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Comment

Relating hippocampal neurogenesis to behavior: the dangers of ignoring confounding variables

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Abstract

Levels of hippocampal neurogenesis are associated with certain learning and memory tasks and with depression-like behavior in rodents. However, simply correlating neurogenesis with behavior can lead to spurious results unless other relevant factors such as age, disease status, or experimental condition are taken into account. A reanalysis of recently published data showed that after adjusting for the age of the rats, there was no significant relationship between the number of doublecortin-positive cells and working memory.

Keywords: Neurogenesis; Memory; Behavior

1. Introduction

A recent paper in this journal by Nyffeler et al. (2008) demonstrated a relationship between the number of new neurons in the dentate gyrus—determined by the number of doublecortin-positive (DCX+) cells—and working memory in rats ($r = -0.68$, $p < 0.005$). A scatterplot of DCX+ cells versus working memory (Fig. 6 in their paper) showed that rats with a greater number of DCX+ cells had better working memory, which is consistent with numerous other studies. The authors were careful to state that there was only an association between the two variables, and while the phrase "correlation does not imply causation" is well known, this seems to be forgotten when (1) there is a plausible biological mechanism which can explain the relationship, and (2) there are many studies showing the same relationship.

This analysis is not appropriate because it does not take into account other relevant variables, which is standard practice in every other scientific field. Simple correlations are common in the neurogenesis literature and not specific to this paper (see Creer et al., 2010 for the most recent example). The research question we should be asking is not "is there an association between neurogenesis and performance on behavioural tasks", but rather "is there an association *after other relevant factors have been taken into account*". This is shown graphically in Fig. 1; the interest is in the relationship between neurogenesis and working memory, but age affects both and cannot be ignored.

2. Methods

Data from Fig. 6 were extracted using g3data (version 1.5.1) software (Frantz, 2000). The same analysis as Nyffeler et al. was performed and the results were identical (to the reported precision), indicating that the data extraction was accurate. The data were then reanalyzed adjusting for the effect of age on working memory. The data, R code (www.r-project.org), and additional analyses are provided as supplementary online material.

3. Results and discussion

When regression lines are fit separately for each group (Fig. 2), the direction of the relationship between the number of DCX+ cells and working memory is reversed for 3 of the 4 groups compared with the original analysis; that is, rats with more DCX+ cells had worse working memory. It may seem unusual that one relationship is found when groups are analyzed separately, and the opposite relationship is

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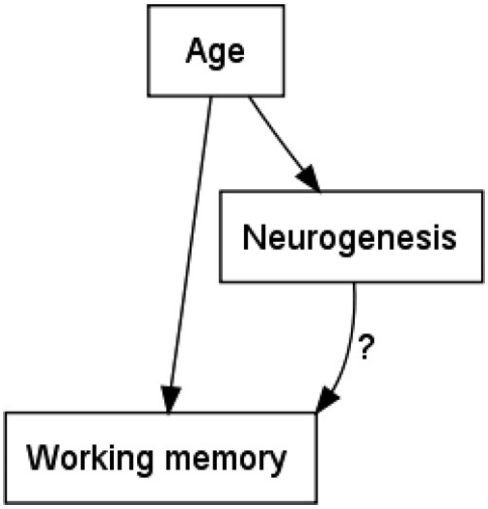

Fig. 1. Path diagram showing hypothesized causal relationships. Age can affect both performance on a working memory task and neurogenesis. Neurogenesis may also have a direct effect on performance, but the effect of age must somehow be taken into account and cannot simply be ignored.

found when groups are combined. This phenomena—referred to as Simpson's paradox—is well-known, has been discussed extensively in the statistics literature, and demonstrates the dangers of omitting relevant variables from the analysis (see http://en.wikipedia.org/wiki/Simpson's_paradox and references therein). Interestingly, when analysis was restricted to old rats only, Bizon et al. (2004) also found that a higher number of proliferating cells in the dentate gyrus (BrdU+ cells) was associated with worse performance on a spatial learning task. More recently, Castro et al. (2010) used structural equation models to tease apart a number of factors, and in one of their models they also found that higher levels of neurogenesis (BrdU+ cells) were associated with more depressive-like symptoms on the forced swim test (other models they examined showed no significant association).

The "good" and "bad" groups at 24 months of age were combined for the reanalysis (because they are the same age). Age was treated as a continuous (rather than categorical) variable because of the greater statistical power this can provide (Lazic, 2008). One simple way to analyze these data is to compare a model with age as the only explanatory variable to a model with both age and the number of DCX+ cells. Whether there is a significant reduction in the unexplained variance when the number of DCX+ cells is included can be formally examined with an *F*-test (Lazic, 2008), and in this analysis, the number of DCX+ cells was not significantly related to working memory ($F[1,13] = 0.004$, $p = 0.95$). Other analyses are possible (see Supplementary Material), but this dataset was not ideal because (1) there were no old rats with many DCX+ cells and young rats with few DCX+ cells, (2) there were different numbers of observations at each age (4, 4, and 8), (3) there was a large range of ages with no observations (between 6 and 24 months), and (4) the sample size and number of ages at which observations were made was small. This is not to criticize the design of the study but just to make clear that the analysis of this particular dataset (by any method) is only suggestive of the relationship between neurogenesis and behavior. However, the main conclusion is that once age is taken into account, there is no evidence that rats with many DCX+ cells perform better on a working memory task than rats with fewer DCX+ cells.

Similar analyses to Nyffeler et al.'s have been performed in other studies, but age is replaced by another experimental variable such as stress, radiation, physical activity, environmental enrichment, or treatment with a drug. Unfortunately, it is difficult to manipulate neurogenesis directly without also affecting other factors which might have a causative influence on behavior (e.g. gene expression, plasticity, long term potentiation [LTP], etc.). It is necessary to measure other relevant variables and take them into account, otherwise observed correlations may be spurious. This issue has been discussed previously in this journal (Baxter and Gallagher, 1996; Breckler, 1993), but not specifically in relation to neurogenesis, and using only hypothetical data as examples. It is hoped that a concrete example with a reanalysis of actual published data will reinforce the point.

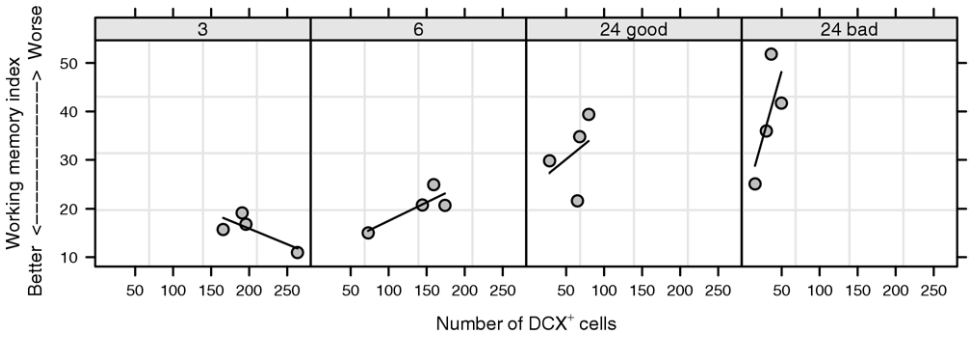

Fig. 2. Number of doublecortin-positive (DCX+) cells versus working memory by age group (3 months, 6 months, and the division of the 24 month group into the "good" and "bad" learners). A regression line is fit for each group separately, and 3 of the 4 groups show the opposite relationship compared with the original analysis, which combined all the age groups.

The suggestion that hippocampal neurogenesis has a causal role in certain learning and memory tasks or on measures of depression-like behavior is accepted with less skepticism than it deserves. Despite all of the studies on neurogenesis and behavior, it is still far from clear the extent to which new neurons are involved in behavioral tasks, mainly because in many studies there are other confounding variables which have not been taken into account, indeed, are often not even measured. Ruling out spurious correlations is a necessary condition for a better understanding of this phenomenon.

Disclosure statement

The author declares no conflicts of interest.

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2010.04.037.

References

Baxter, M.G., Gallagher, M., 1996. Neurobiological substrates of behavioral decline: models and data analytic strategies for individual differences in aging. Neurobiol. Aging 17, 491–495.

Bizon, J.L., Lee, H.J., Gallagher, M., 2004. Neurogenesis in a rat model of age-related cognitive decline. Aging Cell 3, 227–234.

Breckler, S.J., 1993. Age-related behavioral and neurochemical deficits: new data analytic strategies. Neurobiol. Aging 14, 695–697.

Castro, J.E., Varea, E., Marquez, C., Cordero, M.I., Poirier, G., Sandi, C., 2010. Role of the amygdala in antidepressant effects on hippocampal cell proliferation and survival and on depression-like behavior in the rat. PLoS One 5, e8618.

Creer, D.J., Romberg, C., Saksida, L.M., van Praag, H., Bussey, T.J., 2010. Running enhances spatial pattern separation in mice. Proc. Natl. Acad. Sci. U. S. A. 107, 2367–2372.

Frantz, J., 2000. g3data (version 1.5.1) Available at: www.frantz.fi/software/g3data.php.

Lazic, S.E., 2008. Why we should use simpler models if the data allow this: relevance for ANOVA designs in experimental biology. BMC Physiol. 8, 16.

Nyffeler, M., Yee, B.K., Feldon, J., Knuesel, I., 2008. Abnormal differentiation of newborn granule cells in age-related working memory impairments. Neurobiol. Aging, in press.