Neuropsychology Review (2019) 29:181–185
https://doi.org/10.1007/s11065-018-9383-7

REVIEW

Distinct Activity Patterns of the Human Bed Nucleus of the Stria Terminalis and Amygdala during Fear Learning

Kelly Luyck[1] · Travis D. Goode[2] · Haemy Lee Masson[3] · Laura Luyten[4]

Received: 26 January 2018 / Accepted: 13 August 2018 / Published online: 18 September 2018

Abstract

The amygdala and, more recently, also the bed nucleus of the stria terminalis, have been widely implicated in fear and anxiety. Much of our current knowledge is derived from animal studies and suggests an intricate convergence and divergence in functions related to defensive responding. In a recent paper, Klumpers and colleagues set out to examine these functions in a human fear learning procedure using functional magnetic resonance imaging. Their main findings were a role for the bed nucleus of the stria terminalis in threat anticipation, and for the amygdala in threat confrontation. Here, we provide a critical summary of this interesting study and point out some important issues that were not addressed by its authors. In particular, we first take a closer look at the striking differences between both samples that were combined for the study, and, secondly, we provide an in-depth discussion of their findings in relation to existing neurobehavioral models.

Keywords Bed nucleus of the stria terminalis · Amygdala · Fear learning · fMRI · Human · Defensive responses

An organism's survival is dependent on its ability to appropriately respond to anticipated or encountered threats. For example, total immobility might be the optimal reaction for a rat to prevent detection by a falcon, but freezing during an actual attack would likely have lethal consequences (Fanselow 1994). Two brain regions that have been put forward as key mediators of a whole range of behavioral and autonomic defensive responses are the bed nucleus of the stria terminalis (BNST) and the amygdala. Although BNST and amygdala show striking similarities in terms of connections and neurochemical properties (Alheid et al. 1998), a vast number of studies, mainly fear conditioning, suggest their functional distinction. In particular, seminal models have distinguished between BNST and amygdala recruitment based on duration of the threat and fear response (Davis et al. 2010), while others stress the importance of threat proximity (Perusini and Fanselow 2015) or its temporal unpredictability (Luyten et al. 2012; Goode and Maren 2017). These and other views have provided excellent frameworks for studying the roles of BNST and amygdala, although it should be noted that several studies underline that the functional distinction between both structures might be even more complex (Gungor and Paré 2016; Shackman and Fox 2016).

In a recent paper, Klumpers et al. (2017) aimed to shed light on the relative contributions of the BNST and amygdala to human defensive responses, operationalized as episodes of shock anticipation and confrontation in a functional magnetic resonance imaging (fMRI) study. Two large, independent samples consisting of healthy participants underwent a single-session, cued fear conditioning procedure with partial reinforcement, using highly uncomfortable, but not painful, electric shocks to the fingers. The main finding was elevated bilateral BNST activity during shock anticipation in both samples, while the amygdala was not recruited during this phase. During shock confrontation, opposite results were obtained, with stronger activity in the bilateral amygdala as compared with BNST. Although the distinction between both regions as observed in the whole-brain fMRI analyses was marginal from a statistical point of view, a

✉ Laura Luyten
laura.luyten@kuleuven.be

[1] Laboratory of Experimental Neurosurgery and Neuroanatomy, KU Leuven, 3000 Leuven, Belgium

[2] Institute for Neuroscience and Department of Psychological and Brain Sciences, Texas A&M University, College Station, TX 77843-3474, USA

[3] Laboratory for Biological Psychology, KU Leuven, 3000 Leuven, Belgium

[4] Centre for Psychology of Learning and Experimental Psychopathology, KU Leuven, Tiensestraat 102 PB 3712, 3000 Leuven, Belgium

region-of-interest analysis did support these conclusions. According to the authors, these data suggest a shift from BNST to amygdala activity when a threat becomes imminent. In addition, BNST and amygdala showed distinct functional connectivity to other brain structures throughout the experiments, without discerning between threat or safe cues, or shocks. Finally, the authors suggested a role for early-life stress in amygdala, but not BNST, recruitment during defensive responses, as participants (Sample 1 only) with high retrospective childhood maltreatment levels displayed stronger amygdala activation during threat anticipation compared to those with low maltreatment scores. Although interesting, the clinical importance of this correlation (and of the absence of the correlation in BNST) is difficult to interpret, especially in light of the fact that all participants were healthy subjects, explicitly screened to exclude those with prior or ongoing psychiatric disorders. Moreover, the experimental procedure may not have been tailored to detect involvement of the BNST in risk for psychopathology. For example, we can presume that the limited threat intensity and lack of long-term fear memories in Klumpers et al. deviate considerably from the clinical reality, where patients are usually confronted with strong and old emotional memories. While this does not detract at all from human fear conditioning research in healthy subjects, it should be acknowledged that neural activation patterns may differ between healthy participants versus patients or those at risk (Brinkmann et al. 2017a, b; Milad et al. 2013). Beyond the imaging data, the authors reported (in a subset of Sample 2 and outside of the scanner) cardiac deceleration during threat anticipation, whereas actual shock confrontation was associated with acceleration. This was taken as evidence for two distinguishable defensive modes, which were then analyzed separately using fMRI in two rather different datasets. While the combination of fMRI and heart rate measurements is exciting, these data were not collected simultaneously nor during the same time bins, which could have provided more compelling evidence for a direct role of BNST and amygdala in these defensive responses.

Overall, Klumpers et al. (2017) addressed a pertinent research question and provided some fascinating insights by directly contrasting BNST and amygdala involvement in human defensive responses. We especially applaud the effort that they expended on examining the activation of the BNST, a small, deep brain region, which has often been disregarded in prior fMRI studies, notwithstanding the accumulating evidence for its implication in psychopathology (Brinkmann et al. 2017a, b; Lebow and Chen 2016; Luyten et al. 2016). In addition, we praise the authors' efforts to include not one, but two exceptionally large fMRI samples, to enhance the robustness of their findings (Button et al. 2013). In what follows, we will primarily focus on the intriguing distinction between BNST versus amygdala, more particularly on the differences between both datasets and how the present findings relate to existing models.

First, we will discuss the use of two samples by Klumpers et al. (2017), who highlighted it as a strength of their study that the patterns of BNST and amygdala activation were generally consistent in two independent datasets, and rightly so. However, while the authors have primarily focused on the neural commonalities, it is also worthwhile to take a closer look at why brain activity diverged substantially between both samples. For instance, it seems that considerably fewer brain regions were activated in Sample 2 compared to Sample 1 and, more importantly, that both samples displayed conspicuously distinct functional coupling patterns. One possible reason for these outcomes is that both experimental procedures may have resulted in different levels of threat anticipation. Shocks were administered at the offset of a 4-s cue in Sample 1, while they could occur at any time during presentation of a 6-to-12-s cue in Sample 2. Combined with differences in instructions and reinforcement rates (33 and 16% for Sample 1 and 2, respectively), shock unpredictability presumably differed substantially between both procedures. This is especially relevant given the role of the BNST in processing temporally unpredictable threat cues (Alvarez et al. 2011; Goode and Maren 2017; Luyten et al. 2012). Consistent with the hypothesis that unpredictability might have been greater in Sample 2, BNST activity during shock anticipation appeared higher in Sample 2 than Sample 1 (Klumpers et al. 2017, Fig. 3B-C), although this was not statistically evaluated by the authors and it should be kept in mind that there were many procedural differences between both samples. Next, it is noteworthy that some of the participants overlap with those of a separate psychophysiological study, which included startle measurements (Klumpers et al. 2015). It is important to identify which subjects were already exposed to a similar conditioning procedure before imaging, because this may impact fear acquisition data in the scanner. Although it has been suggested that re-exposure to a fear conditioning procedure may have limited influence on threat anticipation as measured by fear-potentiated startle and skin conductance (Klumpers et al. 2010; Zeidan et al. 2012), there are also indications that psychophysiological measures such as skin conductance and neural activation do not always converge (Klucken et al. 2009; LaBar and Cabeza 2006; Tabbert et al. 2006). In addition, startle probes per se can be perceived as rather aversive (Lissek et al. 2005) and may even increase self-reported anxiety (Grillon and Ameli 1998). In other words, re-exposure to a procedure with shocks or startle probes may in itself result in an overall increase in anticipatory anxiety and general stress levels, and thus impact neural activity patterns, which could be especially relevant when investigating BNST recruitment (Crestani et al. 2013; Daniel & Rainnie 2016). Additionally, the authors could have increased the uniformity of their

fMRI data analyses across both samples by reporting the same contrasts for shock anticipation, which was compared with safe cues, and confrontation, which was compared with intertrial intervals. Furthermore, given the existing evidence for a distinct time course of BNST and amygdala activation with regard to stimulus onset (Brinkmann et al. 2017b; Daldrup et al. 2016; Luyck et al. 2018), it would have been preferable to analyze the responses in both samples across the same response duration (either onset data or 4-s periods for both samples). Finally, Sample 2 included both female and male participants ($n = 70$; 49 female), which might induce additional heterogeneity in the imaging data compared to those of all-male Sample 1 ($n = 108$). This is particularly relevant for brain regions with clear sexual dimorphism, such as the BNST and amygdala (Stefanova and Ovtscharoff 2000). Therefore, it would have been informative to evaluate imaging data for both sexes separately.

Our second overarching point concerns the authors' suggestion that their data are in line with existing neurobehavioral models. Before getting to this, we should emphasize that fMRI responses were assessed during a single session of cued fear conditioning, which predominantly reflects acquisition, rather than retrieval and expression of consolidated fear memories. This is quite different from animal studies which often evaluate consolidated fear responses in the absence of shocks. While only briefly mentioned by the authors, this distinction is not trivial. Whereas basolateral and central amygdala have been implicated in various stages of fear acquisition, consolidation, and expression (Fanselow 1994), little is known regarding the role of BNST during fear acquisition (Asok et al. 2017). It is important to note here that, although the participants in Klumpers et al. were given rather explicit instructions about the cue-shock contingencies (particularly in Sample 2), the imaging period cannot be readily equated to the acquisition or retrieval phase of a typical rodent fear conditioning experiment. Whereas instructions in human fear conditioning studies usually give rise to very rapid and robust fear acquisition (Lonsdorf et al. 2017), the recruited neural networks may not be identical to those activated by experience-based associative learning (Mechias et al. 2010) and even differ in the regions of interest examined by Klumpers et al. (e.g. amygdala activation in Atlas et al. 2016). Moreover, apart from the difficult discussion of whether the obtained imaging data may mainly cover acquisition or retrieval, there is no question that the cue-shock association was not fully consolidated on a neural level in this human study (which took less than 30 min). Consolidation into long-term memory presumably takes several hours (McGaugh 2000), which is one of the main reasons for having at least one day in between training and test in most rodent fear conditioning studies. Moreover, the fact that shocks were administered throughout the imaging study makes it more akin to the acquisition than retrieval phase of a typical rodent experiment, at least on a procedural level. Keeping this in mind, we would like to contrast their findings with two important models of amygdala and BNST function (1) the phasic-versus-sustained-fear model of Davis and colleagues, and (2) the predatory imminence model of Fanselow and colleagues. According to Davis and colleagues, BNST activity is evoked by exposure to a long-duration threat cue, but not by short cues such as those used by Klumpers et al. (2017), which would be central amygdala-dependent (Davis et al. 2010).

In our opinion, this discrepancy may be resolved by emerging indications for BNST involvement in certain forms of cued fear (Daldrup et al. 2016), as well as recent conceptualizations of the role of the BNST in temporal unpredictability, regardless of cue duration (Goode and Maren 2017), especially given the pronounced ambiguity inherent to the procedures of Klumpers et al. (2017). The authors themselves already pointed out that their amygdala findings might have been influenced by the low reinforcement rates. Moreover, we hypothesize that the fMRI results in the BNST would also have been very different had the CS undergone 100% reinforcement (see also Alvarez et al. 2011). Additionally, it would be useful to compare BNST or amygdala responses during early and late phases of conditioning to observe whether these responses are consistent during the process of learning. When turning to the second, predatory imminence model, which is based on spatial and temporal threat proximity (Fanselow 1994), the amygdala is recruited upon threat detection ("post-encounter"), whereas actual threat confrontation ("circa-strike") engages different circuits, but not the amygdala. While BNST involvement is less defined, it has been postulated that it may mediate the vigilant pre-encounter phase (Perusini and Fanselow 2015). In contrast, Klumpers et al. (2017) showed BNST, but no amygdala, recruitment during shock anticipation, while shock confrontation resulted in significant amygdala activation. One possible explanation for these inconsistencies might be that the shocks were not as aversive as those used in animal studies, thereby minimizing the contributions of circa-strike circuitry. In apparent contrast, the observations of bradycardia and tachycardia during threat anticipation and confrontation were actually in line with previous findings within the predatory imminence framework (Lang et al. 2000). An interesting approach for future studies might be to use fear-relevant stimuli (e.g., snake pictures) rather than neutral cues (Ohman and Mineka 2001). Such stimuli may produce a shift along the threat imminence continuum, and a different brain activity pattern.

Acknowledgements We acknowledge the financial support of the Research Foundation – Flanders (FWO) (Research Projects G0C9817N and G088216 N), the National Institute of Mental Health (F31MH107113), as well as the European Research Council (CoG 64817). We thank Prof. Bram Vervliet and our colleagues at the KU Leuven Psychology Department for the helpful discussions during the preparation of this manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare no competing financial interests.

References

Alheid, G. F., Beltramino, C. A., De Olmos, J. S., Forbes, M. S., Swanson, D. J., & Heimer, L. (1998). The neuronal organization of the supracapsular part of the stria terminalis in the rat: the dorsal component of the extended amygdala. *Neuroscience, 84*(4), 967–996.

Alvarez, R. P., Chen, G., Bodurka, J., Kaplan, R., & Grillon, C. (2011). Phasic and sustained fear in humans elicits distinct patterns of brain activity. *Neuroimage, 55*(1), 389–400. https://doi.org/10.1016/j.neuroimage.2010.11.057.

Asok, A., Draper, A., Hoffman, A. F., Schulkin, J., Lupica, C. R., & Rosen, J. B. (2017). Optogenetic silencing of a corticotropin-releasing factor pathway from the central amygdala to the bed nucleus of the stria terminalis disrupts sustained fear. *Molecular Psychiatry, 23*(4), 914–922. https://doi.org/10.1038/mp.2017.79.

Atlas, L. Y., Doll, B. B., Li, J., Daw, N. D., & Phelps, E. A. (2016). Instructed knowledge shapes feedback-driven aversive learning in striatum and orbitofrontal cortex, but not the amygdala. *Elife, 5*. https://doi.org/10.7554/eLife.15192.

Brinkmann, L., Buff, C., Feldker, K., Tupak, S. V., Becker, M. P. I., Herrmann, M. J., & Straube, T. (2017a). Distinct phasic and sustained brain responses and connectivity of amygdala and bed nucleus of the stria terminalis during threat anticipation in panic disorder. *Psychological Medicine, 47*(15), 2675–2688. https://doi.org/10.1017/S0033291717001192.

Brinkmann, L., Buff, C., Neumeister, P., Tupak, S. V., Becker, M. P., Herrmann, M. J., & Straube, T. (2017b). Dissociation between amygdala and bed nucleus of the stria terminalis during threat anticipation in female post-traumatic stress disorder patients. *Human Brain Mapping, 38*(4), 2190–2205. https://doi.org/10.1002/hbm.23513.

Button, K. S., Ioannidis, J. P. A., Mokrysz, C., Nosek, B. A., Flint, J., Robinson, E. S. J., & Munafò, M. R. (2013). Power failure: why small sample size undermines the reliability of neuroscience. [research]. *Nature Reviews Neuroscience, 14*(5), 365. https://doi.org/10.1038/nrn3475.

Crestani, C.C., Alves, F.H.F., Gomes, F.V., Resstel, L.B.M., Correa, F.M.A., Herman, J.P. (2013). Mechanisms in the bed nucleus of the stria terminalis involved in control of autonomic and neuroendocrine functions: a review. *Current Neuropharmacology 11*, 141–159.

Daldrup, T., Lesting, J., Meuth, P., Seidenbecher, T., & Pape, H. C. (2016). Neuronal correlates of sustained fear in the anterolateral part of the bed nucleus of stria terminalis. *Neurobiology of Learning and Memory, 131*, 137–146. https://doi.org/10.1016/j.nlm.2016.03.020.

Daniel, S. E. & Rainnie, D. G. (2016). Stress Modulation of Opposing Circuits in the Bed Nucleus of the Stria Terminalis. *Neuropsychopharmacology 41*, 103–125.

Davis, M., Walker, D. L., Miles, L., & Grillon, C. (2010). Phasic vs sustained fear in rats and humans: role of the extended amygdala in fear vs anxiety. [review]. *Neuropsychopharmacology, 35*(1), 105–135. https://doi.org/10.1038/npp.2009.109.

Fanselow, M. S. (1994). Neural organization of the defensive behavior system responsible for fear. *Psychonomic Bulletin & Review, 1*(4), 429–438. https://doi.org/10.3758/bf03210947.

Goode, T. D., & Maren, S. (2017). Role of the bed nucleus of the stria terminalis in aversive learning and memory. *Learning & Memory, 24*(9), 480–491. https://doi.org/10.1101/lm.044206.116.

Grillon, C., & Ameli, R. (1998). Effects of threat and safety signals on startle during anticipation of aversive shocks, sounds, or airblasts. *Journal of Psychophysiology, 12*(4), 329–337.

Gungor, N. Z., & Paré, D. (2016). Functional heterogeneity in the bed nucleus of the Stria terminalis. *The Journal of Neuroscience, 36*(31), 8038–8049. https://doi.org/10.1523/JNEUROSCI.0856-16.2016.

Klucken, T., Kagerer, S., Schweckendiek, J., Tabbert, K., Vaitl, D., & Stark, R. (2009). Neural, electrodermal and behavioral response patterns in contingency aware and unaware subjects during a picture-picture conditioning paradigm. *Neuroscience, 158*(2), 721–731. https://doi.org/10.1016/j.neuroscience.2008.09.049.

Klumpers, F., van Gerven, J. M., Prinssen, E. P., Niklson, I., Roesch, F., Riedel, W. J., et al. (2010). Method development studies for repeatedly measuring anxiolytic drug effects in healthy humans. *Journal of Psychopharmacology, 24*(5), 657–666. https://doi.org/10.1177/0269881109103115.

Klumpers, F., Kroes, M. C., Heitland, I., Everaerd, D., Akkermans, S. E., Oosting, R. S., et al. (2015). Dorsomedial prefrontal cortex mediates the impact of serotonin transporter linked polymorphic region genotype on anticipatory threat reactions. *Biological Psychiatry, 78*(8), 582–589. https://doi.org/10.1016/j.biopsych.2014.07.034.

Klumpers, F., Kroes, M. C. W., Baas, J. M. P., & Fernandez, G. (2017). How human amygdala and bed nucleus of the Stria terminalis may drive distinct defensive responses. *The Journal of Neuroscience, 37*(40), 9645–9656. https://doi.org/10.1523/jneurosci.3830-16.2017.

LaBar, K. S., & Cabeza, R. (2006). Cognitive neuroscience of emotional memory. *Nature Reviews. Neuroscience, 7*(1), 54–64. https://doi.org/10.1038/nrn1825.

Lang, P. J., Davis, M., & Ohman, A. (2000). Fear and anxiety: animal models and human cognitive psychophysiology. *Journal of Affective Disorders, 61*(3), 137–159.

Lebow, M. A., & Chen, A. (2016). Overshadowed by the amygdala: the bed nucleus of the stria terminalis emerges as key to psychiatric disorders. *Molecular Psychiatry, 21*(4), 450–463. https://doi.org/10.1038/mp.2016.1.

Lissek, S., Baas, J. M., Pine, D. S., Orme, K., Dvir, S., Nugent, M., et al. (2005). Airpuff startle probes: an efficacious and less aversive alternative to white-noise. *Biological Psychology, 68*(3), 283–297. https://doi.org/10.1016/j.biopsycho.2004.07.007.

Lonsdorf, T. B., Menz, M. M., Andreatta, M., Fullana, M. A., Golkar, A., Haaker, J., Heitland, I., Hermann, A., Kuhn, M., Kruse, O., Meir Drexler, S., Meulders, A., Nees, F., Pittig, A., Richter, J., Römer, S., Shiban, Y., Schmitz, A., Straube, B., Vervliet, B., Wendt, J., Baas, J. M. P., & Merz, C. J. (2017). Don't fear 'fear conditioning': Methodological considerations for the design and analysis of studies on human fear acquisition, extinction, and return of fear. *Neuroscience and Biobehavioral Reviews, 77*, 247–285. https://doi.org/10.1016/j.neubiorev.2017.02.026.

Luyck, K., Nuttin, B., & Luyten, L. (2018). Electrolytic post-training lesions of the bed nucleus of the stria terminalis block startle potentiation in a cued fear conditioning procedure. *Brain Structure & Function, 223*(4), 1839–1848. https://doi.org/10.1007/s00429-017-1591-z.

Luyten, L., Casteels, C., Vansteenwegen, D., van Kuyck, K., Koole, M., Van Laere, K., & Nuttin, B. (2012). Micro-positron emission tomography imaging of rat brain metabolism during expression of contextual conditioning. *The Journal of Neuroscience, 32*(1), 254–263. https://doi.org/10.1523/JNEUROSCI.3701-11.2012.

Luyten, L., Hendrickx, S., Raymaekers, S., Gabriëls, L., & Nuttin, B. (2016). Electrical stimulation in the bed nucleus of the stria terminalis alleviates severe obsessive-compulsive disorder. *Molecular Psychiatry, 21*(9), 1272–1280. https://doi.org/10.1038/mp.2015.124.

McGaugh, J. L. (2000). Memory–a century of consolidation. *Science, 287*(5451), 248–251.

Mechias, M. L., Etkin, A., & Kalisch, R. (2010). A meta-analysis of instructed fear studies: Implications for conscious appraisal of threat. *Neuroimage, 49*(2), 1760–1768. https://doi.org/10.1016/j.neuroimage.2009.09.040.

Milad, M. R., Furtak, S. C., Greenberg, J. L., Keshaviah, A., Im, J. J., Falkenstein, M. J., Jenike, M., Rauch, S. L., & Wilhelm, S. (2013). Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. *JAMA Psychiatry, 70*(6), 608–618; quiz 554. https://doi.org/10.1001/jamapsychiatry.2013.914.

Ohman, A., & Mineka, S. (2001). Fears, phobias, and preparedness: toward an evolved module of fear and fear learning. *Psychological Review, 108*(3), 483–522.

Perusini, J. N., & Fanselow, M. S. (2015). Neurobehavioral perspectives on the distinction between fear and anxiety. *Learning & Memory, 22*(9), 417–425. https://doi.org/10.1101/lm.039180.115.

Shackman, A. J., & Fox, A. S. (2016). Contributions of the central extended amygdala to fear and anxiety. *The Journal of Neuroscience, 36*(31), 8050–8063. https://doi.org/10.1523/jneurosci.0982-16.2016.

Stefanova, N., & Ovtscharoff, W. (2000). Sexual dimorphism of the bed nucleus of the stria terminalis and the amygdala. *Advances in Anatomy Embryology and Cell Biology, 158*, Iii-x 1-78.

Tabbert, K., Stark, R., Kirsch, P., & Vaitl, D. (2006). Dissociation of neural responses and skin conductance reactions during fear conditioning with and without awareness of stimulus contingencies. *Neuroimage, 32*(2), 761–770. https://doi.org/10.1016/j.neuroimage.2006.03.038.

Zeidan, M. A., Lebron-Milad, K., Thompson-Hollands, J., Im, J. J., Dougherty, D. D., Holt, D. J., et al. (2012). Test-retest reliability during fear acquisition and fear extinction in humans. *CNS Neuroscience & Therapeutics, 18*(4), 313–317. https://doi.org/10.1111/j.1755-5949.2011.00238.x.