investigating how well the set inflection point discriminated groups from each other, we carried a series of *default* Bayesian *t*-tests (Morey & Rouder, 2015; Rouder, Speckman, Sun, Morey, & Iverson, 2009) in which we compared, separate for each day, the mean avoidance of each group, to the mean distance between

the mean responses of Avoiders and Non-Avoiders. We denote the relative support of the data under the alternative hypothesis, compared to the null hypothesis, by BF_{10} .

The size of BF_{10} (detailed results in the Supplemental Table 2) suggests that there is a clear discrimination between classes. Specifically, apart from the Avoiders on the first day ($BF_{10} = 6.595$), all BF_{10} s provided strong ($BF_{10} > 18.755$) or unquestionable ($BF_{10} > 100$) support from the alternative hypothesis (i.e. differences between the mean responses of the group compared to the mean distance between the two groups) compared to the null hypothesis (i.e. no differences between the mean responses of the group compared to the mean distance between the two groups). Collectively, these results suggest that the provided inflection point can be successfully applied to other data sets and meaningfully categorize groups of learners.

Lastly, we tested the split-half reliability of our method by fitting it to two random halves of a subset of data presented in Ramirez, Moscarello, LeDoux, and Sears (2015) (procedural details can be found in the original study). We present the data of 52 animals who undertook an avoidance learning procedure for 3 days.¹ In order to assess the reliability of our results, we split the data randomly into two equal data sets. We visualize the results of each data half on Figure 3 and in Supplemental Figure 2. On Supplemental Table 3 we present the Bayes factors for each half of the data set.

Collectively, the split-half results showed that the analyses of both halves resulted in similar outcomes. Specifically, the proposed inflection point was able to classify rats as Avoiders and Non-Avoiders, with the Bayes factors providing good evidence for most of the groups, and the number of rats per group being largely similar. Collectively, these results show that the suggested inflection point is reliable.

The presented method overcomes the key disadvantages of traditional analyses of SigAA. Specifically, by following our method, researchers are able to detect the expected learning heterogeneity within the tested sample, as well as the predicted step-wise pattern as suggested by

¹Please note that after the third day, some of the rats were infused with muscimol or vehicle into the nucleus accumbens shell, or the nucleus accumbens core. The data collected during and after the treatment were not included in our data analyses and will not be described further.

individual differences analyses and most theoretical models of avoidance learning. As such, our method has many the benefits of the LCGA for SigAA data but comes without requirements for large sample size in LCGA (Hertzog et al., 2006).

Our method can have significant impact on our understanding of mechanisms underlying SigAA as methods such as continuous recordings of neurons can now be utilized to understand change from before to after phase shifts in behavior, and in the relevant brain regions of interest (e.g. with the method of optogenetics or chemogenetics). Further, manipulations can be utilized to determine if they cause a shift between active avoidance and the reactive threat detection state. Both of these approaches are relevant for understanding mechanisms of health and pathology in response to environmental stress, but are not accessible when examining a linear trajectory of change over time.

To make our methodology readily available to every researcher, we have created a user friendly and free software that is available at [urlhttps://utrecht-university.shinyapps.io/aacc/](https://utrecht-university.shinyapps.io/aacc/). Given its intuitive design, every researcher can use our application easily with the relevant outcomes being available in seconds. The wide application of our methodology to SigAA data sets could lead to significant theoretical and clinically relevant advancements.

Despite the advantages of our method over the traditional approach, we acknowledge two potential issues. First, in case of large samples, we would urge researchers to still take advantage of the flexibility of the LCGA analyses. Second, it could be argued that any use of a cut-off (here the inflection point) is debatable, especially when participants perform close to the cut-off scores. At the same time, given the advantages of our method (see above) we do believe that there is little reason not to use our methodology when small sample sizes are used, with larger studies further testing and validating the initial conclusions.

In sum, we have presented a principled way, with a user-friendly software, to classify animals undergoing a SigAA into distinct subpopulations that overcomes many of the limitations of the traditional analytic methods. As we have argued above, the wide application of our methodology to SigAA could lead to significant new theoretical and mechanistic insights.

References

- Bonanno, G. A., Kennedy, P., Galatzer-Levy, I. R., Lude, P., & Elfström, M. L. (2012). Trajectories of resilience, depression, and anxiety following spinal cord injury. *Rehabilitation Psychology*, 57, 236. doi: 10.1037/a0029256
- Galatzer-Levy, I. R. (2014). Empirical characterization of heterogeneous posttraumatic stress responses is necessary to improve the science of posttraumatic stress. *Journal of Clinical Psychiatry*, 75, 950–952. doi: 10.4088/JCP.14com09372
- Galatzer-Levy, I. R., Ankri, Y., Freedman, S., Israeli-Shalev, Y., Roitman, P., Gilad, M., & Shalev, A. Y. (2013). Early PTSD symptom trajectories: persistence, recovery, and response to treatment: results from the jerusalem trauma outreach and prevention study (j-tops). *PLoS One*, 8, e70084. doi: 10.1371/journal.pone.0070084
- Galatzer-Levy, I. R., Moscarello, J., Blessing, E. M., Klein, J., Cain, C. K., & LeDoux, J. E. (2014). Heterogeneity in signaled active avoidance learning: substantive and methodological relevance of diversity in instrumental defensive responses to threat cues. *Frontiers in Systems Neuroscience*, 8. doi: 10.3389/fnsys.2014.00179
- Hertzog, C., Lindenberger, U., Ghisletta, P., & Oertzen, T. v. (2006). On the power of multivariate latent growth curve models to detect correlated change. *Psychological Methods*, 11, 244–252. doi: 10.1037/1082-989X.11.3.244
- Krypotos, A.-M., Effting, M., Kindt, M., & Beckers, T. (2015). Avoidance learning: A review of theoretical models and recent developments. *Frontiers in Behavioral Neuroscience*, 9, 189. doi: 10.3389/fnbeh.2015.00189
- LeDoux, J., Moscarello, J., Sears, R., & Campese, V. (2017). The birth, death and resurrection of avoidance: A reconceptualization of a troubled paradigm. *Molecular Psychiatry*, 22, 24–36. doi: 10.1038/mp.2016.166.
- Lonsdorf, T. B., & Merz, C. J. (2017). More than just noise: Inter-individual differences in fear acquisition, extinction and return of fear in humans—biological, experiential, temperamental factors, and methodological pitfalls. *Neuroscience & Biobehavioral Reviews*. doi:

10.1016/j.neubiorev.2017.07.007

Metz, C. E. (1978). Basic principles of ROC analysis. *Seminars in Nuclear Medicine*, 8, 283–298. doi: 10.1016/S0001-2998(78)80014-2

Morey, R. D., & Rouder, J. N. (2015). BayesFactor: Computation of Bayes factors for common designs [Computer software manual]. Retrieved from <https://CRAN.R-project.org/package=BayesFactor> (R package version 0.9.12-2)

Mowrer, O. H., & Lamoreaux, R. R. (1942). Avoidance conditioning and signal duration—a study of secondary motivation and reward. *Psychological Monographs*, 54, 1–34. doi: 10.1037/h0093499

Ramirez, F., Moscarello, J. M., LeDoux, J. E., & Sears, R. M. (2015). Active avoidance requires a serial basal amygdala to nucleus accumbens shell circuit. *Journal of Neuroscience*, 35, 3470–3477. doi: 10.1523/JNEUROSCI.1331-14.2015

Rouder, J. N., Speckman, P. L., Sun, D., Morey, R. D., & Iverson, G. (2009). Bayesian *t* tests for accepting and rejecting the null hypothesis. *Psychonomic Bulletin & Review*, 16, 225–237. doi: 10.3758/PBR.16.2.225

Solomon, R. L., & Wynne, L. C. (1953). Traumatic avoidance learning: Acquisition in normal dogs. *Psychological Monographs: General and Applied*, 67, 1–19. doi: 10.1037/h0093649

Figure 1. Visualization of the latent groups as identified by Galatzer-Levy et al. (2014). Each point summarizes the mean responses for each group on a single day. Error bars denote standard errors.

Figure 2. A) Mean number of avoids for the second data set Error bars denote standard errors. (B) Individual values for each subgroup, for the second data set.

Figure 3. (A) Mean number of avoids for each subgroup, for the first half of the third data set. Error bars denote standard errors. (B) Mean number of avoids for each subgroup, for the second half of the third data set. Error bars denote standard errors. (C) Individual values for each subgroup, for the first half of the third data set. (D) Individual values for each subgroup, for the second half of the third data set.