The role of Protocadherin 9 in layer 6 of the cortex in sensory-related behavioural tasks

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Published in:
European Neuropsychopharmacology

DOI:
10.1016/j.euroneuro.2019.12.054

Publication date:
2020

Traditionally, most BF functions have been attributed to its cholinergic neurons. The BF cholinergic system has at the extremes 2 relatively distinct subcomponents, the nucleus basalis magnocellularis (NBM) and the medial septal area (MS), which have projections to the frontal cortex and hippocampus, respectively. Degeneration of cholinergic BF neurons is one of the common features of AD. It has been reported that degeneration of BF cholinergic neurons and the decrease of cholinergic projections could be an important factor characterizing the cognitive decline and functional impairment that characterizes this disorder.The BF contains: cortically projecting cholinergic and noncholinergic neurons as well as various interneurons.The most prominent noncholinergic component of the BF corticopetal projection system are the GABAergic corticopetal projections. Substantial evidence suggests that the NBM and MS plays an important role in learning and memory. In contrast to research on the cortical cholinergic input system, little is known about the functions corticopetal GABAergic neurons.

The aim was to investigate the modulation of spatial memory function by the GABAergic cells of the NBM and MS using a new more selective toxin for GABAergic neurons - immunotoxin GAT1-SAP. A total of 36 male rats were used in the present study.

The animals were randomly assigned to MS ($n = 12$) and NBM ($n = 12$) GAT1-SAP lesioned and MS ($n = 6$) and NBM ($n = 6$) shamlesioned groups. All injections of GAT1-SAP (325ng/μl) for immunolesion surgeries or mouse, for control surgeries (Advanced Targetting System, San Diego, USA) were performed stereotaxically. All experiments were approved by the Animal Care and Use Committee of the Center and were in accordance with the principles of laboratory animal care. Animals were tested in a standard Morris water maze, taxing different strutegy (i.e., cue or place) of spatial memory function. The immunotoxic GAT1-SAP lesions of NBM and MS were verified by observing decreased Acetylcholintransferase and parvalbumine staining of the NBM and MS. All statistical analyses were conducted with a significance level of $P < 0.05$.

Immunohistochemical studies showed that injection of GAT1-SAP preferentially reduced GABAergic neurons as compared to cholinergic neurons in the NBM and MS. Behavioral data of the present study suggest that the control and lesioned rats, exhibited corresponding differences in performance during training trials. The control rats, identified as place responders, had significantly more accurate searches on hidden platform days, providing an additional evidence of their effective use of a place learning strategy rather than the NBM and MS-lesioned rats exhibiting a cue strategy in competition trials. The lesioned rats acquired the visible platform version of the water maze task but failed to learn the platform location in space. Present results suggest involvement of the NBM and MS GABAergic neurons in organization of the spatial map-driven behavior and this structures should be viewed as a constituent of the functional system responsible for the cognitive types of spatial memory.

doi: 10.1016/j.euroneuro.2019.12.053

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The role of Protocadherin 9 in layer 6 of the cortex in sensory-related behavioural tasks

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Sensory processing is affected in many neuropsychiatric disorders such as schizophrenia, autism spectrum disorder (ASD), and major depressive disorder [1,2]. The exact neurobiological mechanism and developmental processes underlying sensory processing dysfunction in these neuropsychiatric disorders is unknown. We previously showed that the cell-adhesion molecule Protocadherin 9 (*Pcdh9*) contributes to sensory cortex development and behavioral expressions related to sensory processing in a traditional knockout mouse model [3]. Protocadherins are believed to be involved in neuronal migration, synaptic plasticity, and circuit formation [4]. In the current study, we investigated the functional role of *Pcdh9* in cortex layer 6 in sensory cortex development, sensory processing, and social behavior using a conditional reinstatement mouse model that was constructed to temporally and spatially reinstate *Pcdh9* expression in an otherwise *Pcdh9* knockout background. We were specifically interested in layer 6, because it is implicated in the etiology of ASD [5]. Here, we show the first results of this novel mouse model following reinstatement of *Pcdh9* expression in corticothalamic neurons in cortical layer 6.

The *Pcdh9* conditional reinstatement mouse model was generated by inserting a neo/stop cassette flanked by two lox sites in the first coding exon of *Pcdh9*. These mice were first crossed with *Sox2*-cre mice to validate general *Pcdh9* gene reinstatement and expression. *Sox2*-cre mice have a widespread cre-recombinase expression early on in embryonic development. Using RT-PCR and Western Blot analysis, we were able to show that *Sox2* mediated cre-recombinase leads to the reinstatement of *Pcdh9* gene and protein expression in the conditional reinstatement mouse model. Secondly, we crossed the conditional mouse line with *Ntsr1*-cre mice, with cre-recombinase expression in corticothalamic neurons in cortex layer 6 during early life development. At the moment we are performing Western Blot analyses in the *Pcdh9* reinstatement/*Ntsr1*-cre line to analyse *Pcdh9* expression in these mice. Furthermore, multiple behavioral tests assessing a variety of sensory modalities (e.g., touch, smell) were performed in the cortical layer 6 reinstatement model. Outcome parameters consisted of bedding contextual preference ratio in a bedding preference test (BPT; one-sample T-Test), time to locate food in the buried food test (BFT; one-way ANOVA), and sniffing time in the olfactory habituation/dishabituation test (OHDT; repeated measures ANOVA).

Using the BPT, we observed that mice lacking *Pcdh9* do not a have preference for either of the two bedding materials (mean ratio=0.451; p=0.41). Whereas control littermates (wildtype, non-floxed, *Ntsr1*-cre negative),

(wildtype, non-floxed *Ntsr1*-cre positive), and mice with reinstated *Pcdh9* expression (i.e. floxed, *Ntsr1*-cre positive) do prefer one of the two bedding materials over the other (respectively: mean=0.43; p=0.012; mean=0.398; p=0.012; mean=0.415; p=0.034). These findings indicate that *Pcdh9* expression in layer 6 of the cortex is necessary for expressing contextual bedding preference. Currently, we are investigating whether central odor processing is contributing to the rescue of this cortex layer 6 sensory phenotype by performing the BFT and OHDT. Ultimately, these studies will contribute to our understanding of the functional relationship between early life sensory cortex development, sensory information processing, and behavior.

Disclosure statement: The project leading to this application has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 115916. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA.

References

[1] Hornix, B.E., Havekes, R., Kas, M.J.H., 2018. Multisensory cortical processing and dysfunction across the neuropsychiatric spectrum. Neurosci. Biobehav. Rev. 97, 138–151.
[2] Danjou, P., Viardot, D., Garcés, P., et al., 2019. Electrophysiological assessment methodology of sensory processing dysfunction in schizophrenia and dementia of the Alzheimer type. Neurosci. Biobehav. Rev. 79, 70–84.
[3] Bruining, H., Matsui, A., Oguro-Ando, A., et al., 2015. Genetic mapping in mice reveals the involvement of Pcdh9 in long-term social and object recognition and sensorimotor development. Biol. Psychiatry 78, 485–495.
[4] Peek, S.L., Mah, K.M., Weiner, J.A., 2017. Regulation of neural circuit formation by protocadherins. Cell Moll. Life Sci. 74, 4133–4157.
[5] Willsey, A.J., Sanders, S.J., Mingfeng, L., et al., 2013. Coexpression Networks Implicate Human Midfetal Deep Cortical Projection Neurons in the Pathogenesis of Autism. Cell 155, 997–1007.

doi: 10.1016/j.euroneuro.2019.12.054

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Longitudinal associations between depression, lifestyle and brain structure: a nine-year follow-up MRI study

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Background: Major depressive disorder (MDD) has been associated with smaller regional grey matter brain volumes. However, the longitudinal nature of these abnormalities is still largely unknown as longitudinal studies in MDD are scarce and findings inconsistent. Also, MDD is known to be associated with an unhealthy lifestyle including physical inactivity, short or long sleep, high BMI, smoking and high alcohol consumption, which have also been associated with lower grey matter volume and therefore could partly explain brain structural changes in MDD.
Aim: Investigate the relation between depression, lifestyle and brain structure cross-sectionally and longitudinally over up to 9 years.
Methods: We included persons with MDD and/or anxiety and healthy controls (605 observations from 344 participants) of the Netherlands Study of Depression and Anxiety (NESDA). Hippocampal volume, and rostral anterior cingulate cortex (rACC) and medial orbitofrontal cortex (mOFC) thickness were derived from T1 structural MRI scans using Freesurfer. Generalized Estimating Equations analyses were used to investigate multi-wave cross-sectional associations between depression, lifestyle (BMI, smoking, alcohol consumption, physical activity and sleep duration) and brain structure. Multiple regression analyses were used to investigate associations between baseline depression or lifestyle, or change of these measures, with change in brain structure over 2 (n=178) or 9 years (n=84), and between percentage of time with MDD or anxiety disorders over 9 years to change in brain structure over time.
Results: Cross-sectional analyses across waves show a negative association between depression severity and mOFC (p=0.020) and rACC (p=0.002) thickness, which remained significant when correcting for BMI and other lifestyle factors. BMI, but no other lifestyle indicators, was negatively associated with mOFC (p<0.001) and rACC (p=0.004) thickness, also when correcting for depression. Longitudinal analyses show that thickness of mOFC, rACC and hippocampal volume decreased over time (all p<0.001). Longitudinally, no associations between baseline depression severity, or lifestyle, or change of these measures over time, were observed with change in brain structure over 2 or 9 years. The percentage of time with MDD or anxiety over 9 years was also not related to change in brain structure over time.
Conclusions: Depression severity and BMI were both negatively associated with medial prefrontal brain structure (lower mOFC and rACC thickness), independent of each other and other lifestyle indicators. Associations with hippocampal volume and between brain regions and other lifestyle factors were not significant. As expected, grey matter volume decreased over time. However, this change over time was not significantly associated with (change in) depression or lifestyle. The observed associations with cross-sectional brain structure but not with longitudinal change might suggest that lower prefrontal brain thickness could be a long-term consequence or be related to vulnerability for depression and BMI, however, the limited sample size of longitudinal analyses warrants careful interpretation. We will aim to replicate these findings and further delineate potential associations with lifestyle over the whole lifespan in community-based samples of 3500 persons within the European Lifebrain longitudinal neuroimaging consortium.

doi: 10.1016/j.euroneuro.2019.12.055