Supplementary data

Table 1. **SEQUENCES OF PRIMERS USED**

Gene	**Forward primer 5′–3′**	**Reverse primer 5′–3′**
GAPDH	TGCACCACCAACTGCTTAG	GGATGCAGGGATGATGTTC
GSK-3 alpha	AATCTTGGCCAGTCTGAGCT	TCAGTCCTGGTGAACTGTCC
GSK-3 beta	TCCATTCCTTTGGAATCTGC	CAATTCAGCCAACACACAGC

RNA isolation and RT PCR

The qRT-PCR *was performed in a 25 µl reaction volume containing a 10xPCR Buffer (2.5µl), 25 mmol MgCl2 (4 µl), 10 mmol dNTPs (2µl), specific forward and reverse primers at 20 pmol/µl concentration (1 µl), cDNA (2µl), 5 u/µl Taq DNA polymerase (1 µl) (Beagle, st. Petersburg, Russia), and ddH2O (10 µl). All samples were run in duplicate. Cycling was performed at 95 C° for 5 min followed by a 45-cycle amplification at 95°C for 10s, then at the annealing temperature defined previosuly for 15 s and at the temperature 72°C for 20 s.*

Results of the qPCR measurements were expressed as Ct values, where Ct is defined as the threshold cycle of PCR at which amplified product was 0.05 % of normalized maximal signal. We used the comparative Ct method and computed the difference between the expression of the gene of interest and GAPDH expression in each cDNA sample (2−ΔΔCt method). Results are expressed as folds of expression compared to the mean values of expression in non-stressed control animals (Couch et al., adapted from Livak and Schmittgen 2001).

Table 2. **FLOATING IN THE MODIFIED SWIM TEST: ROLES OF SWIMMING, CONTEXT AND TIMING (Fig. 2)**

FACTOR / EXPERIMENT	**Swimming vs context**	**Role of context**	**Role of timing**
Protocol	Fig. 1 B swimming is replaced by a context exposure on Day 2	Fig. 1 C no swimming, no context exposure on Day 2	Fig. 1 F swimming is on Day 3 instead of Day 5
Outcome: floating on Day 5	↑	=	=

Table 2. Floating in the modified swim test: roles of swimming, context and timing. *In series of experiments, the roles of swimming experience vs. context exposure, a reminder of a context of swimming, and a factor of timing of testing, in an increase of floating behaviour on Day 5 of the modified swim test, were dissected. Mice were exposed to the modified swim test with additional delayed testing on Day 5 or several variants of this protocol presented on Fig. 1. In comparison to the preceding session, the duration of floating behaviour on Day 5 was augmented (↑) or not altered (=) in applied protocols of testing.*

Table 2. **BRAIN mRNA GSK3 BETA IN MODIFIED SWIM TEST: ROLES OF SWIMMING, CONTEXT AND TIMING (Fig. 3)**

FACTOR / EXPERIMENT	**Swimming vs context**	**Role of context**	**Role of timing**
Protocol	Fig. 1 D; swimming is replaced by context exposure on Day 5	Fig. 1 E; no swimming, no context exposure on Day 5	Fig. 1 F; swimming on Day 3 instead Day 5
Outcome: mRNA GSK3 beta	Hip = PFC ↑	=	=

Table 3. mRNA GSK3 beta in the modified swim test: roles of swimming, context and timing. *In series of experiments, the roles of swimming experience vs. context exposure, a reminder of a context of swimming, and a factor of timing of testing, in an increase of mRNA GSK3 beta on Day 5 of the modified swim test, were addressed. Mice were exposed to the modified swim test with additional delayed testing on Day 5 or several variants of this protocol presented on Fig. 1. In comparison to the preceding session, the mRNA GSK3 beta level in the hippocampus (Hip) and prefrontal cortex (PFC) on Day 5 was augmented (↑) or not altered (=) in applied protocols of testing.*

Table 4. **A COMPARISON OF FLOATING AND GSK3 BETA BRAIN ACTIVITES BETWEEN DAY 1-, DAY 2- AND DAY 5-(MODIFIED) SWIM TEST PROTOCOLS**

PARAMETER / TEST SESSION	FLOATING	mRNA GSK3 beta		pGSK3 beta / Total GSK3 beta	
		Hip	PFC	Hip	PFC
Day 1		vs Int =	vs Int =	vs Int =	vs Int =
Day 2	vs Day 1 ↑	vs Int = vs Day 1 =	vs Int = vs Day 1 =	vs Int ↓ vs Day 1 ↓	vs Int ↓ vs Day 1 ↓
Day 5	vs Day 1 ↑ vs Day 2 ↑	vs Int LF ↑, HF ↑ vs Day 1 LF ↑, HF ↑	vs Int LF =, HF ↑ vs Day 1 LF = , HF ↑	vs Int LF ↓, HF ↓ vs Day 1 LF ↓, HF ↓	vs Int LF ↓ vs Day1 HF ↓

Table 4. A comparison of floating and GSK3 beta brain activities between Day 1-, Day 2- and Day 5- (modified) swim test protocols. Total duration of floating, brain *GSK3 beta gene expression and protein levels* were *compared between the groups of mice sacrificed 10 min after testing at Day 1, Day 2 or delayed session on Day 5, in the swim test. mRNA GSK3beta and a* ratio pGSK3 beta / Total GSK3 beta were evaluated in the hippocampus *(Hip)* and prefrontal cortex *(PFC)* of subgroups of mice defined as Low Floaters (**LF**, see manuscript text) and High Floaters (**HF**, see manuscript text) post-testing on Day 2 and Day 5, and were compared to the values determined in intact mice (**Int,** see

manuscript text) and in mice sacrificed at Day 1 of testing. A scheme of testing / sacrifice for each *protocol is presented on Fig. 1A. Evaluated behavioural and molecular measures were augmented (↑),* decreased **(↓)** *or not altered (=) in above-described comparisons.*

Table 5. **EFFECTS OF IMIPRAMINE ON FLOATING AND GSK3 BETA ACTIVITIES IN THE MODIFIED FORCED SWIM TEST**

PARAMETER / TREATMENT	FLOATING	mRNA GSK3 beta		pGSK3 beta / Total GSK3 beta	
		Hip	PFC	Hip	PFC
Day 5 No Drug	vs Day 1 ↑ vs Day 2 ↑	vs Int ↑ vs Day 1 ↑ vs Day 2 ↑	vs Int =	vs Int ↓	vs Int ↓
Day 5 Imi	vs Day 1 ↑ vs Day 2 =	vs Int ↓ vs Con (No drug) ↓	vs Int ↑ vs Con (No drug) =	vs Int = vs Con (No drug) ↑	vs Int ↓ vs Con (No drug) =

Table 5. Effects of imipramine on floating and GSK3 beta activities in the modified forced swim test. Total duration of floating, brain *GSK3 beta gene expression and protein levels* were *compared between the groups of mice sacrificed 10 min after testing at delayed session on Day 5 of the swim test, which either received no drug or were treated with a low dose of imipramine. mRNA GSK3beta and a* ratio pGSK3 beta / Total GSK3 beta were evaluated in the hippocampus *(Hip)* and prefrontal cortex *(PFC)* of these groups and were compared to the values determined in intact mice (**Int,** see manuscript text) and in animals sacrificed at Day 1 and Day 2 of testing. A scheme of testing / sacrifice for each *protocol is presented on Fig. 1G. Evaluated behavioural and molecular measures were augmented (↑),* decreased **(↓)** *or not altered (=) in above-described comparisons.*