Abnormal emotional behavior of mice overexpressing the antiprotease neuroserpin is corrected by co-expression of a tissue-type plasminogen activator transgene.

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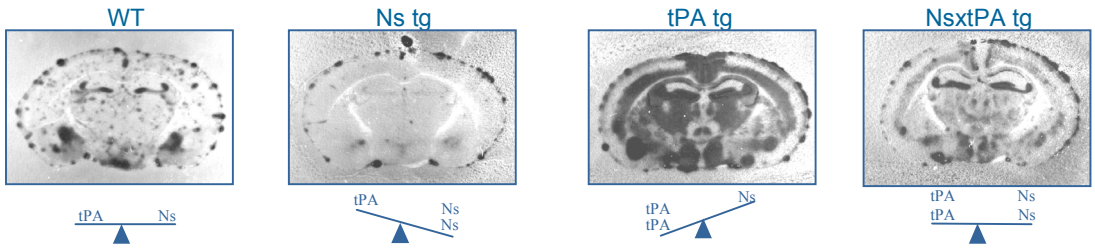
Summary

Increasing evidence suggests that the balance between extracellular proteases, such as tissue-type plasminogen activator (tPA), and antiproteases, such as neuroserpin, is critical for neuronal function and pathology. We have found previously that transgenic mice with neuronal overexpression of tPA under the control of the promoter Thy1.2 (ThytPA) have massively elevated brain tPA activity and improved learning. Mice overexpressing the antiprotease neuroserpin under the control of the same promoter (ThyNs) show decreased brain tPA activity. Recent analysis of their explorative behavior revealed an anxiety-like phenotype. To address the question whether these phenotypes are due to a disturbed balance between tPA and neuroserpin, we generated bi-transgenic mice, by crossing the ThytPA and ThyNs lines. Zymographic analysis shows that tPA activity in bi-transgenic brains is restored to near wild-type level, although activity remains slightly elevated in some regions, such as CA1 or deep layer of the neocortex.

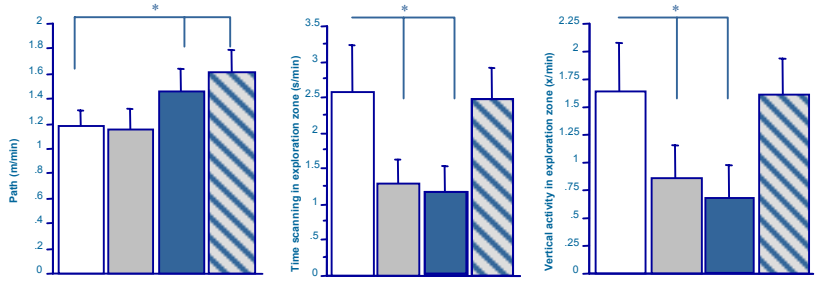
The behavioral phenotype of bi-transgenic mice was compared with littermates bearing only one or none of the transgenes in a battery of tests. When confronted to a novel object, both ThytPA and ThyNs mice showed increased avoidance behaviors, while bi-transgenic mice behaved as wild-type littermates. Thus, normal behavior could be restored if the balance between tPA and its inhibitor was reestablished by over expressing both as a transgene. However, this was not the case when we assessed episodic memory in a water-maze task. As expected, ThytPA mice showed improved performance. By contrast, the ThyNs transgene had no effect on performance in this task. It also failed to diminish the effect of ThytPA transgene in bi-transgenic mice. This suggests that spatial learning is modulated by actions of tPA that are independent of neuroserpin. Taken together, our results provide the first evidence that dysregulation of the balance between proteases and antiproteases in the brain might disturb emotional behavior.

tPA-proteolytic activity in bi-transgenic mice tPAxNs is restored to near normal level



Zymographic analysis:
Activity of tPA was strongly reduced in Ns-overexpressing mice (Ns tg). tPA-overexpressing mice (tPA tg) had strongly increased tPA activity. We cross these two lines to generate here bi-transgenic mice; NsxtPA tg mice In these mice, tPA was restored to a near normal pattern, although activity remained elevated in some regions, such as CA1 or deep layer of the neocortex. The imbalance of tPA and Ns is shown for each mice model.

Manipulations of the balance between tPA and Ns affect emotional behavior



WT, n = 22 | tPA tg, n =18
Ns tg, n =16 | NsxtPA tg, n =21

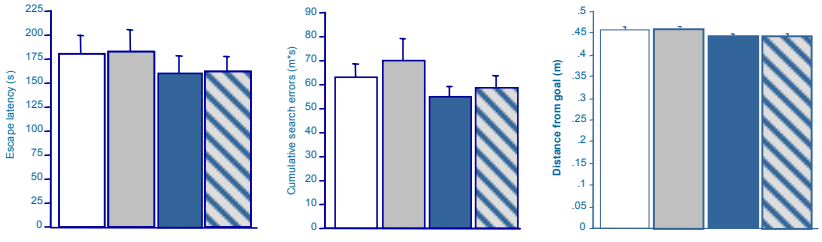
Two-way ANOVA	Ns-effect	tPA-effect	tPAxNs effect
Total path	ns	0.03	ns
In exploration zone:			
Time scanning	ns	ns	0.01
Vertical activity	ns	ns	0.02

Fischer's PLSD	Single transgenic	Bi-transgenic
In exploration zone:		
Time scanning	0.03	ns
Vertical activity	0.05	ns

Novel object test:
An object is introduced in a familiar arena. The parameters of the test are analyzed by two-way ANOVA for Ns or tPA effect and for interaction between Ns and tPA. Mice overexpressing tPA and tPAxNs tg mice show increased total path.

When confronted to a novel object, the tPA tg as well as the Ns tg mice spend less time scanning the object and exhibit reduced vertical activity towards the object. Thus, these mice have reduced object exploration, while mice overexpressing both tPA and Ns behaved as wild-type littermates.

Performance in a spatial learning task is modulated by actions of tPA that are independent of Ns



WT, n = 22 | tPA tg, n =17
Ns tg, n =15 | NsxtPA tg, n =21

Two-way ANOVA	Ns-effect	tPA-effect	tPAxNs effect
Escape latency	ns	0.03	ns
Cumulative search errors	ns	0.03	ns
Distance from goal	ns	0.01	ns

Episodic-like memory in a water-maze task:
Time, cumulative search errors and distance from goal are analyzed by two-way ANOVA for Ns or tPA effect and for interaction between Ns and tPA. Ns expression did not affect these parameters. tPA tg and the NsxtPA tg mice localize the platform in a shorter time, have a reduced number of errors and a reduced distance from the goal. Thus, mice overexpressing tPA show improved performance and their superiority is not abolished by concomitant overexpression of Ns.

Conclusion

Our results on the bi-transgenic mice provide the first evidence that the disturbance of the tPA/neuroserpin balance in the brain modulates emotional behavior. We previously found that both mice lacking or overexpressing Ns have increased avoidance behaviors, suggesting an U-shaped relationship that produces similar behavioral symptoms independently of the direction in which Ns levels are changed. Taken together, our results suggest that normal emotional behavior could be restored if the balance between tPA and its inhibitor is reestablished although at a higher level.

A higher expression of tPA, either in tPA tg or in the tPAxNs bi-transgenic mice, lead to improved episodic-like memory. The phenotype of the bi-transgenic mice may be explained by the residual overexpression of tPA proteolytic activity. Alternatively, we suppose that tPA acts independently of Ns in learning paradigm. This hypothesis is supported by the finding that Ns transgenic mice have normal memory. Further investigation are needed to elucidate whether tPA has a non-proteolytic function.

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