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Emotional memory impairments in a genetic rat model of depression; involvement of 5-HT/MEK/Arc signaling in restoration

Running title:

Memory impairments in a rat model of depression

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Abstract

Cognitive dysfunctions are common in major depressive disorder, but have been difficult to recapitulate in animal models. This study shows that Flinders Sensitive Line (FSL) rats, a genetic rat model of depression, display a pronounced impairment of emotional memory function in the passive avoidance (PA) task, accompanied by reduced transcription of Arc in prefrontal cortex and hippocampus. At the cellular level, FSL rats have selective reductions in levels of NMDA receptor subunits, serotonin 5-HT_{1A} receptors and MEK activity. Treatment with chronic escitalopram, but not with an antidepressant regimen of nortriptyline, restored memory performance and increased Arc transcription in FSL rats. Multiple pharmacological manipulations demonstrated that procognitive effects could also be achieved by either disinhibition of 5-HT_{1A}R/MEK/Arc or stimulation of 5-HT₄R/MEK/Arc signaling cascades. Taken together, studies of FSL rats in the PA task revealed reversible deficits in emotional memory processing, providing a potential model with predictive and construct validity for assessments of procognitive actions of antidepressant drug therapies.

Key words: passive avoidance, MAPK, antidepressant, signal transduction, BDNF, TrkB

1 **Introduction**

2 The clinical impact of emotional dysfunction on cognitive capacity is recognized but difficult
3 to treat. ¹⁻³ Beside depressed mood, the DSM-IV criteria of major depressive disorder (MDD)
4 involve cognitive aspects, *i.e.* diminished ability to think and concentrate, or indecisiveness,
5 with devastating effects on executive functions, short- and long-term learning and memory. ^{2,}
6 ³ These cognitive impairments appear to involve alterations in the neuronal processing of
7 emotional stimuli *i.e.* negative attentional bias including feelings of worthlessness or
8 excessive guilt and recurrent thoughts of suicide. ⁴ Multiple brain areas, involved in
9 emotionality and cognition *i.e.* prefrontal cortex, hippocampus, parahippocampal region,
10 amygdala and striatum, show altered functions in MDD. ^{1, 2, 5} In particular, volume reduction
11 and impaired functionality of hippocampus have been repeatedly reported in MDD. ^{2, 6-7}

12 Studies in depressed patients have linked dysfunction of the central serotonergic system
13 with abnormal cognitive processing of emotional stimuli and indicated that an important
14 component in antidepressant therapy is support of cognitive functions, which can be separated
15 from elevation of mood. ⁸ The clinical efficacy of selective serotonin reuptake inhibitors
16 (SSRI) in MDD is exerted by several of the 14 cloned 5-HT receptors, with 5-HT_{1A}R being
17 the most studied. ⁹ Several studies have described alteration of 5-HT_{1A}R in post-mortem
18 samples from patients with MDD. ¹⁰⁻¹⁵ Interestingly, 5-HT_{1A}R antagonists facilitate learning
19 and counteract memory impairments. ⁹ In addition, 5-HT₄R agonists may have antidepressant
20 ¹⁶ and procognitive effects in learning and memory tasks. ¹⁷

21 The delayed onset of SSRI's and most other antidepressants implicate that therapeutic
22 effects are mediated beyond elevations of monoamines, such as changes of receptor coupling
23 to intracellular signaling cascades and cross-talk with non-monoaminergic mechanisms.
24 Several lines of evidence indicate that BDNF-induced activation of TrkB mediate actions of
25 antidepressants. ¹⁸⁻²¹ TrkB receptors, in turn, stimulate several intracellular cascades including

1 the mitogen-activated protein kinase (MAPK), AKT and phospholipase C (PLC) pathways.²¹⁻
2 ²² In particular, the MAPK pathway is involved in behavioral effects of antidepressants and an
3 important modulator of ionotropic receptor signaling, structural remodeling and consolidation
4 of fear memories.²³⁻²⁵ Synaptic activation of MAPK induces, via CREB and Elk-1, expression
5 of activity-related cytoskeletal-associated protein (Arc/Arg 3.1).^{23, 26-28} Arc expression is
6 induced by long-term potentiation (LTP)-inducing stimuli, requiring NMDAR^{27, 29}, involved
7 in synaptic memory consolidation and enriched in hippocampal dendritic spines where its
8 activation is required for acquisition of contextual and spatial learning.^{26, 30-31} Arc expression
9 is also activated by several other mechanism(s) including BDNF²⁸, protein kinase A (PKA)
10 ³⁰, and pertussis-induced inhibition of G_i-mediated signaling.³² Accordingly, blockade of 5-
11 HT_{1A}R augments the effect of an acute dose of paroxetine on increasing transcription of Arc
12 mRNA.³³ Thus, Arc transcription may be a key nodal point in integration of monoaminergic
13 antidepressant effects with glutamate-driven activity-dependent neuronal adaptations
14 regulating gene transcription associated with information processing and associative learning.

15 The Flinders Sensitive Line (FSL) is a rat strain associated with distinct behavioral and
16 neurochemical features of major depression, including face validity, *i.e.* psychomotor
17 retardation and increased REM sleep. FSL rats display a genetic vulnerability to
18 environmental stressors and have been successfully used for validation of antidepressant
19 effects.³⁴ Interestingly, promotion of hippocampal neuroplasticity, by means of
20 intracerebroventricular injections of bone-marrow mesenchymal stem cells, reverses the
21 depressive-like phenotype of FSL rats.³⁵ LTP is also reduced in the CA1 area of
22 hippocampus, shown during *in vivo* recordings in anesthetized FSL rats³⁶ paralleled by
23 reduced NR1 subunits of the NMDAR in hippocampal synaptosomes.³⁶ FSL rats also have
24 reduced hippocampal volume and dendritic spines, which is reversed by antidepressants.³⁷
25 These alterations may affect emotional memory processes that, indeed, are believed to depend

on enduring remodeling of neuronal morphology and activity-dependent structural plasticity, particularly of glutamatergic dendritic spines, indicating possible cognitive impairments in FSL rats.

In the present study, we performed behavioral, pharmacological, biochemical and histological experiments to examine whether FSL rats exhibit impairments in emotional learning which may respond differentially to various antidepressant therapies and/or selective manipulations of 5-HT neurotransmission.

Materials and methods

Animals and pharmacological treatments

Adult FSL and FRL rats were bred at the Department of Physiology and Pharmacology at Karolinska Institute, and were tested at 2-4 months of age. Rats were kept in standard cages (TypeIV Macrolon[®], 26x42x15cm) with sawdust, at room temperature and relative humidity (45-55%) under a constant light/dark cycle (lights on at 7.00a.m.). Water and food pellets (LactaminR36, Stockholm, Sweden) were available *ad libitum*. Experimental procedures were approved by the local Animal Ethics Committee (Stockholms Norra Djurförsöksetiska Nämnd).

FRL and FSL rats were randomly assigned to groups given either escitalopram, nortriptyline or vehicle administered to standard rat pellets³⁸⁻³⁹ (Fig.S4) (Lactamin AB). Escitalopram, was given at 340mg/kg pellets for 3 weeks, followed by 410mg/kg pellets. Nortriptyline was administered at 330mg/kg pellet.

NAD-299 [(R)-3-*N,N*-dicyclobutylamino-8-fluoro-3,4-dihydro-2*H*-1-benzopyran-5-carboxamide hydrogen (2*R*,3*R*)tartrate monohydrate](1mg/kg s.c.; AstraZeneca R&D, Södertälje, Sweden) and RS67333(1mg/kg, i.p.; Tocris) were administered 30 minutes prior to testing.⁴⁰⁻⁴¹ (±)-8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin](0.3mg/kg s.c.; Sigma,

1 St Louis, MO, USA), and MK-801[(+)-10,11-dihydro-5-methyl-5*H*-dibenzo[*a,d*]-
2 cyclohepten-5,10-imine hydrogen maleate](0.05mg/kg s.c; Sigma, St Louis, MO, USA) were
3 both administered 15 min pretraining. ⁴¹ GR125487[5-Fluoro-2-methoxy-[1-[2-
4 [(methylsulfonyl)amino]ethyl]-4-piperidiny]-1*H*-indole-3-methylcarboxylate sulfamate](10
5 mg/kg i.p.; Tocris) was given 60 min pretraining. ¹⁷ Drugs were dissolved in 2ml/kg of
6 0.9%NaCl. PD184161(Servier, Suresness, France) was administered 60 min prior to testing at
7 a dose of 30 mg/kg i.p and dissolved in 1%Tween80 in 6ml/kg of 0.9%NaCl.

8 *Passive avoidance (PA), Forced-swim test (FST), Open-Field (OF) and Elevated Plus-Maze*
9 *(EPM).*

10 The step-through PA was performed as described earlier ⁴¹ (see supplementary Fig.S1).
11 During PA training, rats were placed in the bright compartment and allowed to explore for
12 120s. Thereafter, the sliding door was opened and once the rat had entered the dark
13 compartment the sliding door was automatically closed and a weak electrical stimulus
14 (0.4mA, 2s scrambled current) was delivered through the grid floor. Twenty-four hours after
15 training, the animal was again gently placed in the light compartment and the latency to enter
16 the dark compartment with all four paws was automatically measured (retention latency) with
17 a cut-off time for testing after 9 min. See supplementary information regarding the procedures
18 for FST and OF(Fig.S4) and EPM(Fig.S5).

19 *Histological measurements of Arc and BDNF transcription and 5-HT receptors*

20 For *in situ* hybridization experiments, rat brains were rapidly dissected after decapitation and
21 immediately frozen at –80°C. Fresh frozen (12µm) coronal cryostat sections were prepared
22 and hybridized with ³⁵S-radiolabeled antisense riboprobes against Arc and BDNF according
23 to a previous protocol. ⁴² For receptor autoradiography procedure with [³H]8-OH-DPAT,
24 [¹²⁵I]cyanopindolol and [³H]GR113808, see supplementary Fig.S2. Densitometric

1 measurements were obtained from autoradiograms using the NIH ImageJ 1.40 software after
2 subtraction of nonspecific binding.

3 *Immunoprecipitation and immunoblotting of total protein and their phosphorylation state*

4 The levels of the studied proteins and their phosphorylation state were assessed by
5 immunoblotting. To preserve protein phosphorylation, rats were sacrificed using focused
6 microwave irradiation (Muromachi Kikai, Tokyo, Japan) with brain regions dissected and
7 frozen at -80°C . Alternatively, rats were decapitated, their head snap frozen, brain regions
8 rapidly dissected out and processed to avoid dephosphorylation events. These procedures
9 have been described earlier.⁴²⁻⁴³ For detailed immunoprecipitation and immunoblotting
10 protocols, see supplementary information in Table S1.

11 *Statistical analysis*

12 The data were analyzed with one-way or two-way ANOVAs with drug treatment, rat strain or
13 brain region as factors in multiple comparisons. For each significant F-ratio, Newman-Keuls
14 test was used for Post-hoc comparison of effects following one-way ANOVA, while
15 Bonferroni Post-hoc test was used after two-way ANOVA. When only two groups were
16 compared (FRL with FSL), a two-tailed unpaired Student's T-test was used. $p < 0.05$ was
17 considered statistically significant.

19 **Results**

20 *Baseline performance in PA and Arc transcription in FSL and FRL rats*

21 FSL rats of both sexes displayed reduced performance in the PA task compared to the control
22 FRL rats (Fig. 1A). To determine biochemical measures corresponding to the deficit in
23 contextual memory, assessment of Arc mRNA levels was made in several brain regions. Arc
24 mRNA expression was specifically reduced in medial prefrontal cortex and in hippocampal

CA1 and dentate gyrus subterritories, but not in parahippocampus, somatosensory cortex, amygdala or thalamus (Fig. 1D-M).

Baseline regulation of glutamate and 5-HT receptors and signaling molecules

Arc mRNA expression is regulated by glutamate and serotonin neurotransmission and, in accordance with reduced Arc mRNA, western blotting showed lower levels of NR1 subunits of the NMDAR complex in prefrontal cortex, hippocampus and parahippocampus of FSL rats (Fig. 2A). The compositions of the different NMDAR subunits determine gating and kinetic properties the NMDAR, with important consequences for synaptic plasticity and memory.⁴⁴⁻⁴⁵ The NR2B subunit is specifically involved in NMDAR interactions with the MAPK cascade.⁴⁶ In FSL rats, NR2A and NR2B subunits were specifically lowered in prefrontal cortex and hippocampus (Fig. 2A). In none of the studied regions, GluR1 subunits of the AMPAR complex were different between FSL and FRL rats (Fig. 2A). Serotonin 5-HT_{1A}R, but not 5-HT_{1B}R or 5-HT₄R, were reduced in all studied regions in FSL rats (Fig. 2A). Likewise, labeling of 5-HT_{1A}R by [³H]8-OH-DPAT was reduced in hippocampal sections from male (Fig. S2A-C) and female (Fig. S2A,D-E) FSL rats, whereas there were no alterations in 5-HT_{1B}R or 5-HT₄R-like binding, using [¹²⁵I]cyanopindolol and [³H]GR113808 respectively (Fig. S2F-O).

In contrast to the reduced levels of NMDAR subunits, their phosphorylation states appeared to be generally increased in FSL rats, possibly as a compensatory mechanism(s). Specifically, NR1 subunits showed significantly increased phosphorylation of Ser⁸⁹⁶, a PKC site, and Ser⁸⁹⁷, a PKA site, in the hippocampus (Fig. 2B). In prefrontal cortices, the phosphorylation at the NR1 subunit at site Ser⁸⁹⁶ was increased and the NR2A subunit exhibited an increased phosphorylation at Ser¹²³², a cdk5 site (Fig. 2B). The phosphorylation states of NR2B subunits at Ser¹³⁰³, a CaMKII site, and Tyr¹⁴⁷², a SRC tyrosine kinase site, were not altered in FSL rats (Fig. 2B). The phosphorylation levels of GluR1 subunits at Ser⁸³¹,

1 a PKC/CaMKII site, and Ser⁸⁴⁵, a PKA site, were not altered (Fig.2B). The total levels of
2 MEK, p42-MAPK and p44-MAPK were increased in prefrontal cortex and hippocampus of
3 FSL rats (Fig.2C). No changes in the phosphorylation of Ser^{217/221}-MEK were found (Fig.2D).
4 However, reduced phosphorylations at Thr^{183/Tyr185}-p42 and Thr^{202/Tyr204}-p44 in hippocampus
5 were found, indicative of a reduced MEK activity in FSL rats (Fig.2D). In contrast, total
6 levels or phosphorylation states of CaMKII, GSK-3 β , AKT or in PSD-95, a postsynaptically
7 enriched protein, were not altered (Fig.2C-D). These data suggest that MEK/MAPK signaling
8 is particularly dysregulated in FSL rats and that decreased MEK activity, perhaps related to
9 reduced NMDAR and 5-HT receptor-mediated transmission, parallels with memory
10 impairments and reduced Arc mRNA expression in FSL rats.

11 *Impairing effect on PA performance by NMDAR blockade in both FSL and FRL rats*

12 NMDAR are critically implicated in the acquisition and consolidation of several types of
13 memories including the PA task ⁹. In agreement with previous reports, treatment with an
14 amnesic dose of MK-801, a non-competitive NMDAR antagonist, of FRL rats severely
15 disrupted retention performance, mimicking the memory impairments seen in FSL rats
16 (Fig.S3 and summary Fig.S7A-C).

17 *Effects of escitalopram and nortriptyline on behavioral performance in PA, FST, OF, EPM*
18 *and transcription of Arc*

19 To test whether the impairment in memory performance in FSL rats could be reversed by
20 antidepressants, FSL and FRL rats were treated chronically with vehicle, escitalopram or
21 nortriptyline. ³⁸⁻³⁹ In FSL rats, escitalopram, but not nortriptyline, reversed the impairment of
22 memory performance in the PA test (Fig.3A). In contrast, escitalopram was without effect on
23 memory performance in FRL rats, comparable with lack of effects of paroxetine in Sprague-
24 Dawley rats ⁴⁷, while nortriptyline impaired performance (Fig.3A). Escitalopram, but not
25 nortriptyline, specifically increased expression of Arc mRNA in the CA1 and dentate gyrus

1 subregions of hippocampus of FSL rats (Fig.3B-E). Escitalopram reduced immobility in the
2 FST in FSL rats.³⁸ Similarly, nortriptyline decreased immobility of FSL rats in the FST
3 (Fig.S4A), via mechanisms unrelated to alterations in locomotion (Fig.S4B-C). Notably, the
4 differential effect of escitalopram and nortriptyline in the PA test could not be correlated to
5 EPM performance, as both agents induced anxiolytic-like effects in control FRL rats
6 (Fig.S4D-E). FSL rats displayed a basal anxiolytic phenotype and nortriptyline was without
7 effect, while escitalopram further reduced anxiety-like behavior (Fig.S4D-E).

8 *Measurements of BDNF mRNA and protein and phosphorylated TrkB under baseline*
9 *conditions and following escitalopram and nortriptyline*

10 Several antidepressant actions are mediated via BDNF and its predominant receptor TrkB and
11 BDNF signaling is reflected by the phosphorylation state of TrkB.¹⁸⁻²² BDNF signaling
12 increases Arc mRNA and protein expression in neurons²³ and could potentially act upstream
13 of Arc under baseline conditions and/or following escitalopram treatment in FSL rats.
14 Immunoblotting revealed regional-specific differences of basal proBDNF, with reduced levels
15 in the hippocampus of FSL rats when compared to FRL rats, but increased levels in the
16 parahippocampal region and unaltered levels in the prefrontal cortex (Fig.S5A). However,
17 basal levels of mature BDNF, as well as total and phosphorylated TrkB, did not differ
18 between the genotypes in the examined regions (Fig.S5A-B).
19 In response to both escitalopram and nortriptyline, FSL rats showed increased expression
20 of BDNF mRNA in the dentate gyrus, but not in the CA1, of hippocampus (Fig.S5C-F). In
21 comparison, no significant alterations of proBDNF or BDNF proteins were found in total
22 hippocampal or parahippocampal homogenates from antidepressant-treated animals
23 (Fig.S5G). Nortriptyline and escitalopram induced treatment-, region- and site-specific
24 changes of TrkB phosphorylation, without affecting total TrkB. Specifically, both nortriptyline
25 and escitalopram increased phosphorylation of TrkB at Tyr⁸¹⁶, whereas nortriptyline, but not

1 escitalopram, increased phosphorylation at Tyr⁷⁰⁵-TrkB, and none of these treatments regulated
2 Tyr⁵¹⁵-TrkB in hippocampus (Fig.S5H). No corresponding alterations were found in the
3 parahippocampal region (Fig.S5H). It should be noted that the antisera towards
4 phosphorylated TrkB can also detect phosphorylated TrkA, and both TrkB and TrkA are
5 found in hippocampal extracts (Fig.S6A-B). In view of this, immunoprecipitations of TrkB
6 were done from hippocampal brain tissue and, subsequently, analyzed for total TrkA and
7 TrkB receptors as well as phosphorylated TrkB (Fig.S6C-E). No TrkA was found in TrkB
8 immunoprecipitates (Fig.S6E). However, immunoreactivity of the antidepressant-responsive
9 Tyr⁸¹⁶-TrkB site was readily detected in TrkB immunoprecipitates (Fig.S6D). In response to
10 treatment with escitalopram and nortriptyline, levels of Tyr⁸¹⁶-TrkB were increased in
11 hippocampal TrkB immunoprecipitates (Fig.S6D,F). Vice versa, in a confirmatory
12 experiment, immunoprecipitation of Tyr⁸¹⁶-TrkB resulted in higher levels of TrkB
13 immunoreactivity in antidepressant-treated rats (Fig.S6C). Notably, no TrkA was found in
14 Tyr⁸¹⁶-TrkB immunoprecipitates (Fig.S6E), indicating that phosphorylation at this site in
15 hippocampus occurs mainly at TrkB.

16 *Effects of selective 5-HT_{1A}R and 5-HT₄R ligands, with and without a MEK inhibitor, on PA*
17 *performance and transcription of Arc*

18 Based on the procognitive effects of escitalopram in the PA test in FSL rats and reduced 5-
19 HT_{1A}R levels, we studied 5-HT receptor subtypes in modulating memory performance in FSL
20 rats. The 5-HT_{1A}R agonist 8-OH-DPAT impaired performance in the PA test in FRL rats
21 (Fig.4A). On the other hand, NAD-299, a 5-HT_{1A}R antagonist, enhanced performance in the
22 PA test in FRL rats and reversed the memory dysfunction in the FSL rats, reminiscent of the
23 action of escitalopram (Fig.4A). Inhibition of presynaptic 5-HT_{1A}R causes an increase in the
24 firing rate of serotonin neurons, while inhibition of postsynaptic 5-HT_{1A}R causes a decrease
25 of inhibitory serotonin transmission in specific circuits.² Next, we pretreated the rats with a 5-

1 HT₄R antagonist, known to mediate its actions at the postsynaptic site. GR125487, at a
2 concentration where it had no intrinsic effect, blocked the effect of NAD-299 indicating that
3 NAD-299 exerts at least some of its effects via stimulation of 5-HT₄R (Fig.4A). The 5-HT₄R
4 agonist, RS67333, also restored memory performance in the PA test (Fig.4A). Both 5-HT_{1A}R
5 ^{9, 48} and 5-HT₄R ⁴⁹⁻⁵⁰ have been shown to regulate the MEK/MAPK cascade and since the
6 biochemical data indicated that MEK/MAPK signaling is altered in FSL rats (Fig.2A-B), we
7 investigated whether the MEK/MAPK signaling cascade is mediating procognitive actions of
8 NAD-299 and/or RS67333. Rats were pretreated with a MEK inhibitor, PD184161 ¹⁹, at a
9 dose where it had no intrinsic effect, prior to treatment with either NAD-299 or RS67333.
10 PD184161 completely blocked the enhancing effects of NAD-299 or RS67333 in the PA test
11 (Fig.4A).

12 We also found that NAD-299 and/or RS67333 could mimic the region-specific
13 stimulatory effects of escitalopram on Arc mRNA in the CA1 and dentate gyrus subregions of
14 hippocampus (Fig.4D,F,H). Moreover, while PD184161 had no effects by itself on Arc
15 transcription in FSL rats (Fig.4C,H) it significantly counteracted the actions of both NAD-299
16 and RS67333 on Arc mRNA (Fig.4E,G,H). It seems that MEK/MAPK signaling is a nodal
17 point in mediating some behavioral and biochemical postsynaptic serotonergic effects
18 critically involved in procognitive effects in the PA paradigm.

20 Discussion

21 A substantial proportion of depressed patients exhibit deficits in several memory tasks,
22 including declarative verbal and recollection memory, executive functions and emotional
23 word processing. ^{2, 6, 8, 14, 21, 51-52} Improvement of cognitive processing and emotional memory
24 function in depression is believed to be important for the treatment outcome in MDD. ^{2, 4} In
25 the present study, we provide data indicating that the genetic FSL rat line of depression may

1 be utilized as a model for studies of reversible impairments in emotional processing and
2 memory, displaying predictive validity for assessment of drugs supporting cognitive
3 performance.^{4, 53} The impairments of memory performance are in accordance with the
4 findings of reduced Arc mRNA specifically in cortical and hippocampal subregions of FSL
5 rats (Fig. 1D-M), consistent with accumulating data indicating that Arc transcription is
6 activated by neuronal stimuli particularly important for encoding of one-trial hippocampal-
7 dependent learning and long-term neuroplasticity involved in consolidation of memories.^{26-27,}
8 ^{31, 54}

9 The marked alterations of glutamatergic receptors in the FSL rat line (Fig. 2AB) agree
10 with a number of neuroimaging and post-mortem histological studies showing glutamatergic
11 abnormalities in MDD^{55, 56}, *e.g.* magnetic resonance spectroscopy studies in patients with
12 MDD demonstrated altered glutamate levels in several cortical subregions.⁵⁷⁻⁵⁹ Accordingly,
13 NMDAR binding and subunits are decreased in cortical regions in depressed patients.⁶⁰⁻⁶⁴
14 Similarly, the core NR1 subunit of the NMDAR was markedly reduced in FSL rats in the
15 prefrontal cortex, parahippocampal region and hippocampal extracts, accompanied with
16 reductions also of the NR2A and NR2B subunits in the prefrontal cortex and hippocampus
17 (Fig. 2A). Selective reductions of several NMDAR subunits in corticolimbic brain regions in
18 FSL rats further indicate that these alterations have severe consequences for synaptic
19 plasticity and account for the impairments of LTP in FSL rats.³⁶ Consistent with findings
20 reported from MDD patients⁵⁵⁻⁵⁶ we observed only modest alterations of GluR1 levels. The
21 phosphorylation state of NMDAR and AMPAR correlates with changes in synaptic strength.
22 ⁶⁵ Despite the lower levels of NMDAR subunits found in FSL rats, phosphorylation levels
23 were in several cases upregulated. Taken together, rather than subtype-specific alterations,
24 regional-specific overall reductions of NMDAR, partially compensated by
25 hyperphosphorylation were found in FSL rats.

1 The specific reductions of 5-HT_{1A}R, but not 5-HT_{1B}R or 5-HT₄R-like binding, using
2 immunoblotting (Fig.2A) and autoradiography receptor binding in FSL rats (Fig.S2A-O)
3 agree with a previous study ⁶⁶ The reduction of 5-HT_{1A}R are consistent with several
4 neuroimaging studies and post-mortem data from patients with MDD. ¹⁰⁻¹⁴ It has however
5 been difficult to distinguish primary effects and confounders in view of variations of 5-HT_{1A}R
6 binding during onset and remission of depression, responses to antidepressants, differences in
7 agonist vs. antagonist labeling, with the mixed results most likely also reflecting the clinical
8 heterogeneity of depressed patients, drug treatment and/or aging ^{12, 14, 15}

9 After chronic antidepressant treatments, a discrepancy was found between escitalopram
10 and nortriptyline on both behavioral outputs and on Arc mRNA expression (Fig.3A-E). The
11 restoring effects on Arc transcription and emotional memory performance of FSL rats are
12 consistent with clinical findings supporting a serotonergic component in recovery of cognitive
13 aspects. ^{4, 8, 67} Indeed, these results in FSL rats show predictive and face validity with data on
14 clinical efficacy for escitalopram and nortriptyline, antidepressants which have been reported
15 to be similar on three conventional depression-rating scales, but differentiate in a superior
16 improvement of cognitive symptoms by escitalopram, while nortriptyline rather improved
17 neurovegetative symptoms. ⁶⁸ Calculation of a proportional impairment-ratio in humans were
18 found to be 3-9-fold higher for tricyclic antidepressants, than those reported for escitalopram,
19 on a range of different psychometric tests for cognitive symptoms. The varying drug-induced
20 cognitive impairment caused by tricyclic agents is attributed to their anticholinergic and
21 antihistaminergic properties, decreasing cognitive performance and arousal, respectively. ⁶⁷

22 Consistent with previous reports on antidepressant regimens, both escitalopram and
23 nortriptyline increased BDNF mRNA in the dentate gyrus, but not CA1, of hippocampus
24 (Fig.S5C-F). ^{20-21, 69} We could not detect any corresponding effects on protein levels of
25 proBDNF and mature BDNF (Fig.S5G). As discussed previously (for reviews see ^{20-21, 69}), a

1 discrepancy between mRNA and protein expression of BDNF as found here may be due to
2 several factors including the omission of subregional analysis of hippocampal BDNF protein,
3 retrograde/anterograde transport of BDNF protein transcribed in the dentate gyrus and the
4 lack of distinct measures of intra- and extracellular BDNF protein.

5 BDNF-mediated signaling can be studied by measures of the phosphorylation state of
6 the TrkB receptor. Binding of BDNF to the TrkB receptor induces its autophosphorylation at
7 Tyr⁷⁰⁵-TrkB in the catalytic domain, which subsequently regulates the phosphorylation state
8 at other tyrosine residues including Tyr⁵¹⁵-TrkB, a Shc binding site, and Tyr⁸¹⁶-TrkB, a
9 PLCγ1 binding site.⁶⁸ The latter phosphorylation site of TrkB is also regulated via
10 transactivation by G-protein coupled receptors, independent of BDNF.⁷⁰ In agreement with
11 Rantamäki, Castrén and co-workers¹⁸⁻¹⁹ we found that both a tricyclic antidepressant,
12 nortriptyline, and, a SSRI, escitalopram, increased phosphorylation at the PLCγ1 binding site
13 Tyr⁸¹⁶-TrkB, but not at the Shc binding site Tyr⁵¹⁵-TrkB in hippocampus (Fig.S5H). It is
14 interesting that studies with phosphomutant TrkB mice have shown that the PLCγ1 binding
15 site is more important for synaptic plasticity than the Shc binding site⁷¹, suggesting that the
16 observed activation of Tyr⁸¹⁶-TrkB by nortriptyline and escitalopram may be of functional
17 significance in mediating long-term antidepressant actions. Since the antisera towards
18 phosphorylated TrkB can also detect phosphorylated TrkA and both these receptors are found
19 in hippocampus (Fig S6A-B), we performed additional experiments to confirm that
20 antidepressants specifically increase phosphorylation at Tyr⁸¹⁶ of TrkB. Indeed, in reciprocal
21 immunoprecipitation experiments with antisera towards either TrkB or Tyr⁸¹⁶-TrkB, both
22 nortriptyline and escitalopram increased Tyr⁸¹⁶ specifically at TrkB without regulating TrkA
23 (Fig.S6C-F). Using direct immunoblotting from snapfrozen hippocampi, we also found that
24 nortriptyline, but not escitalopram, increased Tyr⁷⁰⁵-TrkB at the studied timepoint suggesting
25 a more sustained and/or stronger activation of TrkB by nortriptyline (Fig.S5H). However, it

1 should be noted that we sacrificed the rats during daytime when they consumed less drug-
2 containing pellets and previous work has demonstrated that the autophosphorylation at Tyr⁷⁰⁵-
3 TrkB is transient and most pronounced within one hour after an injection of a tricyclic or a
4 SSRI antidepressant. ¹⁸⁻¹⁹

5 Taken together, the increased BDNF transcription and Tyr⁸¹⁶-TrkB phosphorylation by
6 both nortriptyline and escitalopram in FSL rats correlate well with the antidepressant and
7 anxiolytic effects of these agents, but not with the differential effects on recovery of
8 emotional memory in the PA paradigm. On the other hand, the discrepancy of nortriptyline
9 and escitalopram on Arc transcription corresponds better with their effects on PA memory
10 performance. Arc, rather than BDNF, transcription was therefore chosen as a biochemical
11 correlate in the mechanistic studies on serotonin-mediated modulation of PA using selective
12 ligands. However, it should be noted that a contribution of BDNF/TrkB signaling to the
13 effects of escitalopram on Arc transcription and PA performance cannot be excluded.

14 Given the restoring effects of escitalopram on PA in FSL rats and the alterations of 5-
15 HT_{1A}R levels, we went on to investigate the effects of pharmacological modulation of specific
16 5-HT receptor subtypes. Stimulation of 5-HT_{1A}R impairs learning and memory functions in a
17 range of rodent cognitive tasks. ² This agrees with the pharmacological response to the 5-
18 HT_{1A}R agonist 8-OH-DPAT in FRL rats (Fig.4A and Fig.S7D), resembling the impaired
19 memory performance of untreated FSL rats compared to control FRL rats (Fig.4A and
20 Fig.5A-B). The blunted response of FSL rats to 8-OH-DPAT is consistent with the findings of
21 reduced postsynaptic 5-HT_{1A}R density (Fig.2A). Conversely, the 5-HT_{1A}R antagonist NAD-
22 299 facilitates several types of learning in rodents, attributed to alleviation of 5-HT_{1A}R-
23 activated G_{i/o}-mediated signaling cascades in postsynaptic neurons (Fig.4A). ^{2, 41} Thus, the
24 effects of blockade of pre- and postsynaptic 5-HT_{1A}R resembled the effects of increased 5-HT
25 function by escitalopram (Fig.3A and Fig.5C). Despite an important role of 5-HT_{1A}Rs in

1 modulation of SSRI efficacy, chronic fluoxetine still has antidepressant-like effects in 5-
2 HT_{1A}R KO mice, emphasizing an involvement of other 5-HT receptor subtypes.⁷²
3 Intriguingly, the 5-HT₄R antagonist GR125487 blocked the facilitatory effects of NAD-299
4 on PA performance (Fig.4A), indicating that activation of postsynaptic 5-HT₄Rs is necessary
5 for the restoring effect of 5-HT_{1A}R antagonism on PA performance in FSL rats. Likewise,
6 direct agonist stimulation of 5-HT₄R with RS67333 also restored PA performance (Fig.4A).
7 Thus, these data indicate that not only does 5-HT_{1A}R antagonism disinhibit postsynaptic
8 neurons by alleviating a tonic inhibition mediated by 5-HT, but also enables a shift in
9 endogenous 5-HT transmission towards activation of the 5-HT₄R and downstream signaling
10 cascades (Fig.5C). The effects of chronic SSRI administration on PA performance and Arc
11 mRNA were thus replicated by either 5-HT_{1A}R antagonism or 5-HT₄R agonism
12 (Fig.4A,D,F,H), further supporting that central 5-HT₄R stimulation offers a potential target
13 for improvement of emotional¹⁶ and cognitive processing.¹⁷

14 Systemic pretreatment with the MEK inhibitor PD184161 fully blocked the ability of
15 both NAD-299 and RS67333 to restore memory function (Fig.4A) and induce Arc mRNA
16 expression (Fig.4E,G,H). These data implicate an involvement of the MAPK signaling
17 pathway as a nodal point in restoration of emotional memory consolidation. There is support
18 for G_{i/o}-protein and G_s-protein mediated receptor modulation of the MEK/MAPK cascade.^{9,}
19 ^{23, 48-50, 73} Local increase of cAMP may trigger events that are involved in induction of short-
20 term plasticity and early stages of memory formation, while activation of MAPK signaling
21 pathways may result in more long-lasting effects requiring nuclear gene transcription.^{21, 23}
22 Besides, fear conditioning and LTP-mediated increase of Arc expression in the lateral
23 amygdala is dependent on activation of the MAPK cascade⁷⁴ and hippocampal cAMP/MAPK
24 signaling is involved in long-term consolidation of contextual fear conditioning.⁷⁵
25 Collectively, these findings emphasize a crucial involvement of the MEK/MAPK cascade as a

1 nodal point in 5-HT_{1A}R/5-HT₄R-mediated effects on emotional memory and expression of
2 Arc mRNA (Fig.5D).

3 In conclusion, emotional memory impairments and baseline reductions of Arc
4 transcription were found in the FSL rat line of depression. Memory functions and
5 enhancement of Arc transcription were specifically induced by escitalopram as well as
6 pharmacological treatments increasing postsynaptic 5-HT function via stimulation of
7 cAMP/MAPK signaling cascades. The FSL rat line provides a preclinical model
8 recapitulating reversible deficits of cognitive processing that is disrupted in depression.

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15 16 **Conflict of Interest**

17 The authors declare no conflict of interest.

18
19 Supplementary information is available at Molecular Psychiatry's website
20
21

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4

5

FIGURE LEGENDS

Figure 1. Male and female FSL rats display emotional memory impairments and reduction of Arc mRNA expression specifically in brain regions implicated in cognitive processing. PA emotional memory performance under baseline conditions in male (n=6-8 per group) and female (n=11 per group) FRL and FSL rats (A). Effects on step-through latency at the retention test performed 24 hrs after PA training. Atlas diagrams illustrating coronal sections of mPFC (B) and hippocampal subregions (C). Histograms of Arc mRNA in four to five male (D) and five female (E) FRL and FSL rats per group. Autoradiograms from *in situ* hybridization experiments against Arc mRNA in male FRL (F, G) and FSL (H, I) rats or female FRL (J, K) and FSL (L, M) rats. mPFC: medial prefrontal cortex, PHR: parahippocampal region, CA1: cornu ammonis 1 of hippocampus, DG: dentate gyrus of hippocampus, SCX: primary sensory cortex, AMY: amygdaloid nuclei, TH: thalamic nuclei. Data represent mean \pm SEM. *p<0.05; **p<0.01; ***p<0.001 vs. corresponding FRL control group.

Figure 2. Alterations of total proteins and phosphorylated forms of proteins in FSL rats compared to FRL control rats. Top, illustration of labeling of bars. Histograms of quantification of total protein levels (A and C) and phosphorylated form of the protein normalized to the total level of the same protein (B and D) in PFC: prefrontal cortex; PHR: parahippocampal region and HPC: hippocampal region. Representative Western blots from hippocampal region are shown above each histogram. Values are mean \pm SEM from four to eight animals per group. *p<0.05; **p<0.01; ***p<0.001 vs. corresponding FRL control group.

Figure 3. Differential effect of escitalopram and nortriptyline in restoration of emotional memory impairments and Arc transcription in FSL rats. Effects on step-through latency at the retention test performed 24 hrs after PA training in male FRL and FSL rats (n=9-12 per group) (A). Autoradiograms from *in situ* hybridization experiments against Arc mRNA in rats receiving vehicle pellets (B) chronic nortriptyline (C) and chronic escitalopram (D). Histograms of quantification of the effects of antidepressants on Arc mRNA in six male FSL rats per group (E). CA1: cornu ammonis 1 of hippocampus, DG: dentate gyrus of hippocampus, PHR: parahippocampal region, SCX: primary sensory cortex, AMY: amygdaloid nuclei. Data represent mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. corresponding FRL control group; (A) ### $p < 0.01$ FSL vs. escitalopram; (C) ### $p < 0.01$ nortriptyline vs. escitalopram.

Figure 4. Effects of 5-HT_{1A}R or 5-HT₄R agonists and antagonists and involvement of MEK activity in restoration of emotional memory function and induction of Arc mRNA expression. PA memory performance assessed by step-through latency at the retention test performed 24 hrs after training in male FRL and FSL rats (n=7-18 per group) (A). The lack of error bar for FRL rats treated with NAD-299 was due to that none of the rats returned to the conditioned compartment at testing. Autoradiograms from *in situ* hybridization experiments against Arc mRNA in rats receiving 5-HT ligands alone (upper row) or pretreatment with MEK inhibitor (lower row) or vehicle (B, C), 5-HT_{1A}R antagonist (D, E) or 5-HT₄R agonist (F, G). Primary mechanisms of action: PD184161 - MEK inhibitor; NAD-299 - 5-HT_{1A}R antagonist; RS67333 - 5-HT₄R agonist; GR125487 - 5-HT₄R antagonist; 8-OH-DPAT - 5-HT_{1A}R agonist. Histograms of quantification of the effects on Arc mRNA transcription in six male FSL rats per group (H). CA1: cornu ammonis 1 of hippocampus, DG: dentate gyrus of hippocampus; PHR: parahippocampal region, SCX: primary sensory cortex, AMY: amygdaloid nuclei. Data

1 represent mean \pm SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs. corresponding FRL control
2 group; ## $p < 0.01$; ### $p < 0.01$ vs. FSL NAD-299; X $p < 0.05$ vs. FSL RS67333.
3
4
5 **Figure 5.** Schematic representation of mechanisms involved in effects on emotional memory
6 function and stimulation of Arc transcription. Basal level of transmission in control FRL rats
7 (A). Baseline transmission in untreated FSL rats (B). 5-HT therapy restores emotional
8 memory disturbances and increases Arc transcription (C). Pretreatment with a MEK inhibitor
9 blocks the restoring effects of pharmacological 5-HT therapy on memory performance and
10 expression of Arc mRNA (D). See text besides respective figures for further details.

11

Fig. 1

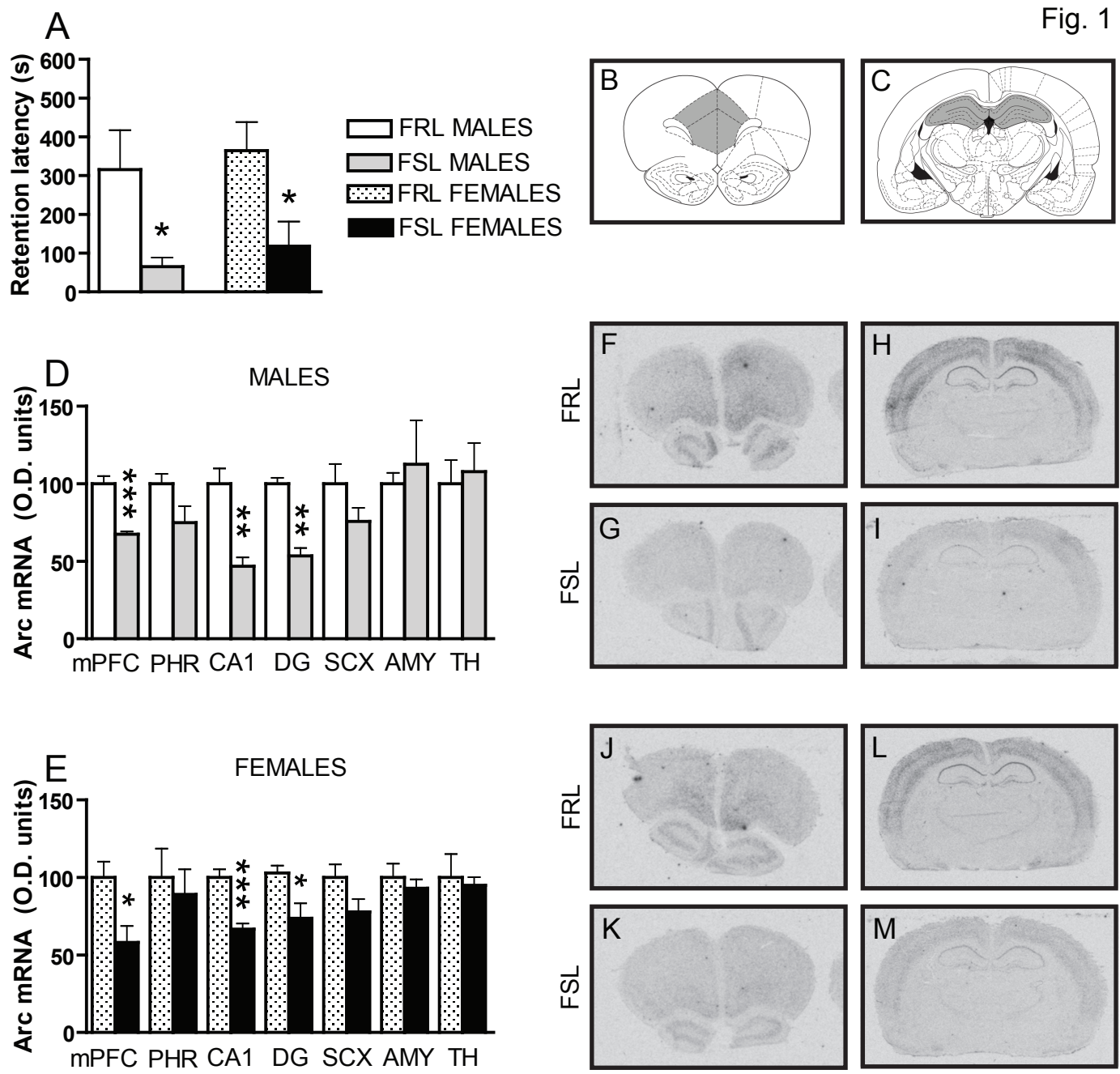


Fig. 2

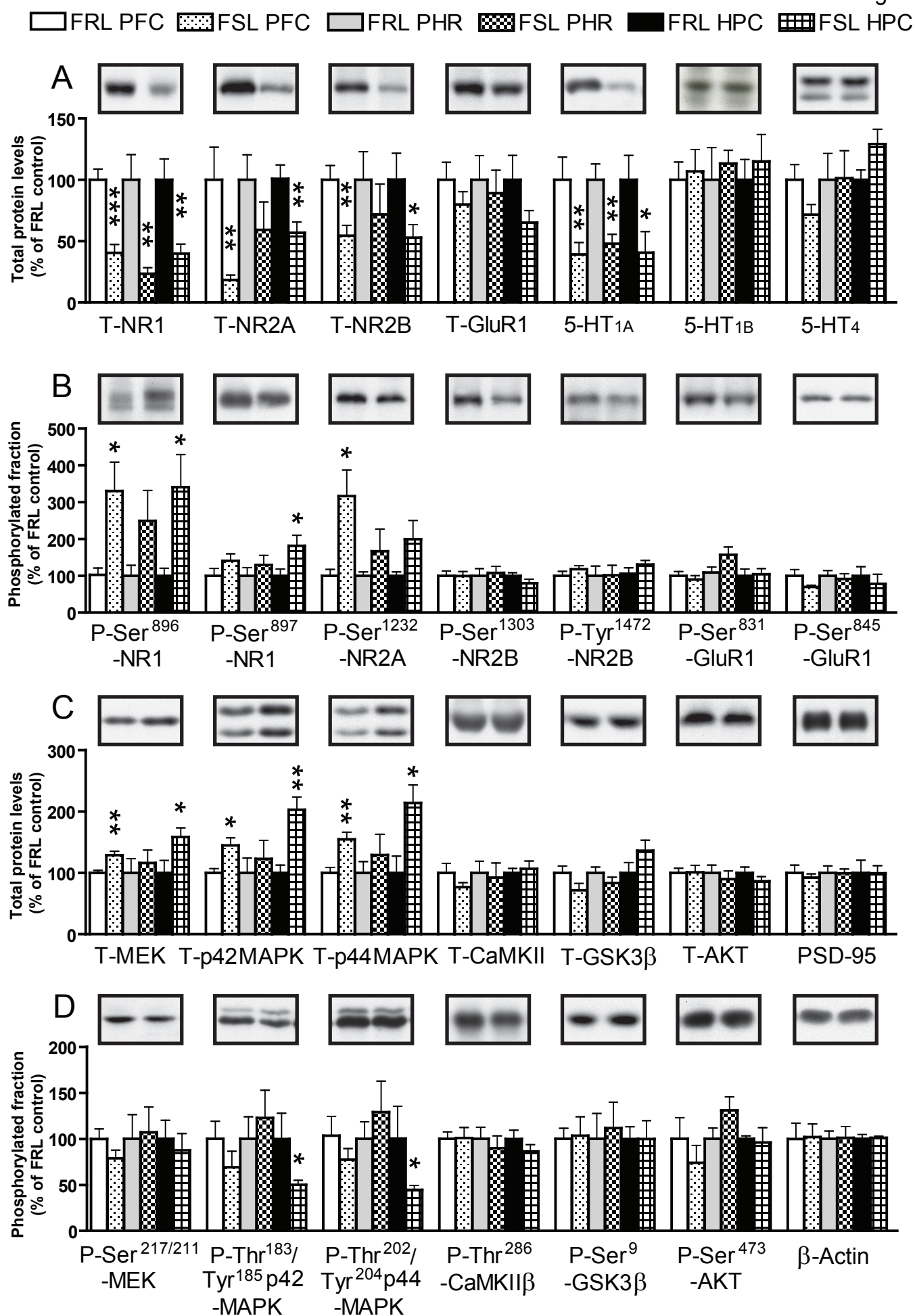


Fig. 3

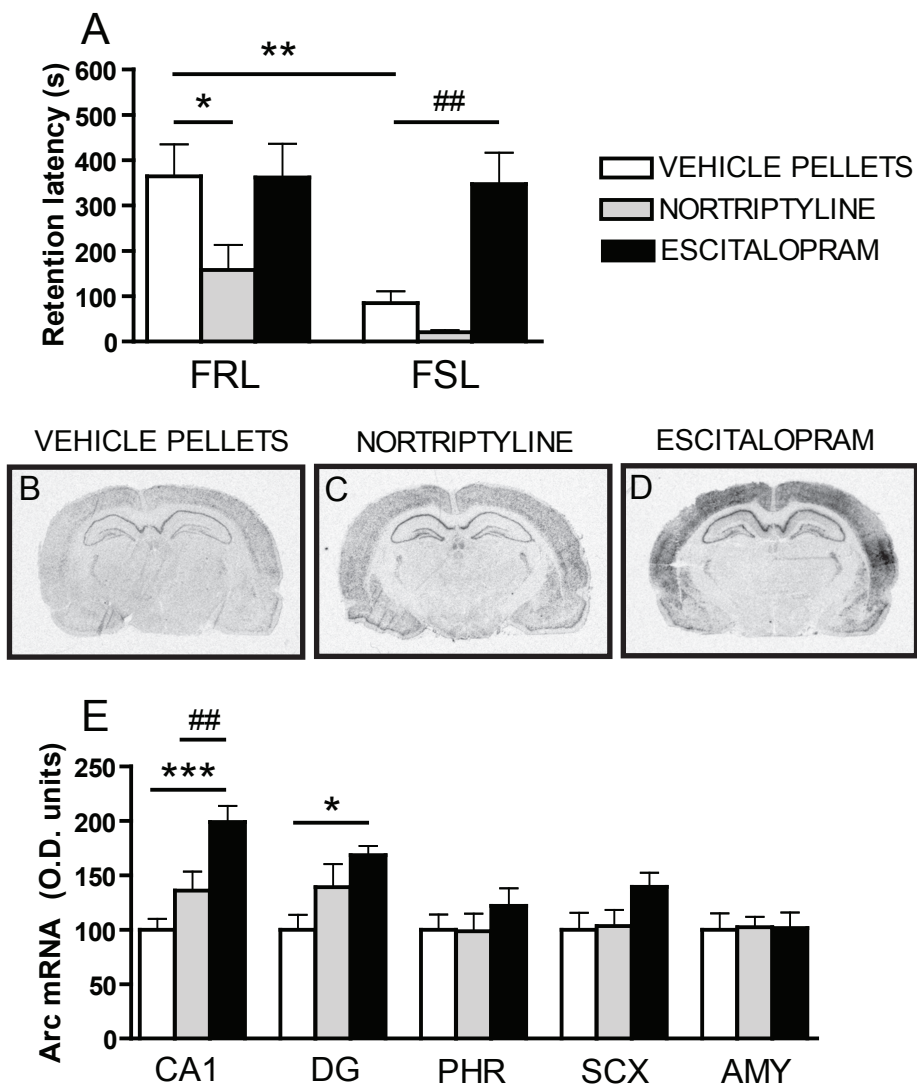


Fig. 4

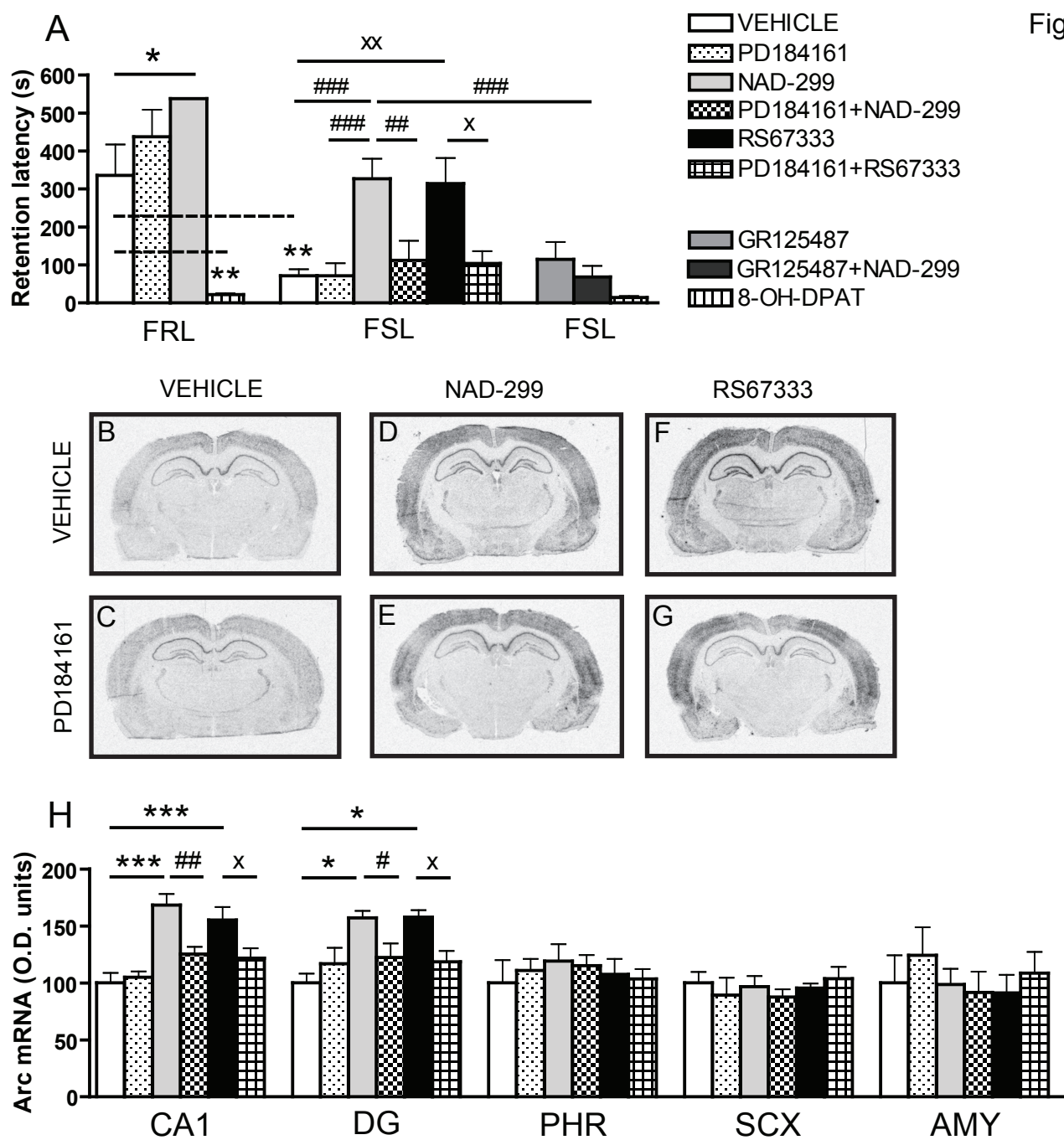


Fig. 5

