

Supplementary Table 11. Phenotypes of Shn-2 KO mice and abnormalities associated with schizophrenia.

	Schizophrenia (1, 2, 3)	Shn-2 KO mice
Positive Signs/Symptoms:	Psychomotor agitation	Increased locomotor activity
Negative Signs/Symptoms:	Social withdrawal	Decreased interaction with a juvenile conspecific, decreased preference for social novelty
	Self neglect	Decreased nest building behavior
Cognitive Signs/Symptoms:	Decreased working memory	Impaired performance in 8-arm radial maze working memory task, impaired working memory in T-maze task
	Deficits in attention/sensorimotor gating	Decreased sensorimotor gating (PPI deficits)
	Inflexibility	Normal performance in reversal learning in T-maze left-right discrimination
Other behavioral signs	Decreased pain sensitivity	Decreased pain sensitivity (5)
	Lack of activity, depressive mood	Increased depression-like behavior in sucrose preference test, Decreased depression-like behavior in forced swim test and tail suspension test (7)
	High prevalence of anxiety disorder/symptomatology (16)	Increased anxiety-related behaviors (4), Increased stay time on open arms in the elevated plus maze (14)
	Increased sensitivity to NMDAR antagonist	Increased sensitivity to MK-801
	Reduction of psychotic agitation by haloperidol (17)	Reduction of increased locomotor activity by haloperidol
	No improvement of PPI by haloperidol (18)	Improvement of PPI by haloperidol
	Reduction of aggression by clozapine (19)	Reduction of increased locomotor activity by clozapine
	Improvement of PPI by clozapine (20)	No improvement of PPI by clozapine
	Poor bilateral transfer (21)	Improved motor coordination in the Rotarod test
Physical signs	Hypercortisolemia (22)	Hypercortisolemia
	Lower body mass index (BMI) (23), no significant BMI difference in male (24), higher BMI in women (24)	Decreased body weight
Physiology (EEG)	Increased delta (25, 26), theta (25) power, Decreased alpha (25, 26), increased gamma power (27), decreased gamma power (28)	Increased Theta wave, decreased Gamma wave
Cortical Thickness	Reduction in frontal lobe and temporal cortex (29), normal (20)	Decreased cortical thickness in PrL and V1
Cortical Cell density	Increase (50, 51), decrease (52, 53), normal (27, 31)	Decrease
Hippocampus Volume	Decrease in bilateral volume (32), Decrease in total volume (33)	Tendency to be large (data not shown)
Parvalbumin	Decrease in hippocampus (34), PFC (35)	Decreased in hippocampus, PFC
GAD67	Decrease in hippocampus (36), increase in DLPFC (37)	Decreased in hippocampus
Myelination/oligodendrocyte	Decreased CNPase (40), decrease myeline water fraction (5)	Decreased CNPase, MBP was decreased
Astrocytes	Increased GFAP (39, 47), increased S100beta (41, 42, 43, 44) decreased GFAP (38)	Increased GFAP, increased S100beta
Microglia	Increased activated microglia (45), microglia activation (46),	No significant change in Iba-1 expression
Dopamine receptor	Decreased D1R in prefrontal (48)	Decreased D1R binding in dentate gyrus
	Increased D2R in striatum (49)	No significant change in D2R binding

References and notes

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- (5) The decreased pain sensitivity in these mice is consistent with reports showing that individuals with schizophrenia are less sensitive to physical pain than unaffected individuals (6).
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(7) Shn-2 KO mice were more mobile than wild type controls in the Porsolt forced swim test (Supplementary Figure 2f). We also performed the sucrose preference test, in which a reduced preference for sucrose is considered to represent anhedonia or depression-like behavior (8). Shn-2 KO mice showed a significantly lower preference for sucrose, which suggests increased depression-like behavior (Figure 8n). Anhedonia is a hallmark of schizophrenia (9). Many strains of schizophrenia model mice, including calcineurin KO mice (10), NR2A KO mice (11), and nNOS KO mice (12), appear to show a reduction in depression-like behavior during the forced swim test. However, these mice are not necessarily less sensitive to stress. Rather, this difference may reflect their hyperactive phenotype and/or their increased sensitivity to stress. It should be noted that depression is not included in the DSM IV diagnostic criteria for schizophrenia, although depressive symptoms are common throughout the course of the illness (13). Schizophrenic patients are likely to comprise several biologically distinct heterogeneous populations, which include patients with or without high levels of depression. Clearly, the characteristics of Shn-2 KO mice do not mimic those of the entire schizophrenic population, but rather those of a specific subset of patients. In this regard, the depression-related behavioral abnormalities shown by Shn-2 KO mice may not be inconsistent with the idea that they represent an animal model of schizophrenia.

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(14) Shn-2 KO mice spent a significantly longer period of time in the open arm during the elevated plus maze test (Supplementary Figure 2c), suggesting decreased anxiety-like behavior. However, there was no significant difference in the anxiety-like behavior in the light/dark transition test between genotypes (Supplementary Figure 2d). During the elevated plus maze test, Shn-2 KO mice spent more time in the open arms and entered the open arms more frequently, which is usually considered a sign of decreased anxiety-like behavior. However, this behavior may simply be attributable to locomotor hyperactivity, and their anxiety-like behavior per se may not differ from that shown by their wild type littermates (it may even be increased). This interpretation stems from our previous findings that: 1) plasma corticosterone levels were significantly greater in KO mice subjected to restraint stress; 2) KO mice subjected to an unfamiliar environment showed behaviors indicative of heightened anxiety, such as backtracking, freezing, stretching, and escaping; and 3) the center stay time in the open field test, a frequently used index of anxiety-like behavior, decreased in Shn-2 KO mice (4). Indeed, plasma corticosterone levels were significantly higher in these mice than in the controls after the elevated plus maze test (Supplementary Figure 2e). Increased time spent in the open arms is sometimes interpreted as “panic-like escaping behavior” in mice showing increased anxiety-like behavior (10, 15), including forebrain-specific calcineurin KO mice, which we previously proposed as an animal model for schizophrenia. It should be noted that anxiety is common throughout the course of the illness (13), although anxiety is not included in the DSM IV diagnostic criteria for schizophrenia. Schizophrenic patients are likely to comprise several biologically distinct heterogeneous populations, which include patients with or without high levels of anxiety. Obviously, the characteristics of Shn-2 KO mice may not mimic those of the entire schizophrenic population, but rather those of a specific subset of patients. In this regard, the anxiety-like behavioral abnormalities shown by Shn-2 KO mice may not be inconsistent with the idea that they represent an animal model of schizophrenia and could be potentially interesting.

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