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ARTICLE

Neural cell adhesion molecule peptide mimetics modulate emotionality: pharmacokinetic and behavioral studies in rats and non-human primates

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Recent evidence highlights the fibroblast growth factor (FGF) family in emotion modulation. Although ligands that activate FGF receptors have antidepressant and anxiolytic effects in animal models, FGF ligands have a broad range of actions both in the brain and the periphery. Therefore, identifying molecular partners that may function as allosteric modulators could offer new avenues for drug development. Since neural cell adhesion molecule (NCAM) activates FGF receptors, we asked whether peripherally administered NCAM peptide mimetics penetrate the brain and alter the behavior of standardized tests that have predictive validity for drug treatments of anxiety or depression. The NCAM peptide mimetic, plannexin, acutely increased and chronically decreased anxiety, but did not have antidepressant effects in rats. Another NCAM peptide mimetic, FGL_L, had acute anxiogenic effects and chronic antidepressant effects in rats. A related NCAM peptide mimetic, FGL_S, had antidepressant effects without modulating anxiety-like behavior, and these antidepressant effects were blocked by an AMPA receptor antagonist. Cisternal cerebrospinal fluid (CSF) levels of FGLs correlated with blood plasma levels in rats and non-human primates, and CSF-to-blood ratios of FGL_S were comparable in both species. Results indicate that NCAM peptide mimetics penetrate the brain and support the suggestion that FGL_S may be a candidate for further development as a novel treatment for major depressive disorder in humans.

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INTRODUCTION

The activation of fibroblast growth factor (FGF) receptors has antidepressant and anxiolytic effects in animal models [1, 2], but FGF ligands are potentially problematic because of their broad effects both centrally and peripherally [3–5]. Therefore, we considered the possibility of activating FGF receptors using molecules other than the typical FGF ligands, especially allosteric modulators that function within the CNS to enhance the effectiveness of FGF signaling. Neural cell adhesion molecule (NCAM) has been crystallized to FGF receptors [6, 7], and it is involved in various FGF-related functions such as neurite extension, neuronal differentiation, cell survival, and synaptic plasticity [8, 9]. Additionally, NCAM polymorphisms have been associated with stress-related mental health disorders in humans [10].

Here we assessed whether NCAM peptide mimetics cross the blood–brain barrier and alter the emotionality. Three NCAM peptide mimetics were examined: plannexin, FGL_L, and FGL_S. Plannexin is a decapeptide (DVRRGIKKTD) that promotes dimerization and *trans*-interactions of NCAM [11]. Plannexin facilitates neurite outgrowth, neuronal survival, and spatial learning with additional positive effects on the hippocampal synaptic functions [12, 13]. FGL_L is a *cis* dimer coupled to a lysine backbone (EVYVVAENQQGKSKA) that binds to FGF receptors [6, 14] and dose dependently increases phosphorylation of FGFR1 [15, 16]. FGL_L enhances spatial learning and memory, promotes synaptogenesis, and prevents stress-induced deficits in the spatial memory and hippocampal granule cell survival [17, 18]. FGL_S (VAENQQGKSKA) is a truncated dimer of FGL_L that improves the spatial memory in NCAM knockout mice [19]. Although NCAM can interact with other signaling mechanisms, these peptide mimetics have only been shown to interact with FGF receptors [20].

NCAM peptide mimetics are involved in neuroplasticity [21–23], but few studies have examined their effects on emotionality. We peripherally administered plannexin, FGL_L, or FGL_S to rats either acutely or chronically and subsequently measured their behavior on standardized tests that have predictive validity for drug treatments of anxiety or depression. To test for psychopharmacological indications, that FGLs penetrates the brain, we determined whether an AMPA receptor antagonist co-administered with FGLs modulates the behavior in rats. We then directly assessed the correlations between FGL_S levels in cisternal cerebrospinal fluid (CSF) and blood plasma of both, rats and non-human primates. Three NCAM peptide mimetics were examined to determine their generalizability and establish a potential target for clinical research. We hypothesized that NCAM peptide mimetics penetrate the brain and have antidepressant effects.

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MATERIAL AND METHODS

A total of 178 adult male Sprague-Dawley rats (*Rattus norvegicus*) and 6 adult male squirrel monkeys (*Saimiri sciureus*) served as subjects. Monkeys were born and raised at the Stanford University Research Animal Facility and were studied in adulthood at 5–10 yrs of age. Adult rats weighing ~250 g were purchased from Charles River Laboratories and acclimated to our facilities for >7 days prior to all studies. Monkeys and rats were socially housed in species appropriate conditions at ~26 °C in 12:12 h light/dark cycles. All procedures were conducted in accordance with state and federal laws, standards of the Department of Health and Human Services, and were approved by Institutional Animal Care and Use Committees.

Behavioral effects of plannexin, FGL_L, and FGL_S

All NCAM peptide mimetics used here were gifts from EnKam Pharmaceuticals. Rats were randomized to treatments with either plannexin (10 mg/kg, i.p. in 0.9% saline), FGL_L (10 mg/kg, s.c. in ddH_2O), FGL_S (10 mg/kg, s.c. in 0.9% saline), or corresponding vehicle solutions that were administered either acutely or chronically as described below. For plannexin, the dose used here prevents reduction in neural progenitor cells associated with status epilepticus [24]. For FGL_L, the dose reverses the working memory deficits induced by phencyclidine [25]. For FGL_S, the dose improves the cognitive impairments in rodents [19]. Sample sizes were $N = 6–12$ per treatment condition, as described in the Supplementary Methods.

For all acute administrations, rats were injected 30 min before testing on the elevated plus-maze (EPM) described below. Behavior on the EPM has predictive validity for drug treatments related to anxiety in humans [26]. After 5 days, these rats were counterbalanced by a group and tested twice on 2 consecutive days by the forced swim test (FST) described below. Behavior on FST has predictive validity for drug treatments related to depression in humans [27]. For FST assessments with acute administration of plannexin or FGL_L, rats were injected 1 and 5 h after Day 1 of FST and then again 30 min before Day 2 of FST. For FST assessments with acute administration of FGL_S, rats were injected 1, 2, 3, 4, and 5 h after Day 1 of FST and then again 30 min before Day 2 of FST. Six, instead of the traditional three, injections between Days 1 and 2 of the FST [28] were used due to concerns that FGL_S may have a shorter half-life than FGL_L.

In a separate experiment, vehicle or FGLs was co-administered with the AMPA receptor antagonist, 2,3-dioxo-6-nitro-1,2,3,4 tetrahydrobenzo[f]quinoxaline-7-sulfonamide (NBQX) disodium salt, or sterile saline vehicle (10 mg/kg, i.p.) 30 min prior to Day 2 of FST [29]. After 24 h, vehicle–vehicle and FGL_S–vehicle animals were tested in the social interaction test. The FGL_S-NBQX animals were served as stimulus rats in the social interaction tests. Briefly, animals were placed simultaneously into an open arena and the time spent interacting (e.g., grooming, sniffing, following, and crawling) by the experimental rats were recorded over 5 min [30].

For chronic administration of plannexin, rats were injected every 48 h between 0800 and 0900 h and then tested for locomotion on Day 13, EPM on Day 14, and FST on Days 15 and 16. The rationale for treating rats every other day with plannexin was based on the evidence of persistent activation of FGF signaling pathways by other NCAM peptide mimetics [15]. For chronic administration of FGL_L, rats were injected every 24 h for 16 days between 0800 and 0900 h and then tested as above. For FGL_L and plannexin, the Day 2 FST results were used in chronic studies. For chronic administration of FGL_S, 14 day osmotic minipumps at a flow rate of 5 µl/h for 12 mg/day were subcutaneously implanted without equilibration to allow an extra day of dosing. These rats were then tested for locomotor behavior on Day 13, EPM on Day 14, and FST on Day 15. For FGL_S, we only

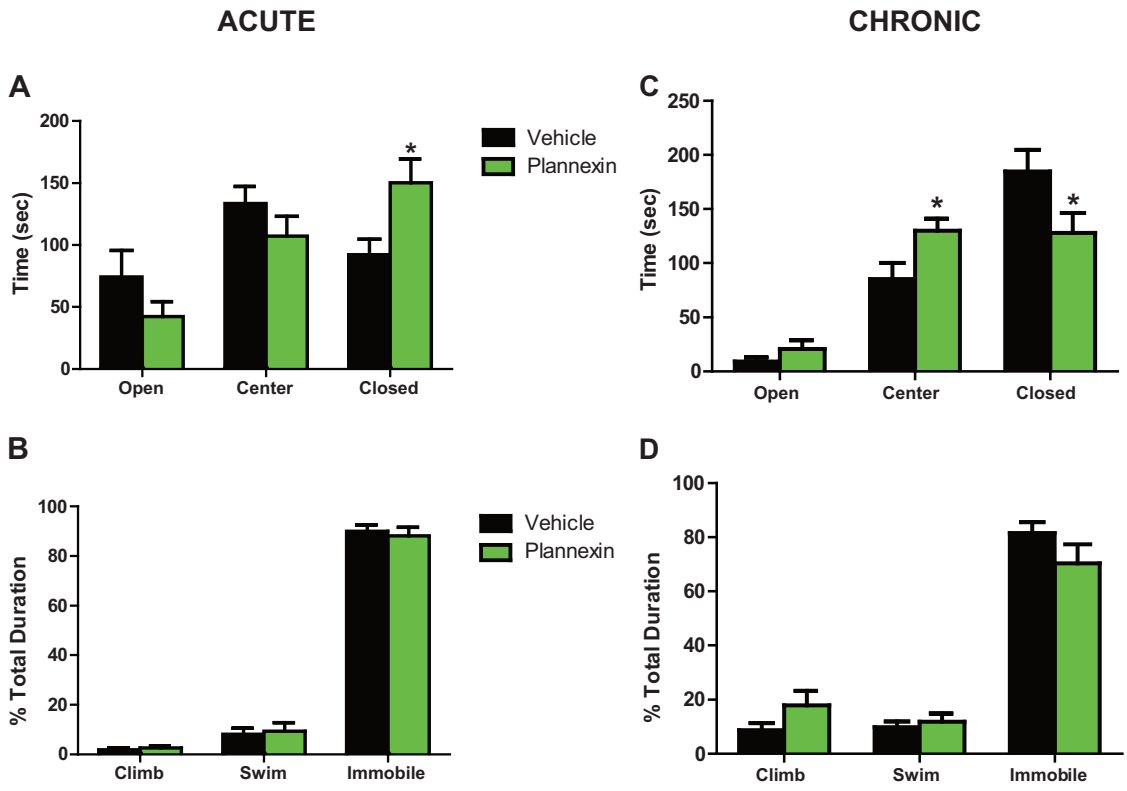

Fig. 1 Behavioral effects of plannexin in rats. **a** Acutely administered plannexin increased anxiety-like behavior on the EPM (*$p < 0.05$). **b** Acutely administered plannexin did not alter the behavior in FST. **c** Chronically administered plannexin had anxiolytic effects on EPM (*$p < 0.05$). **d** Chronically administered plannexin did not alter the behavior in FST

performed Day 1 of FST, as this FST was performed 24 h after the last minipump dose. This approach has previously been validated for chronic dosing [31]. In all cases, behavioral testing was conducted between 0900 and 1200 h. All behavioral measures are described in detail in the Supplementary Methods.

FGL_S in blood plasma and CSF

To extend the behavioral results, blood and cisternal CSF samples were collected from each of the sic randomly selected and experimentally naive rats in three conditions at weekly intervals: (i) undisturbed baseline, (ii) 30 min after a single s.c. injection of 100 mg/kg FGL_S, and (iii) 30 min after the last of the five s.c. injections administered hourly with 100 mg/kg FGL_S. We administered single and multiple injections to compare these two conditions. From another six randomly selected and experimentally naive rats, blood and cisternal CSF samples were collected in the three conditions described above, but at 10 mg/kg FGL_S. From these same six rats, we also collected an additional sample 30 min after a 10 mg/kg FGL_S injection on Day 2 that was preceded 24 h earlier by five 10 mg/kg FGL_S injections administered at hourly intervals on Day 1 for preloading. The single injection and preloading conditions were performed to time-match the rat behavioral testing described above. The cisternal CSF samples were collected under anesthesia and immediately thereafter a blood sample was acquired from the saphenous vein (Supplementary Methods) and placed into EDTA tubes that contained aprotonin (Sigma, 5 µL/mL). Samples were centrifuged at $2300 \times g$ for 10 min at 4 °C and blood plasma fractions were aliquoted for storage at −80 °C. All blood plasma and CSF samples were shipped on dry ice to QPS (https://www.qps.com/) for determinations by liquid chromatography mass spectrometry with an FGL_S detection limit of 50 ng/ml.

Six monkeys not previously treated with NCAM peptide mimetics were each initially administered with a single subcutaneous (s.c.) injection of 10 mg/kg FGL_S diluted in 0.9% saline vehicle. Seven days later, the same six monkeys were administered a single s.c. injection of FGL_S at 100 mg/kg. From each of two monkeys at each dose, cisternal CSF samples were collected under anesthesia at 1, 2, or 4 h after injection of FGL_S in a pseudo-randomized order (Supplementary Methods). Immediately after each CSF sample collection, a blood sample was acquired by femoral venipuncture. The blood and CSF samples were processed for FGL_S determinations as described above.

Statistical analysis

The behavior of the rats was evaluated with analysis of variances (ANOVAs) using SPSS software, except that locomotion and social interactions were evaluated by Student's *t*-tests. Treatment was considered to be a between-subjects factor, and behavioral measures for EPM and FST were within-subjects repeated measures. Whenever there was a non-significant trend for a treatment-by-test measure interaction, Student's *t*-tests were used to evaluate differences between the treatment conditions within each test measure. Fisher's LSD tests were used for post hoc pairwise comparisons, and all test statistics were evaluated with two-tailed probabilities at $p < 0.05$. FGLs in blood plasma and CSF were evaluated with ANOVA using SYSTAT software. For rats, dose was considered to be a between-subjects factor, and the administration condition (single, repeated, preloading) was a within-subjects repeated measure. For monkeys, the sample collection time interval was considered a between-subjects factor and dose was a within-subjects repeated measure. Correlations between blood plasma and CSF levels of FGLs were assessed with Pearson correlation coefficients.

RESULTS

Behavioral effects of plannexin

Acute treatment with plannexin increased anxiety-like behavior on the EPM (Fig. 1a), but did not have antidepressant effects in FST

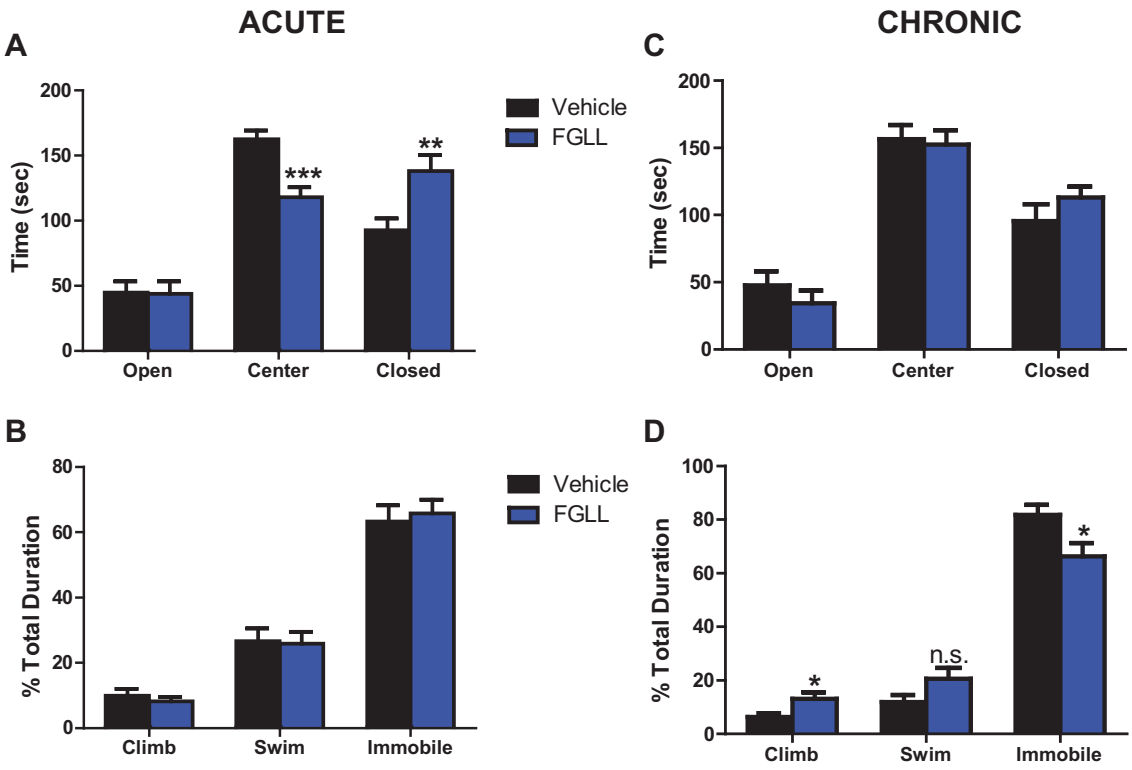

Fig. 2 The behavioral effects of FGL_L in rats. **a** Acutely administered FGL_L increased anxiety-like behavior on the EPM (***$p < 0.000$, **$p < 0.01$). **b** Acutely administered FGL_L did not alter the behavior in FST. **c** Chronically administered FGL_L did not alter the behavior on the EPM. **d** Chronically administered FGL_L had antidepressant effects in FST (*$p < 0.05$)

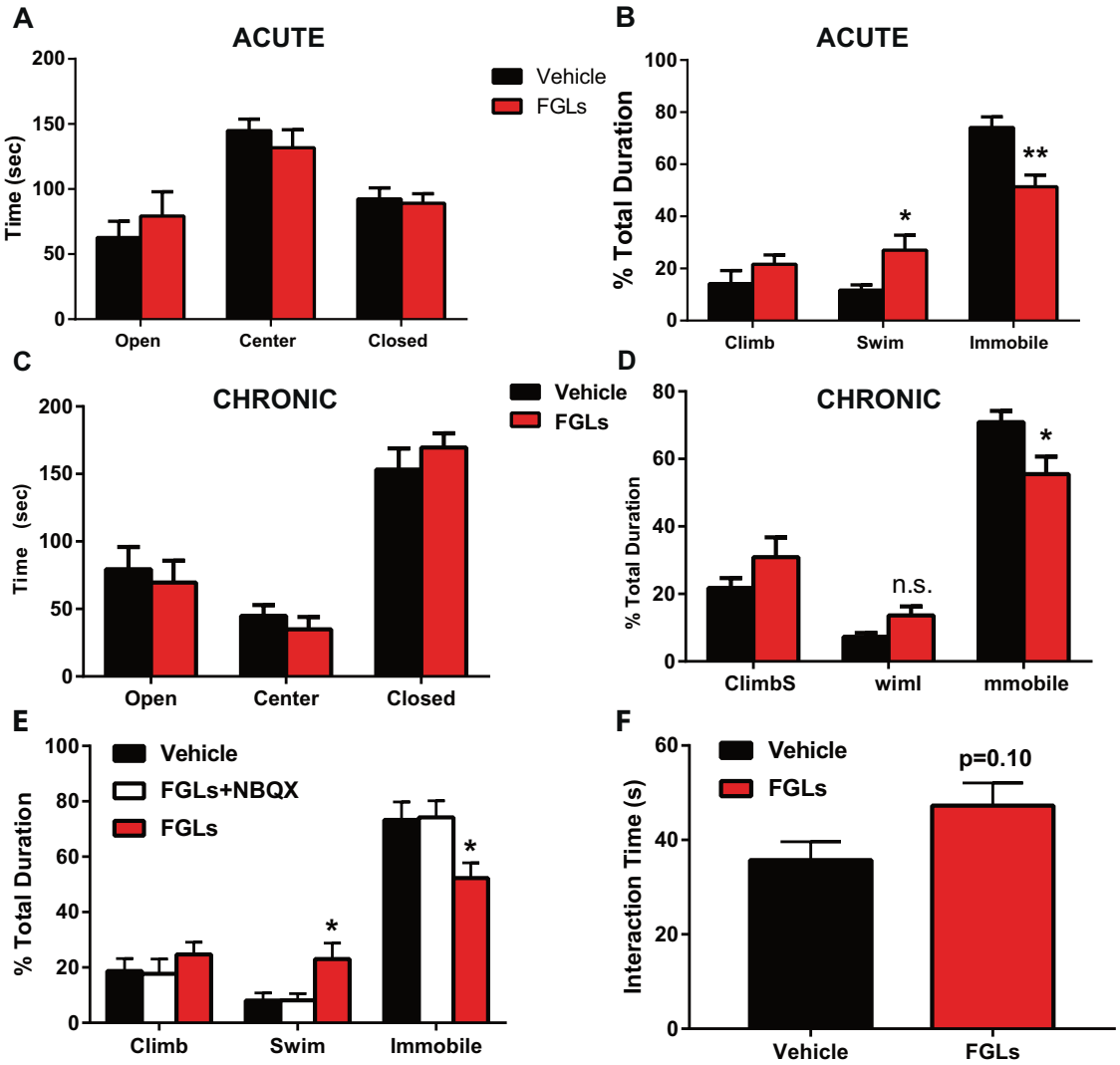

Fig. 3 The behavioral effects of FGL_S in rats. **a** Acutely administered FGL_S did not alter the behavior on the EPM. **b** Acutely administered FGL_S had antidepressant effects in FST (*$p < 0.05$, **$p < 0.005$). **c** Chronically administered FGL_S did not alter the behavior on the EPM. **d** Chronically administered FGL_S had antidepressant effects in FST (*$p < 0.05$). **e** Replication of the FGL_S acute antidepressant effect and blockade by NBQX. FGL_S decreased the immobility in the FST, compared to vehicle and FGL_S + NBQX (*$p < 0.05$). **f** FGL_S was still slightly antidepressant in the social interaction test 24 h later (*$p < 0.05$)

(Fig. 1b). For EPM, there was a main effect for test measure ($F(1,20) = 8.55$, $p = 0.007$) with no main effect of treatment, but the treatment-by-test measure interaction was significant ($F(1,20) = 4.49$, $p = 0.047$). The post hoc comparisons confirmed that acute treatment with plannexin increased the time spent in the closed arms ($p < 0.05$), as depicted in Fig. 1a. For FST (Fig. 1b), acute treatment with plannexin had a main effect for test measure ($F(1,20) = 1513.14$, $p < 0.001$), but no main effect for treatment nor a treatment-by-test measure interaction.

Chronic treatment with plannexin did not affect the locomotor activity (data not shown), but had anxiolytic effects on the EPM (Fig. 1c) without altering the behavior on FST (Fig. 1d). For EPM, treatment main effects were not discerned, but the treatment-by-test measure interaction was significant ($F(1,22) = 5.23$, $p = 0.032$). The post hoc comparisons confirmed that the time spent in the closed arms decreased ($p < 0.05$) and time spent in the center increased ($p < 0.05$), as depicted in Fig. 1c. For FST, there was a main effect for test measure ($F(1,22) = 93.99$, $p = 0.032$), but no main effect of treatment nor a treatment-by-test measure interaction (Fig. 1d). Thus, plannexin was acutely anxiogenic, chronically anxiolytic, and did not have antidepressant effects.

Behavioral effects of FGL_L

Acute treatment with FGL_L increased anxiety-like behavior on the EPM (Fig. 2a), but did not have antidepressant effects in FST (Fig. 2b). For EPM, there was a main effect for test measure ($F(1,22) = 64.03$, $p < 0.001$) and no main effect for treatment, but the treatment-by-test measure interaction was significant ($F(1,22) = 19.45$, $p < 0.001$). The post hoc comparisons confirmed that acute treatment with FGL_L increased the time spent in the closed arms ($p < 0.01$) and decreased the time spent in the center ($p < 0.001$), as depicted in Fig. 2a. In FST, there was a main effect for test measure ($F(1,20) = 1513.14$, $p < 0.001$), but no treatment main effect nor a treatment-by-test measure interaction (Fig. 2b).

Chronic treatment with FGL_L did not affect the locomotor activity (data not shown) nor behavior on the EPM (Fig. 2c), but had antidepressant effects in FST (Fig. 2d). For EPM, there was a significant main effect for test measure ($F(1,22) = 53.41$, $p < 0.001$), but no treatment main effect nor a treatment-by-test measure interaction (Fig. 2c). For FST (Fig. 2d), there was a main effect for test measure ($F(1,22) = 232.55$, $p = 0.000$) and no main effect of treatment, but the treatment-by-test measure interaction was significant ($F(1,22) = 6.91$, $p = 0.015$). The post hoc comparisons

confirmed a decrease in immobility ($p < 0.05$), an increase in climbing ($p < 0.05$), and a non-significant increasing trend for swimming ($p = 0.09$), as depicted in Fig. 2d. Thus, acute treatment with FGL_L was anxiogenic and chronic treatment with FGL_L was antidepressant.

Behavioral effects of FGL_S

Acute treatment with FGL_S did not alter the behavior on the EPM (Fig. 3a), but had antidepressant effects in FST (Fig. 3b). For EPM, there was a main effect of test measure ($F(1,20) = 21.88$, $p < 0.001$), but no main effect of treatment nor a treatment-by-test measure interaction. For FST, there was a main effect of test measure ($F(1,14) = 20.60$, $p = 0.000$) and no main effect of treatment. However, there was a treatment-by-test measure interaction for FST ($F(1,14) = 3.32$, $p = 0.026$). The post hoc comparisons confirmed that acute treatment with FGL_S decreased immobility ($p < 0.005$) and increased swimming ($p < 0.05$), as depicted in Fig. 3b.

Chronic treatment with FGL_S did not alter the locomotor activity (data not shown) nor the behavior on the EPM (Fig. 3c), but had antidepressant effects in FST (Fig. 3d). For EPM, there was a main effect for test measure ($F(1,12) = 83.17$, $p < 0.001$), but no main effect for the treatment or treatment-by-test measure interaction (Fig. 3c). For FST (Fig. 3d), there was a main effect for test measure ($F(1,12) = 243.34$, $p = 0.000$) and no main effect of treatment, but there was a non-significant trend for a treatment-by-test measure interaction ($F(1,12) = 4.53$, $p = 0.055$). Student's t-tests confirmed a decrease in immobility ($t(12) = 2.52$, $p < 0.05$) and a non-significant increasing trend in swimming ($t(12) = 2.13$, $p = 0.06$). Thus, acute and chronic FGL_S had antidepressant effects.

NBQX blocked the acute antidepressant effect of FGL_S, as shown in Fig. 3e. There was a main effect of test measure ($F(1,17) = 59.85$, $p = 0.000$), but no main effect of treatment. However, there was a treatment-by-test measure interaction for FST ($F(2,17) = 3.96$, $p < 0.05$). The post hoc comparisons confirmed increased swimming for FGL_S, compared to vehicle ($p < 0.05$) and FGL_S, compared to FGL_S-NBQX ($p < 0.05$). The post hoc comparisons also confirmed decreased immobility for FGL_S, compared to vehicle ($p < 0.05$) and FGL_S, compared to FGL_S-NBQX ($p < 0.05$). These results suggest that the rapid antidepressant effect of FGL_S may be AMPA receptor-dependent. Twenty-four hours following the last dose of FGL_S, there was still a non-significant trend for FGL_S to have an antidepressant effect on the social interaction test. As shown in Fig. 3f, FGL_S animals spent more time interacting, compared to vehicle ($t(12) = -1.76$, $p = 0.10$).

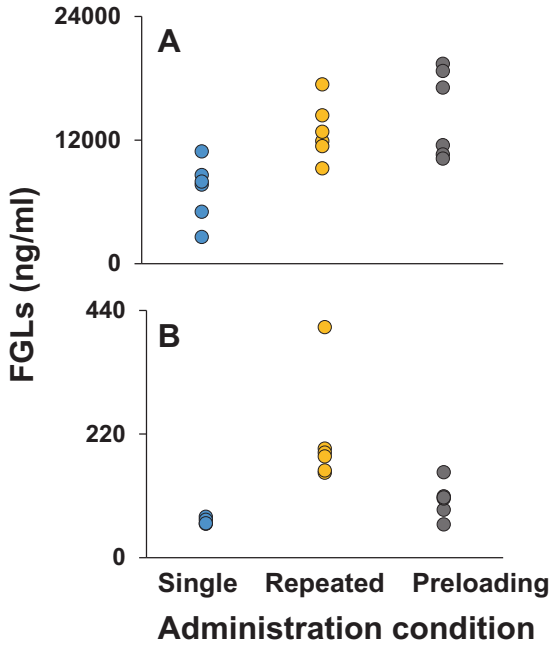

Fig. 4 Preloading amplifies FGLs in blood plasma and CSF. FGL_S levels in **a** blood plasma and **b** CSF collected from rats in three conditions: (i) 30 min after a single-peripheral injection of 10 mg/kg FGL_S, (ii) 30 min after the last of the five repeated peripheral injections of 10 mg/kg FGL_S administered at hourly intervals, or (iii) 30 min after a single-peripheral injection of 10 mg/kg FGL_S administered on Day 2 preceded 24 h earlier by five 10 mg/kg peripheral injections of FGL_S repeated at hourly intervals on Day 1 for preloading. Measurements from individual rats are plotted. Note different scales for y axis in **a** and **b**

FGL$_S$ in blood plasma and CSF

Since NBQX blocked the acute behavioral effect of FGL_S, we sought to determine the FGL_S pharmacokinetics. Blood plasma and CSF samples at baseline in the absence of FGL_S administration were consistently below the assay detection limit, and these results are not presented. Results from the CSF samples that contained visible traces of blood contamination and the corresponding blood plasma samples are also excluded from analysis.

Preloading with five repeated injections of 10 mg/kg FGL_S on Day 1, followed by a single injection of 10 mg/kg FGL_S 24 h later on Day 2 amplified the amounts of FGL_S discerned in rat blood plasma (Fig. 4a) and CSF (Fig. 4b). Significant administration condition effects were discerned for FGL_S in blood plasma ($F(2,10) = 9.3$, $p = 0.005$) and CSF ($F(2,6) = 19.8$, $p = 0.002$). Different degrees of freedom for these results reflect exclusion of CSF samples contaminated with blood (Table S1). The post hoc pairwise comparisons confirmed that FGL_S in blood plasma was significantly greater after preloading ($p < 0.0175$) or repeated administrations ($p < 0.0007$), compared to the single-

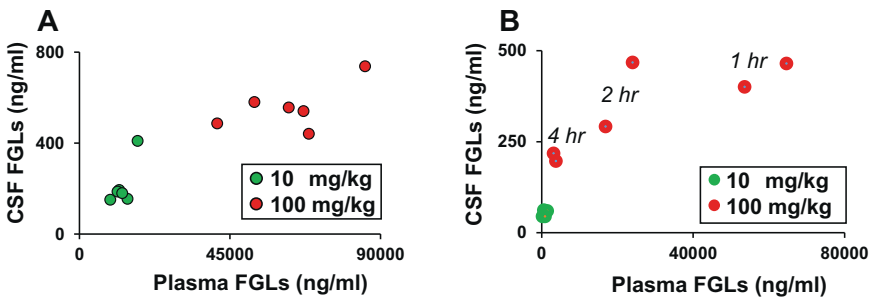

Fig. 5 FGL_S in blood plasma correlates with CSF. **a** Rats were treated with repeated peripheral injections of 10 or 100 mg/kg FGL_S and all samples were collected 30 min after the last repeated FGL_S injection. **b** Monkeys were treated with single-peripheral injections of 10 or 100 mg/kg FGL_S. Time labels represent the interval between FGL_S administration and sample collection

administration condition. Repeated administrations and the preloading condition did not differ for FGL_S in blood plasma (Fig. 4a). FGL_S in CSF was greater after repeated administration compared to single ($p = 0.0014$) or preloading ($p = 0.036$) conditions, but did not differ significantly between preloading and single administration (Fig. 4b).

For FGL_S in rat CSF (Figure S1A), the main effects were discerned for dose ($F(1,6) = 25.7$, $p < 0.0023$) and single versus repeated administration ($F(1,6) = 10.5$, $p < 0.017$). The main effects of dose ($F(1,10) = 221.7$, $p < 0.001$), but not single versus repeated administration, were also detected for FGL_S in rat blood plasma (Figure S1B). After repeated administration, blood plasma FGL_S correlated with CSF FGL_S in rats (Fig. 5a) when both 10 and 100 mg/kg doses were analyzed together ($r = 0.91$, $p < 0.001$). The correlations were comparable for each dose analyzed separately, but smaller samples hampered the attainment of statistical significance (10 mg/kg dose ($r = 0.79$, $p = 0.06$); 100 mg/kg dose ($r = 0.60$, $p = 0.21$)). Similar correlations were also discerned after single administration of FGL_S when data from both doses were analyzed together (($r = 0.72$, $p = 0.043$); data not presented). Doses could not be analyzed separately because of practical considerations for the single-administration condition, where four CSF samples were contaminated with blood (Table S1).

FGL_S in monkey blood plasma (Figure S2A, B) demonstrated the main effects for dose ($F(1,3) = 132.6$, $p = 0.0014$) and sample collection time interval ($F(2,3) = 65.6$, $p = 0.003$), and a dose-by-interval interaction ($F(2,3) = 48.7$, $p = 0.005$). Similar results were obtained for FGL_S in monkey CSF (Figure S2C, D), as confirmed by the main effects for dose ($F(1,2) = 386.2$, $p = 0.003$) and sample collection time interval ($F(2,2) = 27.9$, $p = 0.035$), and a dose-by-interval interaction ($F(2,2) = 30.4$, $p = 0.032$). Blood plasma FGL_S were correlated with CSF FGL_S for all measures of monkeys (Fig. 5b), excluding one CSF sample that was contaminated with blood ($r = 0.85$, $p = 0.001$). A nearly identical correlation for FGL_S in CSF and blood plasma was also discerned in monkeys at the 100 mg/kg FGL_S dose ($r = 0.81$, $p = 0.051$), but the correlation was not significant for 10 mg/kg FGL_S because of practical limitations. At the 10 mg/kg FGL_S dose, one of the six CSF samples was contaminated with blood and three of the five remaining CSF samples fell below the assay detection limit. At the 100 mg/kg FGL_S dose, CSF-to-blood plasma ratios in monkeys at 1, 2, and 4 h postadministration increased significantly over time ($F(2,3) = 32.4$, $p = 0.009$). These results reflect a decline over time in blood plasma FGL_S ($F(2,3) = 56.3$, $p = 0.004$), whereas CSF FGL_S did not change over the same time period ($p = 0.119$, Table S2). In general, CSF-to-blood plasma ratios of FGLs in monkeys (Table S2) were comparable to those in rats (Table S1).

DISCUSSION

This is the first study to demonstrate that NCAM peptide mimetics modulate emotionality in rats. Plannexin acutely increased and chronically decreased anxiety-like behavior, but did not have antidepressant effects. FGL_L had acute anxiogenic effects and chronic antidepressant effects, whereas FGL_S had acute and chronic antidepressant effects without modulating the anxiety-like behavior in rats. Acute antidepressant effects of FGLs were blocked by the AMPA receptor antagonist NBQX. CSF levels of FGLs correlated with blood plasma levels in rats and non-human primates, and the CSF-to-blood ratios of FGL_S were comparable in both species. Taken together, these results indicate that NCAM peptide mimetics penetrate the brain and support the suggestion that FGL_S may be a candidate for further development as a novel treatment for major depressive disorder in humans.

FGL_S is a truncated version of FGL_L, which is known to be safe and well-tolerated in humans with a plasma t_{max} of 1 h [32]. We likewise observed no evidence of toxicity for FGLs in rats or primates. Since the pharmacokinetics of FGL_L for humans are known, and FGL_L may not be ideal for peripheral administration [32], we assessed FGL_S pharmacokinetics in primates and rats. The results indicate that FGL_S pharmacokinetics are similar to those reported elsewhere for FGL_L [32]. Repeated administrations of FGL_S yielded higher levels of FGL_S in CSF and blood plasma, compared to single administration in rats. Similarly, a higher dose of FGL_S yielded higher levels of FGL_S in both CSF and blood plasma, compared to a lower dose in primates and rats. The CSF-to-blood plasma ratios of FGL_S in primates increased over time from administration to sample collection. These results possibly reflect a longer half-life of FGL_S in CSF compared to blood plasma, but additional studies are needed to assess the brain accumulation of FGL_S. Interestingly, the preloading administration of FGL_S in rats increased FGL_S in plasma but not CSF, which suggests that observed antidepressant effects on the behavior are not due to prolonged accumulation of FGL_S in the brain.

In primates, the CSF-to-blood plasma ratios of FGL_S measured 4 h after administration are comparable to the low end of the range reported for known antidepressant medications in humans. For example, imipramine in CSF is approximately 10% of blood plasma levels in humans with major depressive disorder [33]. The calculated CSF-to-blood plasma ratio for venlafaxine is 0.74 [34], and the ratio for mirtazapine (bound and unbound faction) varies from 0.08 to 0.48 with a mean ± SD of 0.16 ± 0.11 [35]. For nortriptyline, the ratio is 0.091 ± 0.025 [36]. Finally, citalopram in CSF from treatment responders and non-responders is approximately 50% of blood plasma levels [37].

Study design limitations should be considered. Since we were limited by amounts of the peptide available, only two different behavioral tests were performed and different administration procedures were used for chronic studies. This hampers direct comparisons between different peptide mimetics. Additionally, higher doses or different routes of administration might yield more robust results. Further research is needed to determine whether different behavioral effects are due to differences in dosing or signaling mechanisms. Although differences in signaling between these peptide mimetics are unknown, NCAM and peptide mimetics differ on other measures [38]. NCAM is involved in neuronal proliferation, e.g., whereas this has yet to be demonstrated for other mimetics, which are involved in outgrowth, survival, and differentiation [13, 38, 39]. The peptide mimetics may also have different responses at different concentrations, or different binding kinetics for FGF receptors, which could influence the behavior. Finally, we matched the monkey and rat doses to help determine a dose for humans, based on the rat behavior and the monkey pharmacokinetic data.

Previously, it was shown that FGL_L can enhance synaptic transmission, an effect mediated by trafficking of AMPA receptors to the synapse [15]. Since FGFR1 is located on the glutamatergic cells [40], it is possible that activation of AMPA receptors may be the mechanism of action for rapid NCAM peptide effects [41]. Since FGL_S was the only peptide mimetic to have acute antidepressant effects, we assessed AMPA modulation of acute FGL_S effects. Our finding that FGLs has rapid effects on the emotionality that may be mediated by AMPA receptors has important implications for accelerating the treatments of affective disorders. It should also be noted that FGL_L is capable of enhancing both positive and negative effects, such as spatial learning and fear conditioning [18]. This finding may partially explain why we observed an acute anxiogenic effect coupled with chronic antidepressant effect. It is possible that antidepressant effects were not observed with plannexin because we did not use a sufficient dose for acute studies or frequent enough administration in chronic studies. FGL_S may be

most useful for therapeutic antidepressant applications because it has behavioral effects after acute and chronic administration. However, it is possible that chronic antidepressant effects of FGL_S were not more robust compared to acute treatment, because the behavioral test was conducted 24 h after the last FGLs dose.

In conclusion, the peripherally administered NCAM peptides penetrate the brain and modulate emotionality. These peptides are top candidates in the search for novel treatments of neurodegenerative disorders, such as Alzheimer's disease [15, 42]. The current study suggests that NCAM peptides not only function as cognitive enhancers, but may be novel therapeutics for the treatment of depressive disorders. FGL_S in particular may be a novel antidepressant. In spite of its relatively short half-life, it has a pharmacokinetic profile similar to some other antidepressant medications, and its accumulation in CSF makes it an attractive candidate for further investigation.

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ADDITIONAL INFORMATION

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