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Validity of 5d-RFSS as a model of depression

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Running title: Validity of 5d-RFSS as a model of depression

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SIGNIFICANCE STATEMENT

The development of valid animal models to model human depression has been a major challenge. One protocol that has been widely used for its presumptive effects to cause depression is 5 consecutive days of forced-swim stress (5d-RFSS) in mice. 5d-RFSS increases floating behavior during consecutive sessions, but whether this is depressive-like behavior or an adaptive response underlying survival is not clear. We subjected two mouse strains (C57BL/6J, BALB/cJ) to 5d-RFSS followed by a battery of reward-related, homeostatic, and behavioral tests. 5d-RFSS increased floating behavior over time but importantly, did not induce emotional, homeostatic, or psychomotor symptoms. These findings suggest that 5d-RFSS has no construct or face validity to model human depression in two mouse strains commonly used in neuropsychiatric research.

ABSTRACT

The development of animal models with construct, face, and predictive validity to accurately model human depression has been a major challenge. One proposed rodent model is repeated forced swim stress on 5 consecutive days (5d-RFSS), which progressively increases floating during individual swim sessions. The onset and persistence of this floating behavior has been anthropomorphically characterized as a measure of depression. This interpretation has been under debate because a progressive increase in floating over time may reflect an adaptive learned behavioral response promoting survival, and not depression (Molendijk and de Kloet, 2015). To assess construct and face validity, we applied 5d-RFSS to C57BL/6J and BALB/cJ mice, two mouse strains commonly used in neuropsychiatric research, and measured a combination of emotional, homeostatic, and psychomotor symptoms indicative of a depressive-like state. We also compared the efficacy of 5d-RFSS and chronic social defeat stress (CSDS), a validated depression model, to induce a depressive-like state in C57BL/6J mice. In both strains, 5d-RFSS progressively increased floating behavior that persisted for at least 4 weeks. 5d-RFSS did not alter sucrose preference, body weight, appetite, locomotor activity, anxiety-like behavior, or immobility behavior during a tail-suspension test compared to non-stressed controls. In contrast, CSDS altered several of these parameters, suggesting a depressive-like state. Finally, predictive validity was assessed using voluntary wheel running (VWR), a known antidepressant intervention. Four weeks of VWR after 5d-RFSS normalized floating behavior towards non-stressed levels. These observations suggest that 5d-RFSS

has no construct or face validity, but might have predictive validity to model human depression.

INTRODUCTION

Major depressive disorder affects about one in six individuals during their lifetime and has an enormous social and financial impact on modern society (Greenberg et al., 2003; Kessler et al., 2005). This psychiatric disorder is diagnosed based on symptoms with considerable heterogeneity and without known highly penetrant genetic causes (Krishnan and Nestler, 2008). There is a general need for animal models to study the pathophysiology of depression and identify therapeutic interventions. However, the development of animal models of depression with construct, face, and predictive validity has been a major challenge (Nestler and Hyman, 2010; Belzung and Lemoine, 2011). Nevertheless, several ethologically valid rodent models of depression have been developed and validated, including chronic unpredictable stress (CUS) and chronic social defeat stress (CSDS) (Willner, 2005; Nestler and Hyman, 2010). Despite the validity of these animal models, lack of ethical approval or other limitations have stimulated investigators to keep exploring additional rodent paradigms that model human depression.

One proposed animal model is the 5-day repeated forced swim stress (5d-RFSS) paradigm, during which mice are forced to swim in a beaker filled with water for 10 minutes during 5 consecutive days. Several studies have reported a progressive increase in floating during consecutive forced swimming sessions that was maintained for at least 4 weeks, and this behavior is commonly interpreted as the

onset and persistence of a depressive-like state (Stone and Lin, 2011; Sun et al., 2011; Serchov et al., 2015). This interpretation has been debated, as increased floating behavior during repeated forced swimming sessions may rather reflect an adaptive learned behavioral response underlying survival (Molendijk and de Kloet, 2015). It has been suggested that to provide more definitive evidence on the presence of a depressive-like state, a combination of emotional symptoms (anhedonia), homeostatic symptoms (sleep, appetite, body weight), psychomotor symptoms (locomotor activity, immobility- and anxiety-like behavior), or direct assessment of the brain's reward circuitry should be measured (Nestler and Hyman, 2010). Emotional, homeostatic, and psychomotor symptoms are hallmarks of human depression and important parameters that can be measured objectively in rodents (Nestler and Hyman, 2010).

5d-RFSS in mice was recently shown to decrease sucrose preference immediately following 5d-RFSS, suggesting anhedonia, and this emotional symptom persisted for at least 4 weeks (Serchov et al., 2015). Maintenance of anhedonia allows for a time window to test and study therapeutic interventions. Here we determined the validity of 5d-RFSS to model human depression using a battery of tests that assessed emotional, homeostatic, and psychomotor symptoms. In other words, does 5d-RFSS induce a depressive-like state in mice? For this purpose, we applied a similar 5d-RFSS protocol as used by Serchov *et al*. to two inbred strains commonly used in neuropsychiatric research: young-adult male stress-resilient C57BL/6J mice and stress-susceptible BALB/cJ mice (Pothion et al., 2004; Farley et al., 2010; Razzoli et al., 2011; Serchov et al., 2015). To thoroughly assess the onset of a depressive-

like state we measured sucrose preference, body weight, food intake, locomotor activity, anxiety-like behavior, and immobility behavior during a tail-suspension test (TST) before and after 5d-RFSS. We also compared the efficacy of 5d-RFSS and chronic social defeat stress (CSDS), a validated model of depression (Kudryavtseva et al., 1991; Krishnan et al., 2007; Nestler and Hyman, 2010; Golden et al., 2011), to induce a depressive-like state in C57BL/6J mice.

Predictive validity relies on the observation that treatment modalities effective in reversing depression in humans should reverse the changes observed in an animal model of depression (McKinney and Bunney, 1969; Belzung and Lemoine, 2011). In line with this, several studies have demonstrated that known antidepressant drugs (imipramine, ketamine, fluoxetine, tranylcypromine) or treatments (repetitive transcranial magnetic stimulation; rTMS) decrease the persistence of increased floating behavior induced by 5d-RFSS (Stone and Lin, 2011; Sun et al., 2011; Serchov et al., 2015), thus suggesting predictive validity. Therefore we also investigated if voluntary wheel running (VWR), a behavioral intervention that mimics human exercise training and has antidepressant action (North et al., 1990; Brene et al., 2007), modulates the persistence of increased floating behavior induced by 5d-RFSS.

MATERIAL AND METHODS

Animals. Experiments were conducted in accordance with the Joslin Diabetes Center Institutional Animal Care and Use Committee. Ten-week old male C57BL/6J (https://www.jax.org/strain/000664) and BALB/cJ

(https://www.jax.org/strain/000651) mice were group-housed and habituated to the Joslin Diabetes Center animal facility for at least 2 days before the onset of experimental treatments. This timeframe was sufficient for body weights to return to stable pre-travel levels (data not shown). Mice were maintained at 23-25°C on a 12-h light/dark cycle (lights on from 06:30) with *ad libitum* access pelleted chow diet (9F 5020 Lab Diet, 23% protein, 55% carbohydrate, and 22% fat, 3.56 kcal/g AFE, PharmaServ Inc.) and water, unless noted otherwise. All experimental groups were body weight-matched at the onset of the experiments. Body weight and available food were measured at indicated time points. All behavioral assessments, except for the two-bottle sucrose preference tests, were performed in an experimental room, and mice were acclimatized to the experimental room for 2 hr before start of behavioral studies.

Five-day repeated forced-swim stress (5d-RFSS). All experimental mice were single-housed from the start of the 5d-RFSS paradigm (**Figure 1)**. We used a recently described 5d-RFSS protocol (Serchov et al., 2015), with one modification: addition of an extra sucrose preference test (i.e. SPT3) during *days* 22 - 25. Stressed mice were forced to swim in an open cylindrical container (diameter, 12 cm; height, 28 cm) containing 19 cm of water (25°C ± 1°C) on 5 consecutive days (*days* 1 – 5; induction phase) and on *day* 37 (test phase; see Figure 1). Individual tests lasting 10 minutes were monitored from the top and scored automatically using the ANY-maze software (version 4.98; Stoelting Co., IL). Immobile behavior sensitivity was set at 65% and the mouse needed to be immobile for 500 msec to initiate scoring of immobility. In general, each mouse was judged to be immobile when it ceased

187 struggling and remained floating motionless in the water, making only movements
188 necessary to keep its head above the water surface (Duman et al., 2008; Cunha et
189 al., 2013). Water was changed between each test. Non-stressed controls were
190 physically handled briefly by the investigator, transferred to a transport cup for 10
191 minutes, and returned to their home cage.
192
193 *Two-bottle sucrose preference test (SPT)*. Mice were given free access to two drinking
194 pipettes in their home cage, one containing 1% (C57BL6/J) or 3% (BALB/cJ) sucrose
195 solution and the other containing water. Fluid consumption was measured in the
196 early afternoon and the position of the pipettes was interchanged daily to prevent
197 a place preference. Sucrose preference is calculated as the percentage of the
198 amount of sucrose solution consumed over total fluid consumption ([sucrose
199 solution intake/total fluid intake] $\times$ 100) and was averaged over all three days of
200 testing.
201
202 *Tail-suspension test (TST)*. Mice were suspended by adhesive tape placed
203 approximately 1 cm from the tip of their tail that was taped to a horizontal holder
204 so that the mouse was suspended 20 cm above a horizontal surface. The mouse-
205 tail was passed through a small plastic cylinder prior to suspension to prevent tail
206 climbing behavior. Individual tests lasting 6 minutes were monitored and scored
207 automatically using the ANY-maze software (version 4.98; Stoelting Co., IL).
208 Immobile behavior sensitivity was set at 70% and the mouse needed to be
209 immobile for 1 sec to initiate scoring of immobility. After the TST, mice were
210 returned to their respective home cages.

211

212 *Open-field test (OFT).* During the light phase mice were placed in a rectangular
213 open field monitoring set-up (59 x 29 cm; walls 30 cm; non-reflecting grey PVC) and
214 locomotor activity was monitored from the top for 6 minutes and scored
215 automatically using the ANY-maze software (version 4.98; Stoelting Co., IL). The
216 center zone was defined as a 20 x 10 cm zone designated in the middle of the open
217 field. Feces produced during the OFT were counted manually. Mice were
218 immediately returned to their respective home cages after the OFT.

219

220 *Chronic social defeat stress (CSDS) and behavioral evaluations*. CSDS was performed
221 as described with a few modifications (Krishnan et al., 2007; Vialou et al., 2010;
222 Golden et al., 2011). In short, all mice were tested during SPT1 (*day* -3 till -0) before
223 the start of 10 consecutive days of CSDS. During each defeat episode,
224 experimental C57BL/6J mice (intruder) were allowed to interact for 10 minutes with
225 an unfamiliar CD1 aggressor (resident), during which they displayed subordinate
226 posturing. Intruders then spent the remainder of each 24h period in the
227 aggressor's cage, separated from the aggressor by a custom-made perforated
228 aluminum partition (sensory housing). Undefeated C57BL/6J controls were housed
229 by pair, one on each side of a perforated aluminum partition (no physical
230 interaction), and were handled daily. For the social interaction test on day 11, time
231 spent in the interaction zone during the first (target absent) and second (social
232 target present) 2.5 minute trials were automatically scored using ANY-maze
233 software (version 4.98; Stoelting Co., IL). Unfamiliar CD1 mice that did not partake

in the defeat episodes were used as social targets. Following the social interaction test, all mice were tested during an OFT (*day* 12) and during SPT2 (*day* 12 till 15).

Voluntary Wheel Running (VWR). Mice were housed without a running wheel (sedentary; SED) or given voluntary access to an active running wheel (VWR; 24 cm diameter; Nalgene, Rochester, NY) for 4 weeks. Wheel revolutions were measured daily using odometers.

Statistical analysis. Data are displayed as mean ± SEM. For all experiments, single comparisons between means were analyzed by unpaired *t*-test, whereas SPT1 and SPT2 were compared using a paired *t*-test. Multiple comparisons between means were analyzed using one- or two-way analysis of variance (ANOVA), with repeated measures where applicable. Daily VWR distances were analyzed using one-way ANOVA, with repeated measures. If appropriate, *post hoc* analyses were made using a Tukey HSD test, with $p < 0.05$ accepted as statistically different.

RESULTS

Effects of 5d-RFSS on depressive-like behavior in C57BL/6J mice. We first tested the hypothesis that 5d-RFSS induces a depressive-like state in C57BL/6J mice using a previously described 5d-RFSS protocol (Serchov et al., 2015). In this paradigm, single-housed mice are stressed by being forced to swim on 5 consecutive days (induction phase; 5d-RFSS) and depressive-like behavior was assessed using a battery of behavioral tests before and after the induction phase (**Figure 1**). Non-stressed C57BL/6J controls were handled daily during the induction phase but

were not forced to swim. C57BL/6J mice that underwent 5d-RFSS demonstrated a progressive increase in floating behavior during the induction phase (**Figure 2A**). During the test phase 32 days later, mice that had undergone 5d-RFSS continued to demonstrate relatively high levels of floating behavior (**Figure 2A**). In line with this persistence, floating behavior scores were significantly greater than non-stressed C57BL/6J controls that had not been forced to swim during the induction phase (**Figure 2A**). When given a choice between water and 1% sucrose before 5d-RFSS (SPT1), C57BL/6J mice showed a typical and strong preference for sucrose (~88%; **Figure 2B**). None of the 5d-RFSS mice developed loss of sucrose preference (i.e. anhedonia) compared to non-stressed C57BL/6J controls or compared to pre-5d-RFSS (SPT1) levels (**Figure 2B**). All experimental mice demonstrated greater immobility scores during TST2 compared to TST1, independent of having been forced to swim (**Figure 2C**). Immobility behavior during the test phase swim session on *day* 37 or during TST2 was not associated with altered general locomotor activity as indicated by similar distance traveled during an OFT (**Figure 2D**). The number of center zone entries or feces produced during the OFT, both indicative parameters of anxiolytic behavior, also did not differ between experimental groups (**Figures 2E, F**). Similarly, time spent in the center zone did not differ between non-stressed C57BL/6J controls and 5d-RFSS mice (38.8 ± 4.7 versus 34.6 ± 4.6 seconds, respectively; $t_{[1,18]} = 0.64$, $p = 0.53$). Five consecutive days of forced swimming had no immediate effect on body weight in 5d-RFSS mice compared to controls that were handled daily but not forced to swim (**Figure 2G**). All C57BL/6J mice showed similar increases in body weight during the 4 weeks following 5d-RFSS, independent of having been forced to swim (**Figure**

2G). In contrast, switching from group-housing to single-housing at the start of the 5d-RFSS paradigm slightly lowered body weight in all C57BL/6J mice (**Figure 2G**). 5d-RFSS did not alter caloric intake acutely during 5d-RFSS (*days* 1 – 5), or in the 4 weeks following 5d-RFSS (*days* 8 – 36; **Figures 2H**, **I**).

Effects of 5d-RFSS on depressive-like behavior in BALB/cJ mice. We next tested the hypothesis that 5d-RFSS induces a depressive-like state in BALB/cJ mice. This strain is particularly sensitive to develop stress-induced anhedonia compared to C57BL/6J mice (Farley et al., 2010; Razzoli et al., 2011). Because our preliminary data indicated that BALB/cJ mice do not generate a sucrose preference to a 1% sucrose solution (data not shown), we used a 3% sucrose solution with this strain. BALB/cJ mice that underwent 5d-RFSS demonstrated a progressive increase in floating behavior during the induction phase (**Figure 3A**). Non-stressed BALB/cJ controls were handled daily during the induction phase but were not forced to swim. During the test phase 32 days later, mice that had undergone 5d-RFSS continued to demonstrate increased floating behavior (**Figure 3A**). In line with this persistence, floating behavior scores were significantly greater than non-stressed BALB/cJ controls that had not been forced to swim during the induction phase (**Figure 3A**). When given a choice between water and 3% sucrose before 5d-RFSS (SPT1), BALB/cJ mice showed a typical and strong preference for sucrose (~81%; **Figure 3B**). None of the 5d-RFSS mice developed loss of sucrose preference (i.e. anhedonia) compared to non-stressed BALB/cJ controls or compared to pre-5d-RFSS (SPT1) levels (**Figure 3B**). Sucrose preference was actually slightly higher during SPT2 - SPT4, independent of treatment of the mice (**Figure 3B**). BALB/cJ

306 mice demonstrated greater immobility scores during TST2 compared to TST1,
307 independent of having been exposed to swim stress (**Figure 3C**). Immobility
308 behavior during the test phase swim session on *day* 37 or during TST2 was not
309 associated with altered general locomotor activity as indicated by similar distance
310 traveled during an OFT (**Figure 3D**). None of the BALB/cJ mice entered the center
311 zone during the OFT (**Figure 3E**). The number of feces produced during the OFT
312 did not differ between 5d-RFSS mice and non-stressed controls (**Figure 3F**). Similar
313 to the C57BL/6J cohort, five consecutive days of forced swimming had no
314 immediate effect on body weight in 5d-RFSS mice compared to controls that were
315 handled daily but not forced to swim (**Figure 3G**). Furthermore, all BALB/cJ mice
316 showed similar increases in body weight during the 4 weeks following 5d-RFSS,
317 independent of having been forced to swim (**Figure 3G**). In contrast, switching
318 from group-housing to single-housing at the start of the 5d-RFSS paradigm slightly
319 lowered body weight in all BALB/cJ mice (**Figure 3G**). 5d-RFSS did not alter caloric
320 intake acutely during 5d-RFSS (*days* 1 till 5), or in the 4 weeks following 5d-RFSS
321 (*days* 8 till 36; **Figures 3H**, **I**).
322
323 *CSDS induces a depressive-like state in C57BL/6J mice*. CSDS is a validated model of
324 depression in C57BL/6J mice (Kudryavtseva et al., 1991; Krishnan et al., 2007;
325 Nestler and Hyman, 2010; Golden et al., 2011). Therefore we next used this animal
326 model as a comparison to determine our ability to induce a depressive-like state in
327 young-adult male C57BL/6J mice. Mice that had undergone ten days of social
328 defeat stress had greater body weight gain compared to non-defeated controls
329 (**Figure 4A**). CSDS mice showed a significant decrease in sucrose preference

during SPT2 compared to pre-CSDS levels (SPT1), whereas non-defeated controls did not (**Figure 4B**). As a second parameter of depressive-like behavior, we also measured social avoidance following CSDS (Krishnan et al., 2007). When tested during a social interaction test on *day* 11, CSDS mice spent less time interacting with a social target than non-defeated controls (**Figure 4C**). Finally, CSDS mice had less center zone entries during an OFT, indicative of increased anxiety-like behavior, and this was independent of total locomotor activity during the OFT (**Figures 4D, E**).

Effects of VWR on persistence of immobility behavior following 5d-RFSS.

Antidepressant drugs and treatments decrease the persistence of increased floating behavior induced by 5d-RFSS (Stone and Lin, 2011; Sun et al., 2011; Serchov et al., 2015), which suggests predictive validity. Therefore we tested if VWR, another known antidepressant intervention (North et al., 1990), could modulate persistence of immobility behavior induced by 5d-RFSS. Following 5d-RFSS, C57BL/6J mice were given voluntary access to running wheels for 28 days (**Figures 5A, B**). VWR after 5d-RFSS lowered the relatively high levels of floating behavior towards non-stressed C57BL/6J control levels (**Figure 5C**). In BALB/cJ mice, VWR was even more effective and fully normalized floating behavior to non-stressed BALB/cJ control levels (**Figures 5C - D**).

DISCUSSION

We assessed if 5d-RFSS has construct, face, and predictive validity to model human depression by determining if 5d-RFSS induces a depressive-like state in C57BL/6J

and BALB/cJ mice. These inbred strains, obtained from a commercial breeder, are commonly used in neuropsychiatric research and have relatively low and high emotionality, respectively (Farley et al., 2010; Razzoli et al., 2011). We thoroughly assessed depressive-like behavior before and after 5d-RFSS by measuring sucrose preference, body weight, food intake, TST immobility behavior, and anxiety-like behavior. Our observations suggest that 5d-RFSS induces an adaptive learned behavioral response, but not a depressive-like state in two mouse strains commonly used in neuropsychiatric research. These findings indicate that 5d-RFSS has no construct or face validity to model human depression. However, because the persistence of the adaptive and learned behavioral response is modulated by known antidepressant drugs (Stone and Lin, 2011; Serchov et al., 2015), rTMS (Sun et al., 2011), and VWR (this study), 5d-RFSS might have predictive validity.

In line with previous reports (Sun et al., 2011; Serchov et al., 2015), 5d-RFSS induced a progressive increase in floating during individual swim sessions in our C57BL/6J and BALB/cJ cohorts, and this increase in floating behavior persisted for at least 4 weeks. Our observations thus confirm that repeated forced-swim sessions can induce an adaptive and learned behavioral response underlying survival in mice (Molendijk and de Kloet, 2015).

C57BL/6J or BALB/cJ mice did not develop loss of sucrose preference (i.e. anhedonia) immediately following 5d-RFSS or during the 4 weeks following 5d-RFSS. In contrast, it has been reported that 5d-RFSS induced anhedonia immediately after 5d-RFSS, and this anhedonia could be rescued following 4 weeks

of enhanced adenosine A_1 receptor expression in the brain (Serchov et al., 2015). Although experimental differences are potential explanations for the contrasting observations, it is difficult to directly compare our findings, as this study did not specify the exact genetic background, age, or gender of the experimental mice for each individual experiment (Serchov et al., 2015). Our observations suggest that wild-type C57BL/6J and BALB/cJ mice obtained directly from a popular commercial breeder do not develop anhedonia following 5d-RFSS, suggesting that genetic background can have important effects on behavioral responses to stressors. Finally, a genetic model used by Serchov *et al*. was raised on doxycycline in their drinking water until weaning, which, as recognized by the authors, makes water extremely bitter. Thus, alterations in drinking behavior or sucrose sensing deficits during adulthood cannot be excluded in these studies. Exposure to CSDS has revealed susceptible and unsusceptible subpopulations in C57BL/6J mice (Krishnan et al., 2007). In our 5d-RFSS cohorts, all C57BL/6J and BALB/cJ mice demonstrated stable and high (>75%) sucrose preferences, suggesting that it is unlikely that our experimental cohorts contained susceptible and unsusceptible subpopulations. To properly assess sucrose preference, we averaged drinking behavior over 3 days. Importantly, all mice showed stable consumption behavior during each SPT. Moreover, sucrose preference was stable over the 3 consecutive days of SPT1, suggesting that initial reactivity to single housing (conducted on the same day as the start of SPT1) did not contribute significantly.

Changes in body weight or caloric intake are important homeostatic symptoms associated with human depression and can be replicated using established animal

models of depression such as CUS and CSDS (Willner, 2005; Nestler and Hyman, 2010). 5d-RFSS did not alter food intake in either strain, both during the 5d-RFSS and in the 4 weeks following the 5d-RFSS. Furthermore, 5d-RFSS had no immediate or delayed effect on body weight compared to non-stressed controls in either strain. In contrast, switching from group-housing to single-housing induced a transient and small decrease in body weight in all mice at the start of the 5d-RFSS protocol. Social isolation can be a mild to severe stressor in mice depending on the duration of social isolation (Wallace et al., 2009), suggesting that acute isolation by single-housing of the experimental mice had a bigger effect on body weight than 5d-RFSS. Body weights were analyzed for 4 weeks following 5d-RFSS, suggesting it is unlikely that body weights will differentiate at a later time point.

All experimental mice demonstrated greater immobility scores during TST2 compared to TST1, again suggesting an adaptive and learned behavioral response. Importantly, TST2 immobility scores were similar between mice that had undergone 5d-RFSS and non-stressed controls. Although the TST is a treatment-based screen with only predictive validity (Nestler and Hyman, 2010), these observations support the notion that 5d-RFSS did not induce depressive-like behavior.

Many stress-based rodent models, including CSDS, also exhibit anxiety-like behavior (Krishnan et al., 2007; Nestler and Hyman, 2010). In contrast, 5d-RFSS did not promote anxiety-like behavior compared to non-stressed controls, as indicated by a similar number of center zone entries, center zone time, or feces produced

426 during the OFT. These observations indicate that 5d-RFSS did not induce anxiety-
427 like behavior often observed in validated rodent models of depression. BALB/cJ
428 mice produced more feces and showed complete avoidance of the center zone
429 during the OFT compared to C57BL/6J mice, confirming their greater emotionality
430 (Farley et al., 2010; Razzoli et al., 2011).
431
432 Taken together, our observations indicate that 5d-RFSS did not induce emotional,
433 homeostatic, or psychomotor symptoms in young-adult male C57BL/6J and
434 BALB/cJ mice obtained from a commercial breeder. In contrast, CSDS induced
435 changes in body weight, a significant decrease in sucrose preference (i.e.
436 anhedonia), social avoidance, and increased anxiety-like behavior in young-adult
437 male C57BL/6J mice. Collectively these symptoms are indicative of a depressive-
438 like state (Krishnan et al., 2007; Nestler and Hyman, 2010). Although we cannot
439 exclude that 5d-RFSS can induce a depressive-like state in older mice or in stress-
440 susceptible genetic models, our findings suggest that 5d-RFSS has no construct or
441 face validity to model human depression in two inbred strains commonly used in
442 neuropsychiatric research. However, because known antidepressant treatments,
443 including drugs (Stone and Lin, 2011; Serchov et al., 2015), rTMS (Sun et al., 2011),
444 and VWR (this study), can modulate persistence of increased floating behavior, 5d-
445 RFSS might have predictive validity to identify novel antidepressant treatment.
446 Finally, initial assessment of depressive-like behavior in rodents can include
447 treatment-based screens, such as the forced-swim test and TST. However, a
448 combination of emotional, homeostatic, and psychomotor symptoms should be
449 measured to provide more definitive evidence of a depressive-like state.

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Figure legends

Figure 1 | 5d-RFSS paradigm. Experimental timeline of 5d-RFSS in C57BL/6J or BALB/cJ mice. Sucrose preference test (SPT1) and tail-suspension test (TST1) were performed on *day* -3 to *day* 0, before stressed mice underwent 10 minutes of forced-swim stress during 5 consecutive days (*day* 1 - *day* 5; induction phase), followed by SPT2 (*day* 5 - *day* 8), SPT3 (*day* 22 - *day* 25), an open-field test (OFT; *day* 36), the last forced swim session (test phase; *day* 37), TST2 (*day* 38), and SPT4 (*day* 38 - *day* 41). Non-stressed controls were not forced to swim during the induction phase but otherwise underwent the same protocol.

Figure 2 | Effects of 5d-RFSS on depressive-like behavior in C57BL/6J mice. (**A**) Immobility time of 5d-RFSS C57BL/6J mice during the induction phase (*day* 1 – *day* 5) forced swim tests (FST; main effect of *time*, $F_{(4,36)} = 8.68$, $p = 0.00005$; *post hoc* test, $^{§}p = 0.006$, $^{§§}p = 0.001$, $^{§§§}p = 0.0002$ versus *day* 1), and of 5d-RFSS and non-stressed control mice during the test phase (*day* 37; $t_{[1,18]} = 3.44$, $^{*}p = 0.003$ versus non-stressed). (**B**) Two-bottle sucrose preference tests (SPT) before 5d-RFSS (SPT1) and after 5d-RFSS (SPT2 - SPT4). (**C**) Tail-suspension test (TST) immobility time before 5d-RFSS (TST1) and after 5d-RFSS (TST2; main effect of *time*, $F_{(1,18)} = 9.14$, $p = 0.007$; *post hoc* test, $^{§}p = 0.007$ versus TST1). (**D**) Distance traveled ($t_{[1,18]} = 0.43$, $p = 0.67$), (**E**) number of center zone entries ($t_{[1,18]} = 1.01$, $p = 0.33$), and (**F**) number of feces produced during the open-field test (OFT; $t_{[1,18]} = 0.63$, $p = 0.53$). (**G**) Change in body weight (%) from *day* -3 to *day* 36 (main effect of *time*, $F_{(6,108)} = 90.48$, $p < 0.00001$; *post hoc* test, $^{§}p = 0.02$, $^{§§}p = 0.0001$ versus *day* -3). Food intake during (**H**) induction phase (*days* 1 – 5; $t_{[1,17]} = 0.77$, $p = 0.45$) and (**I**) during *days* 8 – 36 ($t_{[1,18]} = 0.71$, $p = 0.49$). NS, not significant. n = 10/group for all experiments.

Figure 3 | Effects of 5d-RFSS on depressive-like behavior in BALB/cJ mice. (**A**) Immobility time of 5d-RFSS BALB/cJ mice during the induction phase (*day* 1 – *day* 5) forced swim tests (FST; main effect of *time*, $F_{(4,36)} = 6.29$, $p = 0.0006$; *post hoc* test, $^{§}p = 0.02$, $^{§§}p = 0.002$ versus *day* 1), and of 5d-RFSS and non-stressed control mice

during the test phase (*day* 37; $t_{[1,18]} = 3.08$, $^{*}p = 0.007$). (**B**) Two-bottle sucrose preference tests (SPT) before 5d-RFSS (SPT1) and after 5d-RFSS (SPT2 – 4; main effect of *time*, $F_{(3,54)} = 12.67$, $p < 0.00001$; *post hoc* test, $^{§}p = 0.002$, $^{§§}p = 0.0002$ versus SPT1). (**C**) Tail-suspension test (TST) immobility time before 5d-RFSS (TST1) and after 5d-RFSS (TST2; main effect of *time*, $F_{(1,18)} = 5.06$, $p = 0.04$; *post hoc* test, $^{§}p = 0.04$ versus TST1). (**D**) Distance traveled ($t_{[1,18]} = 0.74$, $p = 0.47$), (**E**) number of center zone entries, and (**F**) number of feces produced during the open-field test (OFT; $t_{[1,18]} = 0.79$, $p = 0.44$). (**G**) Change in body weight (%) from *day* -3 to *day* 36 (main effect of *time*, $F_{(6,108)} = 36.96$, $p < 0.00001$; *post hoc* test, $^{§}p = 0.002$, $^{§§}p = 0.0001$ versus *day* -3). Food intake during (**H**) induction phase (*days* 1 – 5; $t_{[1,18]} = 0.07$, $p = 0.94$) and (**I**) during *days* 8 –36 ($t_{[1,18]} = 1.36$, $p = 0.19$). NS, not significant; ND, not detected. n = 10/group for all experiments.

Figure 4 | CSDS induces a depressive-like state in C57BL/6J mice. (**A**) CSDS mice had greater weight gain compared to non-defeated control (CON) mice (*days* 1 – 11; $t_{[1,14]} = 2.28$, $^{*}p = 0.039$). (**B**) CON mice did not show a significant decrease in sucrose preference during two-bottle sucrose preference test 2 (SPT2) compared to SPT1 ($t_{[1,7]} = 1.96$, $p = 0.1$). CSDS mice demonstrated lower sucrose preference during SPT2 compared to SPT1 (before CSDS; $t_{[1,7]} = 3.16$, $^{*}p = 0.016$). (**C**) CSDS mice spent less time in interaction zone with target present during social interaction test on *day* 11 ($t_{[1,14]} = 5.04$, $^{*}p = 0.00018$). (**D**) Locomotor activity did not differ significantly between experimental groups during open-field test (OFT) on *day* 12 ($t_{[1,14]} = 1.01$, $p = 0.33$). (**E**) CSDS mice enter OFT center zone less than CON mice ($t_{[1,14]} = 2.99$, $^{*}p = 0.01$). NS, not significant. n = 8/group for all experiments.

Figure 5 | Effects of VWR on persistence of immobility behavior following 5d-RFSS. (**A**) Daily VWR distance (*left*; $F_{(27,243)} = 5.89$. $p < 0.00001$) and cumulative VWR distance (*right*) of C57BL/6J mice. (**B**) VWR (28d) following 5d-RFSS lowered immobility scores during the test day swim session towards levels of non-stressed SED mice ($F_{2,27} = 18.45$, $p = 0.00001$; *post hoc* test, $^{*}p = 0.00013$ versus non-stressed SED, $^{+}p = 0.0034$ versus non-stressed SED, $^{§}p = 0.059$ versus 5d-RFSS SED). (**C**) Daily VWR distance (*left*; $F_{(27,243)} = 4.37$. $p < 0.00001$) and cumulative VWR distance (*right*)

of BALBc/J mice. (**B**) VWR (28d) following 5d-RFSS normalized immobility scores during the test day swim session to levels of non-stressed SED mice ($F_{2,27} = 4.41$, $p = 0.02$; *post hoc* test, $^{*}p = 0.026$ versus non-stressed SED, $^{\S}p = 0.068$ versus 5d-RFSS SED). n = 10/group for all experiments.