Chapter 5: Stress vulnerability promotes an alcohol dependence-like phenotype in a preclinical model of sustained depression

Manuscript in preparation

Major depression and alcohol-related disorders frequently co-occur. Depression severity weighs on the magnitude and persistence of the secondary alcohol use disorder, with severe implications for disease prognosis. Here, we investigated whether depression vulnerability drives propensity to alcohol dependence at the preclinical level. We used the social defeat-induced persistent stress (SDPS) model of chronic depression in combination with operant alcohol self-administration (SA). Male Wistar rats were subjected to social defeat (5 episodes) and prolonged social isolation (~8 weeks) and subsequently classified as SDPS-prone or SDPS-resilient based on their affective and cognitive performance. Using a 5-month operant alcohol SA paradigm, acquisition, motivation, extinction and cue-induced reinstatement of alcohol-seeking were examined in the two subpopulations. SDPS-prone animals showed home cage alcohol preference, increased alcohol SA, excessive motivation to acquire alcohol, impulsive alcohol-seeking, extinction resistance and increased cue-induced relapse. In SDPS-resilient rats, prior exposure to social defeat increased alcohol SA in absence of any other alcohol dependence-like phenotype. Our data revealed that depression proneness confers vulnerability to alcohol, emulating patterns of alcohol dependence seen in human addicts, and that depression resilience to a large extent protects from the development of this alcohol-related pathology. Furthermore, our data suggest that stress exposure alone, independently of depressive symptoms, alters alcohol intake in the long-term.

“That’s the problem with drinking, I thought, as I poured myself a drink. If something bad happens you drink in an attempt to forget; if something good happens you drink in order to celebrate; and if nothing happens you drink to make something happen.” — Charles Bukowski
Introduction

Major Depressive Disorder (MDD) is characterized by i) persistent low mood, ii) loss of interest or inability to experience pleasure (anhedonia) and iii) mild cognitive impairment. MDD is amongst the most detrimental psychiatric disorders, due to its high prevalence, substantial health burden and limited treatment response. MDD commonly co-occurs with alcohol use disorder (AUD), defined by alcohol preoccupation, alcohol craving and recurrent episodes of relapse to alcohol use, complicating its clinical profile and treatment. Approximately 1 out of 5 individuals diagnosed with MDD also suffers from AUD, a 4-fold incidence increase vs. healthy individuals. In the majority of comorbid cases, MDD precedes the onset of alcohol dependence. Notably, in epidemiological studies, chronicity and severity of primary MDD appears to be a risk factor for developing secondary AUD. Furthermore, comorbidity with MDD predicts greater severity of alcohol dependence.

Exposure to severe and/or repeated stress is a well-established trigger of depressive symptoms, as observed both at the clinical and the preclinical level. Response to stress is considered a major factor modulating the magnitude of the depressive state, a hypothesis that is substantiated by an accumulating body of preclinical data examining individual variability to the effects of stress. Notably, susceptibility to stress is characterized by dysregulation of the brain reward pathways and is accompanied by severe reward-associated behavioral deficits. In addition, stress-susceptible animals display facilitation of drug-seeking behaviors, as observed in increased alcohol, amphetamine and cocaine intake and sensitization to the effects of cocaine and amphetamine.

Together, clinical and preclinical data support interplay between stress, depression-severity and subsequent vulnerability to substance abuse and dependence. Previously, we developed a rat paradigm that models primary depression and secondary AUD. Using social defeat-induced persistent stress (SDPS), we demonstrated that animals displaying a sustained depressive-like phenotype showed propensity to alcohol-related pathology, as reflected in excessive motivation to consume alcohol and heightened relapse rate.

In the present study, we investigated whether individual variability to the effects of SDPS relates to subsequent vulnerability to alcohol-related pathology, and whether resilience to the effects of SDPS protects from the development of an alcohol dependence-like phenotype. We employed...
specific readouts that mimic the characteristics of AUD, as defined by the Diagnostic and Statistical Manual of Mental Disorders (5th edition). This approach was selected as it represents the essential diagnostic criteria of addictive pathologies in humans and is sensitive to identify addiction vulnerability at the preclinical level. In particular, we measured i) appetitive approach to alcohol, assessing excessive alcohol consumption; ii) incentive motivation for alcohol-taking, emulating extreme preoccupation and alcohol craving; iii) impulsivity towards alcohol-signifying cues, representing compulsive alcohol-seeking; iv) extinction resistance, mimicking difficulties in cut down on alcohol use; and lastly v) reinstatement of alcohol-seeking, reflecting relapse to alcohol use following abstinence.

Animals, Methods and Materials

Animals & social defeat-induced persistent stress (SDPS)

Paired-housed male Wistar rats (Harlan CPB, Horst, Netherlands) 6–7 weeks old, weighing <200 g upon arrival were habituated (2 weeks), and exposed to social defeat-induced persistent stress (SDPS) followed by an operant alcohol self-administration (SA) paradigm, as previously described. In brief, SDPS animals (n=48) were subjected to 5 daily social defeat encounters and control animals (n=32) were exposed to an empty defeat cage. From the first defeat session or empty cage exposure onwards, all animals were single-housed and remained in social isolation for the rest of the experimental conditions, in absence of further sensory interaction with the stressor (residents). All experimental manipulations were conducted during the dark phase of a reversed 12-h light-dark cycle (lights on at 19:00 h). For the whole experimental period, animals received food and water ad libitum. All experiments were approved by the VU University Amsterdam Animal Users Care Committee.

Selection of SDPS-prone vs. resilient groups

SDPS rats were assigned to either prone (SDPS-Pro) or resilient (SDPS-Res) subgroups following a two-step cluster analysis of performance in social approach-avoidance (SAA) and in object place recognition (OPR) tasks, assessed in weeks 5 and 9 post-defeat, using the Schwarz’s Bayesian criterion (for details see Supplemental Methods and Supplemental Figure 1). From the emerging SDPS-prone and -resilient groups, data obtained from 10 rats (n=5 per subgroup) were used to describe alcohol-related effects of SDPS in the general population, thus were not included in the alcohol SA analysis presented here. Control animals were divided in two equally performing groups (balanced average
performance in SAA and OPR tests) and a total of 16 control rats participated in the experiments described below.

Alcohol exposure

*Home-cage consumption* – All animals were habituated to alcohol consumption using the two-bottle free/limited-access paradigm as previously described. In brief, rats were exposed to gradually elevating alcohol concentrations (2–12% v/v) in the home cage for a total of 5 weeks. During the first 3 weeks of habituation in the home-cage, alcohol was allowed for 24-h, followed by an alcohol free day before the next concentration increment. During the last 2 weeks, alcohol availability was limited to 1 h/day, to prime rats to the subsequent 1-h self-administration sessions. Water bottles were presented in parallel with alcohol, and were used to estimate alcohol preference vs. total liquid consumption. Alcohol and water bottles position was alternated between days / sessions to avoid development of preference.

*Cue-coupled alcohol SA – Fixed Ratio* – Rats were trained to nose-poke for a 0.20 ml 12% alcohol reward in 1-h sessions given every other day. Alcohol delivery (US) was accompanied by discrete audiovisual stimuli (CS, 4-s active hole illumination and tone presentation) and was followed by a 15-s time-out period, during which nose-poking has no programmed consequences. Different reinforcement schedules (fixed ratio, FR) were used (FR1–3). In total, animals were subjected to 15 FR1, 5 FR2 and 5 FR3 sessions. Each FR increment was implemented after animals had reached a stable performance, i.e., when there were no significant differences in responding between the last two sessions of each reinforcement schedule.

*Cue-coupled alcohol SA – Progressive Ratio* – Animals were subjected to five 2-h progressive ratio (PR) sessions, during which the effort (number of nose-pokes) to obtain a reward was progressively increased according to: response ratio = \((5e^{0.2 \times \text{reward number}}) – 5\), rounded to the nearest integer.

*Cue-coupled alcohol SA – Time-Out performance* – Following PR, rats were re-trained to FR1 schedule (9 1-h sessions), to minimize between-group differences that could affect subsequent analysis of extinction performance. To decipher SDPS effects on time-out performance, animals were subjected to 4 additional FR1 sessions (sessions 10–13) that included a double time-out interval (30-s).

Depression vulnerability promotes alcohol seeking
Cue-coupled alcohol SA - Extinction and Relapse - Extinction training consisted of 1-h exposure to the training context in absence of alcohol and alcohol-associated cues. Following 15 daily sessions, operant responding was successfully extinguished (<6 active responses session) and all animals participated in a 30-minute cue-induced reinstatement session, at the start of which a single 0.20 mL alcohol reward was delivered.

Statistical analyses
All behavioral data during alcohol SA, including FR, PR, extinction and relapse, were analyzed using repeated measures analysis of variance (ANOVA). When P-values reached level of significance (P<0.05), further analysis was performed using one-way ANOVA, paired or unpaired student’s t-test and post-hoc Tukey-HSD multiple comparisons. Homogeneity of variance was estimated and Hyunh-Feldt correction or non-parametric Kruskal-Wallis H test were implemented in case of assumption violation. All statistics were performed using IBM SPSS Statistics 21. In the alcohol SA paradigm, one animal (control) was excluded from statistical analysis as behavioral outlier (>2xSD from mean) in >50% of the FR3 and >50% of the PR sessions.

Results
Effects of SDPS on affective state and cognition
Selection of SDPS-prone and -resilient groups - Following two-step cluster analysis, two divergent groups were identified, as reflected by their performance in SAA and OPR tests over a period of 9 weeks after exposure to social defeat (Supplemental Fig. 1). The SDPS-resilient population coped with defeat and isolation stress and did not develop any of the affective or cognitive deficits commonly seen after SDPS. In contrast, the SDPS-prone population showed long-lasting deterioration of affective performance, reflected in social withdrawal, accompanied by severe impairments in spatial memory, which worsened over time. For experimental details see Supplemental methods. At week 10 after defeat, animals proceeded to the alcohol paradigm.

Effects of SDPS on alcohol-taking and -seeking
Acquisition of operant alcohol self-administration - During the 24-h free-access schedule in the home cage, similar alcohol consumption between control, SDPS-prone and SDPS-resilient animals was observed (Supplemental Fig. 2), and all three groups consumed ~1 g/kg of 12% alcohol by the end of the free-access period (control, 1.04±0.2; SDPS-prone, 1.44±0.3; and SDPS-resilient, 1.08±0.2 g/kg).
Notably, analysis of preference for the alcohol over the water solution during the entire free-access period showed a significant group effect (repeated measures ANOVA, F_{GROUP}(2,32)=3.34, P=0.048). This was driven by increased preference for alcohol as exhibited by the SDPS-prone animals, when compared with control (P=0.015) and SDPS-resilient (P=0.096) groups, whereas no difference between the latter two was detected (P=0.503) (Supplemental Fig. 2). These differences disappeared during the subsequent 1-h limited access schedule, and no significant between-group effects were observed in either absolute consumption (F(2,34)=1.11, P=0.894); or preference (F(2,34)=0.51, P=0.607) for the 12% alcohol solution (Supplemental Fig. 2). Together, SDSP-prone animals showed a moderate facilitation of alcohol consumption in the home cage that developed at >10 weeks from the last defeat exposure.

Following home-cage alcohol habituation, animals were subjected to operant alcohol self-administration (Figure 1a). Already in the first SA session, animals learned to discriminate between the active and the inactive hole, preferring the alcohol-associated one: Paired t-test, FR1_{active} vs. FR1_{inactive}, control, t(14)=5.95, P<0.001; SDPS-prone, t(9)=3.45, P=0.007; and SDPS-resilient, t(9)=2.77, P=0.022 (Figure 1b, Supplemental Fig. 3). Analyses of FR1–3 performance (Figure 1b) for each reinforcement schedule separately, revealed an overall effect of training, indicative of the increase in responding following each change in schedule. In addition, an overall effect of defeat on active responding during FR was observed in all 3 ratios; defeated animals, independently of subgroups, displayed increased number of responses in comparison with controls, as observed previously. No training x group effect was seen in any of the FRs tested, and no differences between SDPS-prone and SDPS-resilient animals were observed (Table 1).

Analysis of the alcohol consumption data led to similar results, as in all 3 reinforcement schedules, the two SDPS groups showed similar performance and both gained higher number of rewards in comparison with controls (Supplemental Fig. 3). The average number of inactive responses per session was similar between the three groups in all reinforcement schedules given, supporting the view that task responding was alcohol-specific and excluding general psychomotor deficits long-term following social defeat (Supplemental Fig. 3). Together, the FR1–3 acquisition data reflected an SDPS-driven escalation of responding for an alcohol reward, which persisted, and was even exaggerated under more demanding...
Figure 1. SDPS facilitates acquisition of operant alcohol self-administration.

a) At ~4 months from defeat and following alcohol habituation at the home cage, all animals were subjected to a cue-coupled alcohol self-administration paradigm, starting with acquisition at FR. Different reinforcement schedules were used (FR1-FR3).

b) Analysis of the number of responses to the active, alcohol-delivering hole during FR1 revealed significant training and group effects, as both SDPS groups displayed increased responding as compared with controls. Similarly, during FR2 and FR3 training schedules, the two SDPS groups exhibited enhanced responding for alcohol vs. controls. Although SDPS-prone animals showed relatively higher response rates, no group difference between the two SDPS groups was observed. Repeated measures ANOVA across the 3 reinforcement schedules, main time (t) and group (g) effects are depicted; pairwise group comparisons are indicated (vertical lines); *P<0.05; **P<0.01.

Progressive ratio (PR) – After the last FR session we implemented PR training to study whether a similar increase in demand of reinforcement was evident in these SDPS subgroups as observed previously152,155 (Figure 2a). Analysis over the 5 PR sessions showed no effect of training for the number of active responses (repeated measures ANOVA: F_{PR}(3.40,108.84)=2.07, P=0.100, Table 2). A significant group effect was observed (F_{GROUP}(2,32)=3.42, P=0.045; Figure 2b), in absence of training x group interaction (F_{PRxGROUP}(6.80,108.84)=0.72, P=0.653). Pairwise comparisons revealed that the SDPS-prone group showed significantly higher number of responses for the alcohol reward vs. controls (P=0.016). No overall difference in responding between SDPS-resilient and control animals (P=0.128), nor between the two SDPS groups (P=0.375), were
Table 1. SDPS facilitates acquisition of operant alcohol self-administration. Repeated measures ANOVA was employed to analyze alcohol acquisition during self-administration in the operant chambers. Fifteen FR1, five FR2 and five FR3 sessions were provided, and performance of SDPS-prone (n=10), SDPS-resilient (n=10) and control (n=15) groups was analyzed. Statistics for main training and group effects, as well as training x group interaction for each reinforcement schedule are summarized. Pairwise comparisons among the three groups and correspondent P-values are reported.

<table>
<thead>
<tr>
<th>Training</th>
<th>FR1</th>
<th>FR2</th>
<th>FR3</th>
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<tbody>
<tr>
<td>Training</td>
<td>F(8,84,219.03)=&lt;5.05</td>
<td>F(2,64,84.61)=8.47</td>
<td>F(3,43,109.72)=5.02</td>
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<tr>
<td>Group</td>
<td>P=0.001</td>
<td>P=0.001</td>
<td>P=0.002</td>
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<tr>
<td>F(2,32)=7.32</td>
<td>F(2,32)=3.95</td>
<td>F(2,32)=7.11</td>
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</tr>
<tr>
<td>P=0.002</td>
<td>P=0.029</td>
<td>P=0.003</td>
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</tr>
<tr>
<td>Training x Group</td>
<td>F(13,69,219.03)=0.91</td>
<td>F(5,29,84.61)=1.14</td>
<td>F(6,86,109.72)=1.62</td>
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<tr>
<td>P=0.547</td>
<td>P=0.348</td>
<td>P=0.138</td>
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<tr>
<td>Post-Hoc</td>
<td>Con vs. SDPS-Pro</td>
<td>Con vs. SDPS-Res</td>
<td>SDPS-Pro vs. SDPS-Res</td>
</tr>
<tr>
<td>Con vs. SDPS-Pro</td>
<td>P=0.001</td>
<td>P=0.029</td>
<td>P=0.001</td>
</tr>
<tr>
<td>Con vs. SDPS-Res</td>
<td>P=0.012</td>
<td>P=0.021</td>
<td>P=0.013</td>
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<tr>
<td>SDPS-Pro vs. SDPS-Res</td>
<td>P=0.183</td>
<td>P=0.901</td>
<td>P=0.413</td>
</tr>
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</table>

detected. Accordingly, a significant group effect in average break points over the 5 PR sessions was observed (one-way ANOVA, F(2,34)=3.90, P=0.031). Post-hoc comparisons revealed that this effect was driven by a strong increase in break points displayed by the SDPS-prone animals (P=0.009 vs. control), an effect that was absent in SDPS-resilient rats (P=0.160 vs. control) (Figure 2c). No difference in break points between the two SDPS groups was observed (P=0.234). Taken together, PR data confirmed that SDPS enhances motivation for alcohol-seeking and suggested that, to a large extent, SDPS-resilience prevents these motivational deficits.

Re-training in FR1 – Following PR, all animals were subjected to FR1 re-training (13 1-h sessions; reFR1) in order to normalize preexisting group differences at the start of extinction. Analysis of the first 9 reFR1 sessions revealed a significant training effect (repeated measures ANOVA: F_9FR1(5,80,185.48)=19.56, P<0.001), as all groups of animals gradually reduced their responding for an alcohol reward (Supplemental Fig. 4). A significant group effect (F_3GROUP(2,32)=6.78, P=0.003) and a trend for group x training interaction (F_9FR1xGROUP(11,59,185.48)=1.63, P=0.089) pointed towards differential group performance over time. Post-hoc analysis further affirmed that, similar to acquisition in FR1, both SDPS groups showed enhanced responses as compared with controls (SDPS-prone P=0.001; SDPS-resilient, P=0.040, respectively). No overall difference between the
Figure 2. SDPS vulnerability increases motivation for alcohol intake.

a) Following acquisition of alcohol SA, all animals were subjected to 5 progressive ratio sessions in which motivation for alcohol was assessed. b) Analysis of active responding revealed a main group effect, as SDPS-prone animals displayed significantly higher number of responses vs. controls. No difference between the two SDPS groups, or between the SDPS-resilient and control animals was observed. c) Similarly, break points (maximum FR reached, averaged over 5 PR sessions) confirmed an SDPS-induced dysregulation of the motivational response. Importantly, this effect was seen only in the SDPS-prone rats, as SDPS-resilient animals did not differ from controls. Repeated measures ANOVA across the 5 PR sessions main group (g) effect and pairwise group comparisons are depicted (vertical lines, b); one way ANOVA main group (g) effect and post-hoc comparisons are indicated (c); *P<0.05.

Table 2. SDPS vulnerability increases motivation for alcohol intake.
Repeated measures ANOVA was employed to analyze active responding during training in progressive ratio (PR) reinforcement schedule. Animals were subjected to a total of 5 PR training sessions. Statistics for main time and group effects, as well as time x group interaction are summarized. One-way ANOVA was employed to analyze the average breaking point (AvBP) reached in the 5 PR sessions provided. Post-hoc comparisons among the three groups and correspondent P-values are reported.

<table>
<thead>
<tr>
<th>Time</th>
<th>Group</th>
<th>Time x Group</th>
<th>Post-Hoc</th>
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<tbody>
<tr>
<td>PR1-5</td>
<td>F(3,40,108.84)=2.07</td>
<td>F(2,32)=3.42</td>
<td>F(6,80,108.84)=0.72</td>
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<tr>
<td></td>
<td>P=0.100</td>
<td>P=0.045</td>
<td>P=0.653</td>
</tr>
<tr>
<td>AvBP</td>
<td>F(2,34)=3.90</td>
<td>P=0.031</td>
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Con vs. SDPS-Pro; P=0.016
Con vs. SDPS-Res; P=0.128
SDPS-Pro vs. SDPS-Res; P=0.375

Con vs. SDPS-Pro; P=0.009
Con vs. SDPS-Res; P=0.160
SDPS-Pro vs. SDPS-Res; P=0.234
SDPS-prone and SDPS-resilient group was detected \((P=0.190)\). Notably, an initial carry-over effect in responding after PR was observed in the SDPS-prone group, which displayed higher number of active responses when compared with both control \((P=0.001)\) and SDPS-resilient \((P=0.047)\) groups at the first reFR1 session. Together, re-training in FR1 further indicated a stable SDPS-triggered increase in alcohol-taking that was more prominent in the SDPS-prone rats.

**Time-out performance** – Initially during acquisition \((FR1–3)\), we observed an SDPS-induced increase in responding during time-out periods, in which reward delivery was omitted \(FR1, F_{GROUP}(2,32)=6.80, P=0.003; FR2, F_{GROUP}(2,32)=3.38, P=0.046; FR3, F_{GROUP}(2,32)=4.81, P=0.015\;\text{(Supplemental Fig. 3)}\). This effect was predominantly observed in the SDPS-prone group \((FR1, P=0.001; FR2, P=0.049; \text{and } FR3, P=0.005 \text{ vs. control})\), and to a lesser extent in the SDPS-resilient group \((FR1, P=0.068; FR2, P=0.029; \text{and } FR3, P=0.056 \text{ vs. control})\). To further dissect this behavioral phenotype, we introduced a 30-s time-out interval following each reward, for the last four re-FR1 sessions \((sessions 10–13, \text{Figure 3a})\). Analysis of active responses showed no training effect \((repeated measures ANOVA: F_{FR1}(1.77,56.73)=1.63, P=0.220)\) and no training x group interaction \((F_{FR1GROUP}(3.55,56.73)=0.35, P=0.824)\), suggesting that responding for alcohol was not affected by the change in the duration of the time-out period. Notably, a significant group effect was observed \((F_{GROUP}(2,32)=3.65, P=0.037)\) due to increased responding in the SDPS-prone group when compared with controls \((P=0.013)\), and a trend vs. SDPS-resilient \((P=0.061)\). No group difference was detected between SDPS-resilient animals and controls \((P=0.617)\;\text{(Figure 3b, Table 3)}\).

SDPS-prone animals displayed enhanced active responding, and thus gained higher number of rewards. As this allowed for a larger chance for time-out responding, we went on to examine the relationship between time-out responses and the number of actual rewards obtained. To control for the preexisting difference in responding, we analyzed the ratio between time-out responses and total rewards gained under this 30-s time-out interval \((\text{Figure 3c})\). This revealed a significant training \((repeated measures ANOVA: F_{RATIO}(3,96)=6.01, P=0.001)\) and group \((F_{GROUP}(2,32)=5.29, P=0.010)\) effect, in absence of a training x group interaction \((F_{RATIOGROUP}(6,96)=0.22, P=0.970)\). Pairwise comparisons showed that SDPS-prone rats exhibited increased ratios compared with both control \((P=0.003)\) and SDPS-resilient \((P=0.033)\) animals. No difference between the two latter groups was seen \((P=0.454)\). Together, prolongation of the time-out period, during which alcohol delivery is omitted,
increased premature responses selectively in the SDPS-prone group, indicating that depression vulnerability induces an impulsive-like alcohol-directed phenotype.

Figure 3. SDPS vulnerability unmasks impulsive-like alcohol-seeking.

a) After PR training, all animals were subjected to re-training in FR1 for 13 1-h sessions (cf. Supplemental Fig. 4). During the last 4 reFR1 sessions (10–13), a 30-s time-out interval was implemented following each alcohol reward, doubling the original time-out period. b) Analysis of the active responses over the 4 sessions revealed a main group effect, as SDPS-prone animals displayed significantly increased responses vs. controls. No difference between SDPS-resilient and control groups was observed. Similarly, no group difference between the two SDPS groups were detected, although SDPS-prone animals showed considerably higher rates of responding vs. SDPS-resilient rats. c) To correct for pre-existing group differences in the chance of time-out responding, the ratio of time-out responses to actual rewards was calculated. Analysis of the ratio over 4 reFR1 sessions showed that the SDPS-prone group reached significantly higher number of time-out responses, when compared both to control and SDPS-resilient groups. No difference in the ratio of time-out responses to actual rewards between control and SDPS-resilient groups was observed. Together, SDPS-prone rats exhibited premature responding for alcohol, indicative of impulsive-like alcohol-seeking, whereas SDPS resilience prevented this effect. Repeated measures ANOVA across the 4 reFR1 sessions main time (t) and group (g) effects and pairwise group comparisons (vertical lines) are indicated; *P<0.05; **P<0.01.
Table 3. SDPS vulnerability unmasks impulsive-like alcohol-seeking.
Repeated measures ANOVA was employed to analyze time-out responses during re-training to FR1, using an increased time-out (t.o.) interval. Four reFR1 sessions were provided, and group performance in respect to total active responses and the ratio between t.o. responses and rewards gained was analyzed. Statistics for main training and group effects, as well as training x group interaction are summarized. Pairwise comparisons among the three groups and correspondent P-values are reported.

<table>
<thead>
<tr>
<th>Total Active Responses</th>
<th>Time</th>
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<tr>
<td>F(1,77,56.73)=1.56</td>
<td>F(2,32)=3.65</td>
<td>F(3,55,56.73)=3.35</td>
<td>Con vs. SDPS-Pro; P=0.013</td>
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<tr>
<td>P=0.222</td>
<td>P=0.037</td>
<td>P=0.824</td>
<td>Con vs. SDPS-Res; P=0.617</td>
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<table>
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<tr>
<th>Ratio (t.o./rewards)</th>
<th>Time</th>
<th>Group</th>
<th>Time x Group</th>
<th>Post-Hoc</th>
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</thead>
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<tr>
<td>F(3,96)=1.10</td>
<td>F(2,32)=5.29</td>
<td>F(6,96)=0.22</td>
<td>Con vs. SDPS-Pro; P=0.003</td>
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<tr>
<td>P=0.001</td>
<td>P=0.010</td>
<td>P=0.960</td>
<td>Con vs. SDPS-Res; P=0.454</td>
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Extinction – Extinction training took place following re-exposure to FR1 (Figure 4a). First, analysis of overall extinction performance during the whole training period revealed a significant time effect in absence of time x group interaction (repeated measures ANOVA, F_{EXT1-15}(5.74,183.69)=21.66, P<0.001; F_{EXT1-15xGROUP}(11.48,183.69)=64.71, P=0.302), as all groups reacted to alcohol unavailability by gradually decreasing their active responses (Figure 4b). A significant main group effect was detected (repeated measures ANOVA, F_{GROUP}(2,32)=7.21, P=0.003), driven by the increased responding of SDPS-prone rats when compared with controls (P=0.001) and their resilient counterparts (P=0.026). No difference in responding between the latter two groups was observed (P=0.229). To further dissect the temporal component of the observed variation in extinction performance between the three groups, active responding was analyzed in 3 bins of 5 extinction sessions, representing each week of training in-between no-training weekend days (Figure 4b). Repeated measures ANOVA showed significant time and group effects for the first two extinction training bins, i.e., EXT1-5 and EXT6-10 (Table 4), confirming the delayed extinction learning displayed by the SDPS-prone group. No between-group difference in the last training week (EXT11-15) was observed (F_{GROUP}(2,32)=2.35, P=0.112), indicating that, by the end of extinction training period, all three groups performed similarly extinguishing heir responding for an alcohol reward (Figure 4b and Supplemental Fig. 5). Together, extinction data indicated that SDPS led to an initial delay in extinction learning that was more pronounced in the SDPS-prone group. Notably, SDPS-proneness resulted in persistent responding despite alcohol unavailability, a behavioral aspect not seen in resilient animals.
Figure 4. SDPS vulnerability delays extinction learning and facilitates reinstatement of alcohol-seeking.

a) After re-training in FR1, all animals were provided with 15 1-h daily extinction sessions, during which uncoupling of the context (operant chambers) and the alcohol delivery was achieved. Following extinction training, all animals were subjected to a single cue-induced relapse test. b) Main training and group effects indicated differential extinction performance across the training period and among the three groups. Analysis of responding per training bin (3x5 extinction sessions) showed that the SDPS-prone group exhibited delayed extinction learning in the first two weeks (sessions 1-5 and 6-10), as reflected in increased active responses vs. controls and SDPS-resilient rats. Controls and SDPS-resilient animals responded similarly, illustrating that SDPS effect on extinction were SDPS-prone-specific. Analysis of the remaining extinction sessions (11-15) showed that all 3 groups were successfully extinguished by the end of the training period. c) Presentation of cues previously associated with reward delivery reinstated alcohol-seeking in all 3 groups, as compared to their responding during the last 3 extinction sessions (indicated in b, dashed square). SDPS-prone animals showed increased relapse when compared with controls. No between-group differences in SDPS-resilient vs. control or SDPS-prone vs. SDPS-resilient groups were observed. Repeated measures ANOVA main time (t) and group (g) effects (b,c) and pairwise group comparisons (vertical lines, b) are indicated; One-way ANOVA main group effect is indicated (c); *P<0.05; **P<0.01.
Cue-induced reinstatement – Following extinction of the context of alcohol delivery, a cue-induced reinstatement test was implemented in order to examine the effects of individual SDPS variability to alcohol relapse (Figure 4a). The average number of active responses during the last 3 extinction sessions (EXT13-15; one-way ANOVA, F_{GROUP}(2,32)=1.58, P=0.222) was compared to responses gained during the relapse test. A significant session effect was detected following presentation of alcohol-associated cues, in absence of session x group interaction (repeated measures ANOVA: F_{RELAPSE}(1,32)=30.72, P<0.001; F_{RELAPSE, GROUP}(2,32)=1.28, P=0.293), as all animals increased responding compared with their extinction performance (paired t-test: controls, t(14)=3.14; P=0.007; SDPS-Prone, t(9)=3.59; P=0.006; and SDPS-Resilient, t(9)=2.70; P=0.024) (Figure 4c and Supplemental Fig. 5). Notably, a main group effect was observed (repeated measures ANOVA: F_{GROUP}(1,32)=3.71, P=0.035), which was due to increased relapse rate in SDPS-prone animals vs. control (P=0.011) and a trend vs. SDPS-resilient (P=0.072) groups. The two latter groups performed almost identical in respect to their responding in the relapse test (P=0.520). Analysis of relapse performance alone confirmed that SDPS vulnerability triggered increased reinstatement of alcohol seeking (one-way ANOVA, post-hoc: P=0.031 vs. controls; P=0.092 vs. SDPS-resilient group; and controls vs. SDPS-resilient group, P=0.730). Taken together, the relapse data pointed to an SDPS-induced facilitation of reinstatement of alcohol seeking, as shown previously. Importantly, this effect was selectively seen in the SDPS-prone individuals, as SDPS-resilience protected from the excessive relapse to alcohol seeking.

### Table 4. SDPS vulnerability delays extinction learning and facilitates reinstatement of alcohol-seeking.

Repeated measures ANOVAs were employed to analyze extinction of alcohol seeking. Given an overall time effect (cf. Figure 4b) in active responding, extinction performance was analyzed in 3 bins of 5 extinction sessions, representing each week of training in-between no-training weekend days. Statistics for main time and group effects, as well as time x group interaction for each training bin are summarized. Post-hoc comparisons among the three groups and correspondent P-values are reported.

<table>
<thead>
<tr>
<th></th>
<th>EXT1-S</th>
<th>EXT6-10</th>
<th>EXT11-15</th>
<th>EXT-REL</th>
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<tr>
<td><strong>Time</strong></td>
<td>F(2,33)=103.41; P=0.001</td>
<td>F(2,33)=115.84; P=0.002</td>
<td>F(2,33)=117.98; P=0.005</td>
<td>F(2,33)=30.72; P=0.001</td>
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<tr>
<td><strong>Group</strong></td>
<td>F(1,32)=6.45; P=0.004</td>
<td>F(1,32)=6.41; P=0.007</td>
<td>F(1,32)=1.35; P=0.112</td>
<td>F(1,32)=1.71; P=0.195</td>
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<tr>
<td><strong>Time x Group</strong></td>
<td>F(2,48)=103.43; P=0.001</td>
<td>F(2,48)=115.84; P=0.001</td>
<td>F(2,48)=117.98; P=0.001</td>
<td>F(2,48)=1.28; P=0.293</td>
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<tr>
<td><strong>Post-Hoc</strong></td>
<td>P=0.001</td>
<td>P=0.006</td>
<td>-</td>
<td>P=0.011</td>
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<tr>
<td>Con vs. SDPS-Pro</td>
<td>P=0.001</td>
<td>P=0.006</td>
<td>-</td>
<td>P=0.011</td>
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<tr>
<td>Con vs. SDPS-Res</td>
<td>P=0.007</td>
<td>P=0.007</td>
<td>-</td>
<td>P=0.520</td>
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<tr>
<td>SDPS-Pro vs. SDPS-Res</td>
<td>P=0.089</td>
<td>P=0.036</td>
<td>-</td>
<td>P=0.072</td>
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Depression vulnerability promotes alcohol seeking
Discussion
In the present preclinical study, we examined the effects of depression vulnerability on alcohol-seeking and taking behavior. This was to establish whether SDPS-proneness, which is associated with primary depressive-like symptoms, promotes secondary alcohol use disorder, two phenotypes that are often comorbid in human subjects. We used SDPS in rats to model a depressivelike state that is sustained for at least 6 months following exposure to stress, mimicking chronic depression in humans. Our approach allowed for measuring features of alcohol dependence in depression-prone vs. depression-resilient individuals, drawing parallels to the human disease as described by the DSM-5.

In the population of patients diagnosed with lifetime depression, comorbid alcohol abuse reaches a striking 40%, indicating common genetic and/or environmental causes. In agreement with this, we previously showed that SDPS, when coupled with operant alcohol self-administration, promotes AUD-like behaviors, as reflected in excessive motivation for alcohol and increased relapse rate. Here, we extend these findings by showing that animals selected for depression susceptibility exhibit greater vulnerability to alcohol in terms of impulsive alcohol seeking and delayed extinction of alcohol-related learning. Furthermore, in our model, depression resilience subdued the emergence of specific alcohol-related deficits, including exacerbated motivation and impulsive-like approach to alcohol, aberrant extinction and aggravated alcohol-seeking during reinstatement. Of note, stress exposure, in the form of short-term defeat stress in combination with chronic sub-threshold isolation stress, resulted in lasting increase in instrumental alcohol-taking in depression-resilient individuals, in which affective and cognitive deficits are absent.

Exposure to SDPS precipitates alcohol-taking vulnerability
In humans, depression-susceptibility primes the development of an alcohol-vulnerable phenotype, which is characterized by core manifestations of AUD, including increased intake. We showed that SDPS-prone rats displayed preference for the alcohol solution compared with control and resilient animals, already in the home cage, an effect not observed in the general population of SDPS rats. Increased preference for alcohol in SDPS-prone rats was evident for the whole 24-h free access period (5 weeks, 6 alcohol concentrations). This phenomenon, selectively seen in rats that display severe depressive-like symptoms (cf. Supplemental Figure 1), could indicate an attempt for self-medication, as it has been long hypothesized based on the anxiolytic properties of alcohol.
Preclinical literature supports detrimental effects of stress in alcohol consumption and alcohol-seeking, although there is a complex interplay between biological factors governing stress responses and the methodological variations in stress application and alcohol exposure\textsuperscript{338,339}. We previously reported that exposure to the SDPS paradigm facilitated acquisition of alcohol self-administration in demanding fixed reinforcement schedules in the general population\textsuperscript{155}. Here, we replicated this finding, in fact showing that SDPS increases alcohol intake independently of the emergence of depressive-like symptoms, namely, affective and cognitive deficits. In particular, we report that SDPS-resilient animals, which exhibit no difference in the SAA and OPR tasks as compared with controls, showed increased alcohol acquisition during fixed-ratio responding. This SDPS-induced facilitation of operant alcohol intake has important clinical implications as it indicates that exposure to brief but severe social stress in combination with milder prolonged stressors can render an individual vulnerable to alcohol intake, independently of its measurable depressiogenic effects. This is in agreement with clinical studies implicating stress coping styles, i.e., an individual’s response to perceived stress, in the development of alcohol dependence\textsuperscript{340,341}. It is noteworthy that, although no significant differences between the two SDPS groups were observed during acquisition of self-administration, SDPS-prone rats showed considerable enhancement of active responding for alcohol compared with their resilient counterparts. This surfaced in particular when more effort was required for the acquisition of an alcohol reward, i.e., during FR3, acting as prelude to PR performance.

SDPS-susceptibility is accompanied by excessive motivation towards alcohol

In depression, anhedonia, including disruptions in normal anticipatory response and in goal-directed behavior, is considered a core symptom\textsuperscript{22} that has been employed to assess depression-susceptibility\textsuperscript{156}. At the preclinical level, progressive ratio (PR) responding has been extensively used to dissect the effects of depressive-like state in motivation towards natural\textsuperscript{152} and drug-related rewards\textsuperscript{142}. In drug addiction, persistent preoccupation and heightened motivation to acquire the drug of abuse are central to diagnosis\textsuperscript{3}. Consequently, PR schedules have been employed to assess the incentive value of drugs of abuse both in humans and in rodents\textsuperscript{228,343} and are considered essential in prediction of addiction-proneness at the preclinical level\textsuperscript{136}. We previously showed that SDPS dramatically increased alcohol break points and that the SDPS-induced depressive state, as manifested in social avoidance 5 weeks after defeat, was predictive of a high motivational drive to seek alcohol\textsuperscript{155}. Here, we demonstrated that SDPS-prone rats showed a
similar, yet exaggerated response to PR training, confirming the crucial interaction between depression-proneness and alcohol-vulnerability. In support of this, resilience to SDPS subdued alcohol-related motivational overdrive, as SDPS-resilient rats demonstrated PR performance similar to controls. This further indicates the conducive role of depression susceptibility on alcohol dependence-like manifestations, in this case, excessive alcohol preoccupation and craving.

SDPS-susceptibility elicits extinction-resistance

The depressive-like state was accompanied by resistant extinction learning, which was carried on for the first two weeks of extinction training. In particular, SDPS-prone rats showed delayed discontinuation of responding, as compared both with controls and their resilient counterparts. These data suggested that depression-vulnerability promotes a unique dependence-like phenotype, namely, persistent alcohol-seeking when alcohol unavailability is announced. This delayed incorporation of contextual updates corresponds well to over-generalization of conditioned stimuli, as it is hypothesized in depression. In favor of this notion, mice exposed to repeated social defeat stress display delayed fear extinction and exhibit generalization of fear. Alternatively, delayed extinction performance could reflect depression-induced deficits in cognitive flexibility, as observed in depressed patients. Impaired reversal learning, especially when it requires the inhibition of behavioral patterns driven by affective information, has been observed in the clinic. At the preclinical level, exposure to social defeat during adolescence is associated with deficits in reversal learning during adulthood. Importantly, these deficits depend on social context, as they were reversed following social housing but maintained in adults that, similar to our paradigm, remained in social isolation.

Depression-resilience limited the emergence of delayed extinction. Particularly, during the first week of extinction training, SDPS-resilient animals performed midway of the two other groups, mimicking the effect of SDPS in a general Wistar population. From then onwards, extinction responding in SDPS-resilient individuals mirrored the one of control rats, indicating that in these animals, extinction of previously learned but currently inappropriate behavioral patterns is intact. This is in accordance with the notion that resilience to severe stress necessitates facilitation of the extinction of non-relevant information, i.e., adaptive extinction learning. Notably, facilitation of reversal learning, namely, a swift from learned responses towards the most adaptive ones, is observed following administration of tricyclic antidepressants and of
selective serotonine reuptake inhibitors. Thus it is possible that in SDPS-resilient animals, extinction learning is mediated via adaptations of the serotoninergic and noradrenergic systems that promote cognitive/behavioral flexibility.

SDPS-susceptibility prompts impulsive-like behavior and intensifies alcohol reinstatement

Impulsivity, defined as behavioral disinhibition that leads to premature or inappropriate conduct, although not a prominent characteristic of MDD, has been linked to depression severity. Behavioral loss of control and cognitive impulsivity are shown to predict suicidal tendencies in depressed individuals. Impulsivity has been long identified as a major vulnerability factor in the development of abusive drug-intake patterns and in transition from recreational to compulsive drug use. Notably, a strong association between impulsivity and alcohol abuse is reported, with abstinent alcohol-dependent individuals demonstrating increased anticipatory responding and heightened premature responses predicting binge drinking. Our current data suggest that depression-vulnerability unmasked an impulsive-like phenotype, reflected in increased time-out responding during reFR1 training. This escalation in alcohol-seeking was observed after doubling the time interval before a subsequent alcohol reward was available, indicating an inability of the SDPS-prone rats to withhold active responding.

In addition, SDPS-proneness was accompanied by heightened reactivity to alcohol-signifying cues, provoking excessive reinstatement of alcohol-seeking, a common characteristic of animals displaying addictive-like behavior and in SDPS-induced chronic depression. In accordance with the effect of SDPS proneness in time-out responding, aggravated relapse could reflect reduced cognitive control, i.e., an inability in refraining from alcohol-seeking and -taking. This dysfunctional inhibitory control towards alcohol has been observed in ethanol-preferring mice, and mice selected for decreased inhibitory control show, similar to the SDPS-prone subpopulation, escalated motivation for alcohol, increased time-out responding and increased relapse following presentation of alcohol signifying cues. Furthermore, it is possible that these deficits are exaggerated by dysregulation of the brain’s reward system, which is present in the depression-susceptible but not in the unsusceptible subpopulation, leading to maladaptive responsivity to alcohol-paired cues. Animals resilient to the effects of SDPS showed neither the impulsive-like phenotype nor heightened relapse, further indicating that adaptability to adverse life events ameliorates the expression of addiction-like phenotypes.
Is SDPS-proneness a common denominator in depression- and addiction-vulnerability?

Currently, the cause and the temporal order of the development of comorbid MDD and AUD is lively debated\textsuperscript{357}, highlighting the role of alcohol abuse as a risk factor for depression and vice versa\textsuperscript{358}. In this discussion, genetic and environmental factors, as well as their interaction, are considered crucial for the emergence of comorbidity\textsuperscript{36,38,359}. We report that individual variability to the effects of chronic stress, which precipitates or precludes the development of depression, is related to the emergence or absence of alcohol dependence-like manifestations, respectively. In our model, maladaptive stress coping, which leads to propensity to primary depression, exaggerates secondary AUD-like behaviors. Notably, stress resilience limits the progression of both phenotypes. Our data might be explained by 1) a common (epi-)genetic predisposition underlying the two diseases, 2) depression as a factor that confers vulnerability to alcohol abuse, 3) a combination of the previous.

Based on the home cage data, susceptibility to the effects of (sub)chronic stress in the SDPS paradigm is associated with increased alcohol preference, as this is observed selectively in the SDPS-prone individuals. This might reflect a genetic vulnerability to alcohol\textsuperscript{360} in the same individuals that show depression susceptibility\textsuperscript{359}, especially when these individuals are exposed to adverse environmental conditions\textsuperscript{361,362}. Based on time-out responding data, vulnerability to the effects of SDPS brought forward an alcohol-related impulsive-like phenotype. It would be of interest to examine whether this phenotype is drug-specific or whether it is extended to non-drug, natural rewards, such as sucrose or food.

Preclinical studies illustrated an causative role of impulsivity in addiction-like behaviors such as compulsive drug-seeking\textsuperscript{363}, extinction resistance\textsuperscript{364} and increased relapse\textsuperscript{365,366}, which are all associated with SDPS proneness. Thus, it is possible that trait impulsivity is a common factor conferring vulnerability to both depression and alcohol abuse, following exposure to severe / chronic stress. However, human studies have shown that negative mood impacts on impulsive choice, thereby increasing problematic alcohol use\textsuperscript{367}. These data support a mediating\textsuperscript{368} and not causal role of impulsivity in depression-AUD association. Our own observations that guanfacine, an agent used against attention deficit-hyperactivity disorder (ADHD), ameliorates the effects of SDPS on alcohol in the general population\textsuperscript{155} are in support of this notion.
An alternative explanation is that by selecting SDPS-prone individuals, a pre-selection of high impulsive individuals takes place. As mentioned before, impulsivity is associated with increased alcohol consumption, both in humans and in rodents. Vice-versa, high-alcohol preference is linked to increased impulsivity, similar to what observed in the SPDS-prone group. These data suggest a common genetic background for impulsive trait and alcohol abuse, which could be unmasked by severe stress, such as in SDPS.

Collectively, our data support the hypothesis that depression susceptibility promotes addiction-like behaviors in the rat, fulfilling the diagnostic criteria for alcohol dependence as described in the DSM-5. Furthermore, depression-resilience limits the emergence of the full comorbid phenotype, protecting from changes in extinction learning and inhibitory control. The SDPS model can be used to screen for depressed individuals with propensity to alcohol abuse and identify those that will develop dependence-like phenotypes, such as impulsivity. In turn, this can be used as a starting point for further research into (epi)genetic vulnerability factors, as well as molecular mechanisms that underlie the comorbid phenotype.
**Supplemental Material**

**Social defeat-induced persistent stress (SDPS)**

Male Long-Evans rats, (Charles River, UK, weighing >500 g) that were paired-housed with age-matched tube-ligated females (Wistar, Harlan) were used as residents. SDPS rats were exposed to five 15-minute long daily defeat sessions, which included a 5-minute fight phase. A different resident was matched to each Wistar rat per day. Social defeat episodes were monitored by two researchers and the latency to submission during the fight phase was recorded for each rat in each of the five sessions provided. Due to the large number of animals participating in the experiments, social defeat and subsequent behavioral assessment were performed in 3 consecutive weeks, as rats arrived in the vivarium in 3 independent batches, separated by 1 week each.

**Assessment of the depressive-like state**

Before participating in any behavioral measurement, all animals were transferred to the video-recording room and habituated to the test arena (plastic, opaque, 79 x 57 x 42 cm) for at least 10 minutes during 3 consecutive days. Animals were subjected to the Social Approach-Avoidance (SAA) and the Object Place Recognition (OPR) tasks at different time points, as indicated in Figure 1a. All video recordings were analyzed with Viewer² software (BiObserve GmbH, Bonn, Germany).

**Social approach-avoidance test (SAA)** – Approach-avoidance behavior was estimated using an unfamiliar Long-Evans adult male rat (resident) as previously described. In order to examine the development and progression of social withdrawal the weeks after social defeat, all animals were exposed to 5 consecutive SAA tests: the week before social defeat (Baseline -bl); following the defeat week (acute -w1); at week 5 (w5); at week 9 (w9) and at 6 months (6mth) following the last defeat exposure. In brief, rats were habituated and allowed to explore two empty target boxes (TBs, perforated, metal, 16 x 7 x 8 cm) located in the opposite sides of the testing arena (sampling phase). Subsequently, an unfamiliar resident was introduced to one of the TBs and rats were allowed to explore and interact with the target, in absence of direct physical contact (testing phase). Interaction index was calculated as time spent in active zone (resident zone)/ total exploration time (resident + neutral zone), in a 5-minute test. Active and inactive zones were randomly assigned, in all tests provided and between groups, to avoid development of preference.
Object place recognition (OPR) – Hippocampal-dependent short-term memory was assessed by the object place recognition task using a 15-minute retention interval as previously described\textsuperscript{[252,255]}. In order to examine the development and progression of cognitive impairments after SDPS, all animals participated in three OPR tests, given the week before social defeat (baseline, bl), in week 5 (w5) and in week 9 (w9) following the last defeat exposure. In brief, following habituation, rats were allowed to explore two identical objects (cylinders or cubes, metal, 8 x 8 x 35 cm), located in two opposite corners of the arena (sampling phase). After a 15-minute time interval, both objects were replaced with 2 identical ones, and one was displaced to a different position. Discrimination between the spatial locations of the two objects was used to assess spatial memory (exploration index = time spent in novel location / total exploration time (novel + familiar location)) in a 4-minute test. The position of novel and familiar locations and the choice of object shapes was random in all tests provided and between groups, to avoid development of preference.

Selection procedure – For two-step cluster analysis the number of resulting clusters was determined automatically, to avoid a biased subject selection due to a fixed number of emerging subgroups. Both SAA and OPR were weighted equally for final group assignment, as the criterion for susceptibility or resilience required to include both affective and cognitive aspects of the depressive-like phenotype. As such, cluster analysis was conducted for the two time-points that both SAA and OPR data were available, i.e., week 5 and week 9.

Statistical analyses
All behavioral data collected from SAA, OPR tests were analyzed using repeated measures analysis of variance (ANOVA). When P-values reached level of significance (P<0.05), further analysis was performed using one-way ANOVA, paired or unpaired student’s t-test and post-hoc Tukey-HSD multiple comparisons. Homogeneity of variance was estimated and Hyunh-Feldt correction or non-parametric Kruskal-Wallis one-way ANOVA were implemented in case of assumption violation. Preference in interaction and exploration indexes (SAA, OPR) was estimated against a fictive group representing performance at chance levels, while retaining the same variation as the experimental groups\textsuperscript{[259]}. All statistics were performed using IBM SPSS Statistics 21. During assessment of the depressive-like state, the tracking software was erroneously terminated, thus datasets for the following tests were incomplete: ORR\textsubscript{bl}, n=2; OPR\textsubscript{w5}, n=3.
Supplemental Figure 1. Development of affective and cognitive deficits in SDPS-prone and SDPS-resilient rats over time. a) Experimental time-line. Wistar rats were exposed to 5 daily defeat sessions (week 0) and were subsequently single-housed for a period of ~6 months. The week before defeat (w-1), acutely after (w1), at 1 (w5), 2 (w9) and 6 months (w24) following defeat, SDPS effects in social approach-avoidance (SAA) and object place recognition (OPR) tasks were assessed. Based on SAA and OPR performance at weeks 5 and 9, SDPS rats were clustered into SDPS-prone (Pro) or SDPS-resilient. b) Approach-avoidance behavior was examined in 5 SAA tests provided overtime. Before defeat, no preexisting differences were observed between groups. At SAA w5, the SDPS-prone group failed to display preference for the social target an effect not observed in either controls or SDPS-resilient rats. In the months following defeat, SDPS-prone rats showed a decrease in interaction index, indicating the development of social withdrawal that persisted up to 6 months. No differences between control and SDPS-resilient groups were observed. c) Short-term spatial memory was assessed in 3 OPR tests given overtime. Prior to defeat no between-group differences were observed. Already at 1 month from defeat, SDPS-prone animals showed inability to retain spatial information for a displaced object, which was exaggerated at the 2 months test. Both control and SDPS-resilient groups displayed intact memory retention. Surprisingly, at the 2 months OPR test, SDPS-resilient rats showed improved memory retention vs. controls. Repeated measures ANOVA main time (t) and group (g) effect, time x group (t x g) interaction and pairwise comparisons are indicated. Dashed line represents chance levels (0.50) of interaction (SAA) or exploration (OPR). *no preference for the social target (SAA) or the displaced object (OPR) **P<0.05; ***P<0.001.

Supplemental Figures
Supplemental Figure 2. Habituation of alcohol intake in the home-cage. Controls and the two SDPS groups were habituated to progressively increasing concentrations of alcohol (2-12%) in the home-cage, for a period of 5 weeks, using a two-bottle paradigm. a) Consumption of alcohol during 24-h free access, normalized for weight, revealed that starting from 6% onwards SDPS-prone rats displayed a relative increase in consumption of alcohol, albeit that no significant overall difference was observed between the 3 groups. b) Analysis of alcohol preference over water during 24-h free access (see a), depicted as percentage of alcohol/total liquid consumed. A clear facilitation of alcohol consumption selectively in the SDPS-prone rats was observed when analyzing the whole free-access period. Between-group differences were most prominent at 2% and 12% alcohol concentration. SDPS-resilient and control groups showed similar preference for alcohol in all concentrations provided. c) Consumption of 12% alcohol during 1-h limited access (average of 11 sessions). No difference in alcohol intake was observed between the 3 groups. d) Similarly, no between-group difference in preference for the alcohol solution was observed, as all 3 groups drank similar amounts of alcohol vs. water during the 1-h sessions. Repeated measures ANOVA main time (t) and group (g) effect are indicated; one-way ANOVA post-hoc group comparisons are depicted (b); *P<0.05.
Supplemental Figure 3. Operant behavior during acquisition of alcohol self-administration. After habituation in the home-cage, all animals were trained to respond for an alcohol reward under fixed reinforcement schedules (FR1-3). a) Significant effects of time were seen in all three FRs, as alcohol consumption was adjusted in response to changes in reinforcement schedules. Similar to the number of active responses per session (cf. Figure 1), both SDPS groups gained increased number of rewards as compared with controls, in all three FR schedules provided (FR1, SDPS-prone, P=0.002; SDPS-resilient, P=0.025 vs. control; FR2, SDPS-prone, P=0.031; SDPS-resilient, P=0.027 vs. control; FR3, SDPS-prone, P=0.001; SDPS-resilient, P=0.011 vs. control). No between-group differences were seen for SDPS-prone vs. SDPS-resilient groups (FR1, P=0.375; FR2, P=0.961; FR3, P=0.474). b) A significant time effect was
observed for inactive responses in FR1, as all animals reduced responding to the inactive hole, indicating consolidation of the task, i.e., preference for the active, alcohol-associated hole (c.f. Figure 1).

A time effect was seen for FR3, as all groups slightly increased their responses, reflecting changes in the reinforcement schedule. No between-group differences were observed in any of the schedules provided (FR1, F<sub>GROUP (2,32)=245.56, P=0.096; FR2, F<sub>GROUP (2,32)=17.20, P=0.588; and FR2, F<sub>GROUP (2,32)=46.11, P=0.174), illustrating that SDPS had no gross effects in animals discriminative ability (active vs. inactive hole) or motoric skills. c) Analysis of time-out (unreinforced) responses during FR1 revealed significant time and group effects, as SDPS-prone rats displayed increased responses vs. controls (P=0.001). Trends for between-group differences were detected in all other comparisons (SDPS-resilient vs. controls, P=0.068; SDPS-prone vs. SDPS-resilient, P=0.116). During FR2, SDPS increased overall time-out responses (SDPS-prone vs. control, P=0.049; SDPS-resilient, P=0.029 vs. control), and no difference between the two SDPS groups (P=0.829) was observed. Finally, during FR3, significant time and group effects were seen, as the SDPS-prone group reached a higher number of responses when compared with controls (P=0.005). SDPS-resilient animals displayed a trend for higher time-out responses vs. controls (P=0.056) and no difference between the two SDPS groups was observed (P=0.369). Although not corrected for enhanced active responding after SDPS, time-out data pinpointed to an SDPS effect on premature responding for alcohol, as function of the magnitude of the depressive-like state. Repeated measures ANOVA main time (t) and group (g) effects and pairwise comparisons (vertical lines) are indicated; *P<0.05; **P<0.01.

Supplemental Figure 4. Active responding during re-training in FR1 Following PR schedules, and before extinction of the alcohol-associated context, all animals were subjected to 13 reFR1 training sessions. During the first 9 reFR1 sessions, all rats reduced active responding, as the less demanding training schedule was introduced. Similar to initial acquisition in FR1, a significant group effect was observed, as both SDPS groups reached higher number of responses when compared with controls (P=0.001 and P=0.040, respectively). A trend for time x group interaction was observed due to enhanced responding displayed by the SDPS-prone rats in reFR1 session 1, which showed carry-over effects in performance after PR (vs. control, P=0.001; vs. SDPS-resilient, P=0.047). Repeated measures ANOVA main time (t), group (g) and time x group (t x g) effects and pairwise comparisons (vertical lines) are indicated. *P<0.05; **P<0.01; †significant difference between SDPS-prone vs. both SDPS-resilient and control groups.
Supplemental Figure 5. Extinction of operant alcohol self-administration.
Following re-training in FR1, all animals were subjected to 17 1-h extinction sessions, during which alcohol availability was omitted (c.f. Figure 4). Group performance at the last reFR1 and last extinction sessions are depicted here. By the end of the extinction training, all groups were successfully extinguished responding to the alcohol-associated hole, as compared with their own performance during reFR1, reaching less than 5 active responses (control, 4.9±0.7; SDPS-prone, 7.2±1.0; and SDPS-resilient, 6.1±1.2). Paired t-tests are indicated; *P<0.05; **P<0.01.