Restoring Mood Balance in Depression: Ketamine Reverses Deficit in Dopamine-Dependent Synaptic Plasticity

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Background: One of the most novel and exciting findings in major depressive disorder research over the last decade is the discovery of the fast-acting and long-lasting antidepressant effects of ketamine. Indeed, the therapeutic effects of classic antidepressants, such as selective serotonin reuptake inhibitors, require a month or longer to be expressed, with about a third of major depressive disorder patients resistant to treatment. Clinical studies have shown that a low dose of ketamine exhibits fast-acting relatively sustained antidepressant action, even in treatment-resistant patients. However, the mechanisms of ketamine action at a systems level remain unclear.

Methods: Wistar-Kyoto rats were exposed to inescapable, uncontrollable footshocks. To evaluate learned helplessness behavior, we used an active avoidance task in a shuttle box equipped with an electrical grid floor. After helplessness assessment, we performed in vivo electrophysiological recordings first from ventral tegmental area dopaminergic (DA) neurons and second from accumbens neurons responsive to fimbria stimulation. Ketamine was injected and tested on helpless behavior and electrophysiological recordings.

Results: We show that ketamine is able to restore the integrity of a network by acting on the DA system and restoring synaptic dysfunction observed in stress-induced depression. We show that part of the antidepressant effect of ketamine is via the DA system. Indeed, injection of ketamine restores a decreased dopamine neuron population activity, as well as synaptic plasticity (long-term potentiation) in the hippocampus-accumbens pathway, via, in part, activation of D1 receptors.

Conclusions: This work provides a unique systems perspective on the mechanisms of ketamine on a disrupted limbic system.

Key Words: Dopamine, ketamine, learned helplessness, nucleus accumbens, synaptic plasticity, ventral tegmental area

Major depressive disorder (MDD) is the most common mental disorder in the United States (1). A recent advance shows that a single low dose of ketamine, a functional noncompetitive *N*-methyl-D-aspartate (NMDA) antagonist, relieves symptoms in treatment-resistant depression within hours and its effects can last for up to 10 days (2), and repeated injections induce sustained antidepressant action with mild side effects (3).

Cellular mechanisms of ketamine involve the rapid induction of synaptic proteins in the prefrontal cortex and the hippocampus of rats (4,5). Ketamine rapidly reverses the stress-induced deficit in spine density (6) by activation of the mammalian target of rapamycin signaling pathway (4,6,7). However, mechanisms at a systems level remain unclear.

We focused on two systems in the learned helplessness (LH) model of stress-induced depression, the first being the dopaminergic (DA) reward system of the ventral tegmental area (VTA), in which dysfunctions (8) are thought to lead to the core MDD symptom of anhedonia that is also found in the LH model (9). Second, the ventral subiculum of the hippocampus (vSub), which is involved in context-dependent regulation of behavior and stress integration (10), was examined due to its potential involvement in ruminative behavior, a condition associated with an abnormal focus on internal states (11,12). Therefore, stress-induced disruptions of vSub-nucleus accumbens (NAc) contextual focus could drive an organism to a ruminative state (13). Mental rumination itself is not measurable in rats; however, LH can be maintained over time by processes that may be similar to those occurring in rumination (14). We thus proposed to investigate the impact of LH on DA neuron activity and synaptic transmission in the vSub-NAc pathway and how this system is influenced by ketamine administration.

Methods and Materials

Animals

Adult male Wistar-Kyoto rats (300–350 g; Charles River Laboratories, Wilmington, Massachusetts) were used for their susceptibility to LH (15). Rats were housed singly on a reversed 12-hour dark/light cycle (lights on: 7:00 P.M.) with food and water ad libitum. All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.

Behavioral and electrophysiological experiments are detailed in the Supplemental Methods & Materials in Supplement 1.

Learned Helplessness Paradigm

In the LH paradigm (16,17), inescapable stress occurred on day 1 in one chamber of a two-chamber shuttle box (Med Associates, St. Albans, Vermont). Control animals (no-shock) were placed in the shocking chamber in parallel without shocking.

Helpless behavior and the effect of repeated injections of ketamine were assessed using an active avoidance task on days 2, 3, and 4. Failure was recorded if no crossing was made during the shock. The criterion of 40% failures to escape and 8-second latency to escape (17) was used to discriminate between nonhelpless and helpless rats.

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A subanesthetic and subanalgesic (18) dose of ketamine (dissolved in saline, 5 mg/kg, intraperitoneal [IP]) or saline (1 ml/kg, IP) was injected 20 minutes or 2 hours before the beginning of the active avoidance task. Ketamine was injected either repeatedly (three times, on days 3, 4, and 5) or acutely after testing for active avoidance on day 2. For acute injection, on day 3, one group of rats was tested for behavior and another was used for electrophysiological recordings.

Extracellular Recordings

Recordings were performed in chloral hydrate anesthetized rats (400 mg/kg, IP) 24 hours after the last active avoidance task and as previously described (19–23).

VTA Recordings. Microelectrodes were lowered through the VTA (anteroposterior [AP] −5.5 to −5.9 mm, mediolateral [ML] +.6 to +1.0 mm from bregma and dorsoventral [DV] −6.5 to −9.5 mm from dura) (24–26). Three parameters of activity were measured: 1) population activity (Figure S1 in Supplement 1); 2) basal firing rate; and 3) the proportion of action potentials occurring in bursts (24). Electrophysiological identification of dopaminergic neurons in the VTA is shown in Figure S2 in Supplement 1.

Ketamine (5 mg/kg, IP) or saline (1 ml/kg, IP) was injected 20 minutes, 2 hours, or 24 hours before the beginning of the first track.

NAc Recordings. Microelectrodes were lowered through the NAc (AP +1.5 mm from bregma; ML +1.1 mm from midline; DV −5 to −7.5 mm from the dura). Single-pulse (intensity 1 mA; pulse-width .25 msec) and high-frequency stimulation (HFS) (50 Hz; 2 seconds at suprathreshold) were applied to the fimbria [AP −1.6 mm from bregma; ML +1.3 mm from the midline; DV −4.5 mm from the dura (20)]. The D1 antagonist SCH23390 (.5 μg/.5 μL) or Dulbecco's phosphate buffered saline was infused locally into the NAc at a rate of .5 μL per minute via a 33-gauge cannula. Ketamine was injected IP (5 mg/kg, in saline).

Histology

Recording electrode placement was verified via electrophoretic ejection of Chicago Sky Blue dye (Sigma-Aldrich, St. Louis, Missouri) into the recording site and stimulation electrode placement was verified by delivering a 10-second pulse at 200 μA. Rats were euthanized with a lethal dose of chloral hydrate (additional 400 mg/kg) and brains were removed following decapitation. The tissue was fixed in 8% paraformaldehyde for at least 48 hours and then transferred to a 25% sucrose solution for cryoprotection. Once saturated, brains were frozen and sliced coronally at 60 μm thick using a cryostat (Leica Frigocut 2800; Leica, Bannockburn, Illinois) and mounted onto gelatin-chromalum coated slides. Tissue was stained with a combination of neutral red and cresyl violet.

Analysis

For behavior, results were expressed as mean number of failures (± SEM) and mean latency to escape (± SEM) recorded over 25 trials each day. Two-way analysis of variance (ANOVA) followed by a Dunnett's *t* test was performed with treatment as the between-subject factor and session as the within-subject factor.

Electrophysiological data were analyzed using a one-way ANOVA (DA recordings) and a one-way ANOVA with repeated measures (NAc recordings) followed by the Holm–Sidak test, with time as the within-subject factor. When the normality test failed, a one-way ANOVA on ranks (Kruskal-Wallis H-test) was performed. Multiple comparisons were analyzed using a two-way ANOVA followed by the Holm–Sidak test, with treatment as the between-subject factor and time as the within-subject factor.

Results

Repeated Injections of Ketamine Restore Escape Behavior in Helpless Rats

Rats received inescapable shocks on day 1 and were tested for escape behavior on 3 consecutive days before electrophysiological recordings (Figure 1A, B). As previously reported (17,27), inescapable

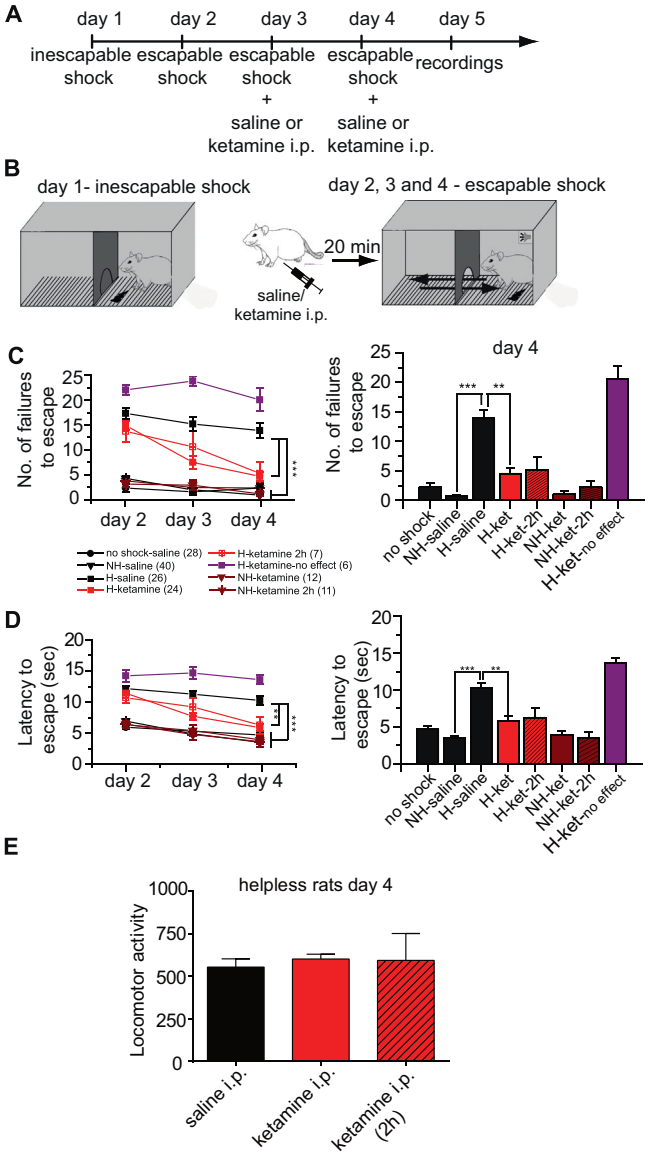

Figure 1. Learned helplessness is reversed by repeated injections of ketamine. **(A)** Experimental timeline. **(B)** Helplessness paradigm. **(C)** Number of failures to escape across 3 consecutive days of escapable shock sessions (left). Data for the escapable session on day 4 are summarized in bar graphs (right). Rats fall into two groups: those showing escape (triangles, nonhelpless; circles, no-shock) and those failing to escape (squares, helpless). Ketamine (red) causes helpless rats to show escape behavior. **(D)** Latency to escape across 3 consecutive days of escapable shock sessions, showing results consistent with escape failures (left). Data for the escapable session on day 4 are summarized in bar graphs (right). Red, purple, and brown represent data for ketamine. **(E)** There was no difference in locomotor activity measured in both sides of the shuttle box during the escapable shock session on day 4 in helpless rats following saline or ketamine 20 minutes or 2 hours after (striped bar) the injection. $^{**}p < .01$; $^{***}p < .001$. Error bars represent SEM. H, helpless rats; i.p., intraperitoneal; ket, ketamine; NH, nonhelpless rats.

shocks induce helpless behavior in ≈ 50% of the rats (nonhelpless rats: 87 of 172 rats or 50.6%; helpless rats: 85 of 172 rats or 49.4%). Between no-shock ($n = 28$) and nonhelpless rats ($n = 40$), there was no difference in the number of failures to escape ($F_{1,27} = .645$, $p = .429$) as well as the latency to escape ($F_{1,27} = .042$, $p = .840$). Helpless rats ($n = 26$) showed reduced escape and higher latency to escape compared with nonhelpless rats (failures: $F_{1,25} = 115.265$, $p < .001$; latency: $F_{1,25} = 71.954$, $p < .001$; Figure 1C, D). Escape failure in helpless rats was reversed by prior administration of ketamine (20 minutes) (Figure 1C, D left, red; summary Figure 1C, D right). There was a significant difference between helpless rats treated with ketamine in comparison with saline injection on days 3 and 4 (Holm-Sidak post hoc) (failures: $F_{1,23} = 21.433$, $p < .001$; latency: $F_{1,23} = 12.931$, $p < .001$). Prior administration of ketamine (20 minutes) had no effect on the behavior of nonhelpless rats in comparison with saline injection (failure: $F_{1,11} = .048$, $p = .504$; latency: $F_{1,11} = .291$, $p = .6$; Figure 1C, D left, brown). This is consistent with previous studies showing that low dose of ketamine does not interfere with active avoidance task performance (28,29).

In some cases, ketamine had no effect on escape behavior in helpless rats (6 of 85 rats). There was no difference in latency or number of failures to escape before and 20 minutes after ketamine injection (latency: $F_{2,5} = 1.928$, $p = .196$; failures: $F_{2,5} = 1.761$, $p = .221$; $n = 6$).

Although the time course of drug action in rats is not directly comparable with that in humans, given clinical data showing that ketamine antidepressant effects are typically observed 2 hours after the injections (2), ketamine effects on escape behavior in helpless and nonhelpless rats were tested 2 hours before the active avoidance tasks. For both groups, there was no difference in the number of failures to escape and the latency to escape in comparison with the 20-minute injection (helpless rats failures: $F_{1,6} = .024$, $p = .882$; latency: $F_{1,6} = .067$, $p = .819$; nonhelpless failures: $F_{1,10} = .261$, $p = .621$; latency: $F_{1,10} = .016$, $p = .902$). This effect in helpless rats was significant on day 4 but not on day 3 (Holm-Sidak post hoc).

Locomotor activity measured in helpless rats in the shuttle box on day 4 of the testing was not different between preinjection of saline and preinjection of ketamine (2 hours and 20 minutes) ($F_{1,54} = .379$, $p = .686$; Figure 1E), ruling out ketamine-induced hyperactivity confounding measures of escape.

Ketamine Restores Dopaminergic Population Activity in the VTA of Helpless Rats

One of the core symptoms of MDD is anhedonia or the reduction of pleasure, which has been described in the LH model (9) and manifests as hyposensitivity to rewarding events; this likely involves dysfunction of the DA system (8,30). In the current study, we examined whether DA activity was different in helpless and nonhelpless animals (Figure 2A–D). Nonhelpless rats that

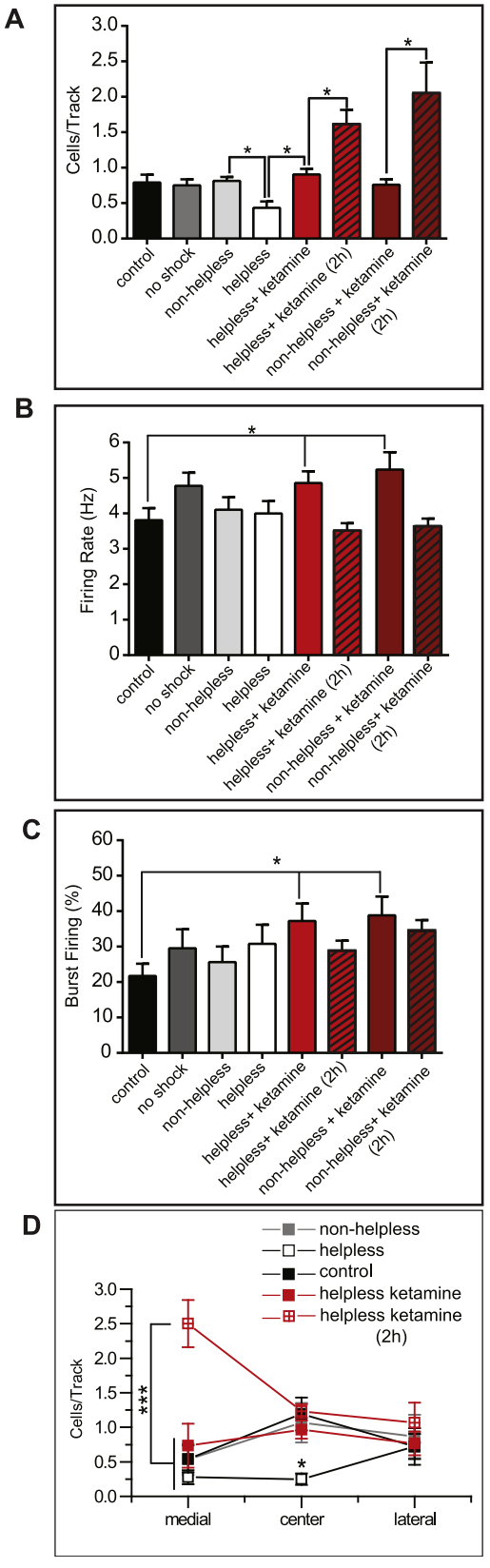

Figure 2. Helpless rats show selective decrease in dopamine neuron population activity that is reversed by repeated injections of ketamine. **(A)** Number of spontaneously active dopamine (DA) neurons per electrode track. Only helpless rats showed a 50% decrease in number of active DA neurons (white bar), which was reversed by ketamine 20 minutes postinjection (red bar). Two hours after the injection, a significant increase in the population activity of nonhelpless and helpless rats is observed (striped bar). In contrast, there was no change in firing rate **(B)** and burst firing **(C)** between helpless and nonhelpless rats; ketamine administration 20 minutes before recordings produced its typical increase in DA neuron rate and bursting in both groups, which is not seen 2 hours after the injection (striped bar). **(D)** Helpless rats show a decreased number of spontaneously active DA neurons located in the central, but not in the medial and lateral tracks, in the ventral tegmental area (white squares) compared with control rats (black squares), nonhelpless rats, and helpless rats treated with ketamine 20 minutes before recordings. Ketamine 2 hours postinjection induces an increase in population activity in the medial tracks. *$p < .05$, ***$p < .001$. Error bars are ± SEM. Red and brown bars represent data with injection of ketamine.

received daily saline injections ($n = 5$ rats, 32 neurons) exhibited an average of .84 ± .05 spontaneously active DA neurons per electrode track that fired at an average rate of 4.1 ± .4 Hz and with 25.6 ± 4.4% of the action potentials occurring in a burst discharge pattern, which is consistent with data obtained from home cage control rats ($n = 6$ rats, 31 neurons; cells per track: $F_{1,10} = .268$, $p = .616$; firing rate: $F_{1,61} = .166$, $p = .685$; burst firing: H = .104, $p = .747$; Figure 2A–D) and no-shock animals ($n = 4$ rats, 23 neurons; cells per track: $F_{1,8} = .444$, $p = .524$; firing rate: $F_{1,53} = 1.662$, $p = .203$; burst firing: H = .671, $p = .413$; Figure 2A–C). Helpless rats ($n = 6$ rats, 24 neurons) showed a significant decrease (approximately 50%) in DA neuron population activity compared with control ($F_{1,10} = 6.713$, $p < .05$) and nonhelpless rats ($F_{1,9} = 13.455$, $p < .01$) (Figure 2A). No significant differences were observed in average firing rate (control versus helpless: $F_{1,52} = .145$, $p = .705$; nonhelpless versus helpless: $F_{1,53} = .0397$, $p = .843$; Figure 2B) or burst firing (control versus helpless: H = 1.407, $p = .236$; nonhelpless versus helpless: H = .700, $p = .403$; Figure 2C) across the population of neurons recorded. Injection of ketamine (20 minutes) compared with saline restores the decreased population activity in helpless rats ($F_{1,9} = 14.33$, $p < .01$) with no change in firing rate ($F_{1,50} = 3.119$, $p = .083$) or burst firing (H = .866, $p = .352$). In nonhelpless rats, ketamine ($n = 5$ rats, 30 neurons) had no effect on the population activity ($F_{1,8} = .756$, $p = .410$), firing rate ($F_{1,59} = 3.424$, $p = .07$), or bursting activity (H = 3.553, $p = .06$) compared with saline. However, in comparison with home cage control animals, ketamine injection increased firing rate and bursting for both helpless (firing rate: $F_{1,58} = 4.091$, $p < .05$; bursting: H = 6.663, $p < .05$) and nonhelpless rats (firing rate: $F_{1,59} = 4.947$, $p < .05$; bursting: H = 6.067, $p < .05$). This is consistent with previous studies showing that noncompetitive NMDA receptor antagonists increase DA firing in the VTA (31).

Ketamine injected 2 hours before the beginning of DA recordings induced a significant increase in population activity in helpless ($n = 5$ rats, 65 neurons) and nonhelpless rats ($n = 5$ rats, 77 neurons) in comparison with ketamine injected 20 minutes prior (1.6 ± .2 cells per track; $F_{1,8} = 10.945$, $p < .05$; $n = 5$ in helpless rats versus 2.1 ± .4 in nonhelpless rats: H = 6.86, $p < .01$; $n = 5$; Figure 2C). However, it had no effect on firing rate or burst firing in helpless and nonhelpless rats compared with saline injection (nonhelpless rats, firing rate: H = 1.25, $p = .264$; burst firing: H = 2.374, $p = .123$; helpless rats, firing rate: $F_{1,86} = 1.407$, $p = .239$; burst firing: H = .003, $p = .958$) (Figure 2B, C).

Ventral tegmental area DA neurons of helpless rats showed a decreased population activity primarily in the central VTA (Figure 2D) with no differences in medial and lateral tracks compared with control and nonhelpless rats (medial: $F_{2,14} = .744$, $p = .493$; central: H = 9.417, $p < .001$; lateral: $F_{2,14} = .102$, $p = .903$). Injection of ketamine (20 minutes) induced an increase in the number of cells per track in the center tracks ($F_{1,9} = 22.358$, $p < .01$; compared with saline) without affecting medial and lateral tracks (medial H = 2.37, $p = .177$; lateral H = .035, $p = .931$). In contrast, ketamine (2 hours) also increased DA cells per track in the medial track over that of ketamine administered 20 minutes prior (medial: $F_{1,8} = 14.295$, $p < .001$; versus lateral $F_{1,8} = 1.161$, $p = .31$; and central $F_{1,8} = 2.560$, $p = .148$).

Therefore, ketamine (20 minutes) in helpless rats restored escape behavior and DA activity in the VTA, mainly in the center tracks of the VTA. However, 2 hours after injection, ketamine also induced an overactivation of the medial tracks.

Ketamine Has Sustained Action on Escape Behavior and Activity in the VTA

Given that clinical studies show that ketamine exerts effects that are sustained for 24 hours (2), we tested the effects of a single injection of ketamine (after the end of the escapable session on day 2) on escape behavior (day 3; Figure 3A). Helpless rats ($n = 6$) showed reduced escape and higher latency to escape compared with nonhelpless rats ($n = 6$ rats) (failures: $F_{1,5} = 64.961$, $p < .001$; latency: $F_{1,5} = 82.732$, $p < .001$; Figure 3A), which was reduced by ketamine injected 24 hours before testing (failures: $F_{1,10} = 6.027$, $p < .05$; latency: $F_{1,10} = 5.438$, $p < .05$; Figure 3A, red, $n = 6$) but was still significantly different compared with nonhelpless rats (failures: H = 8.966, $p < .01$; latency: H = 8.308, $p < .01$). In a separate group of rats, electrophysiological recordings were performed on identically treated rats on day 3. Nonhelpless rats that received saline ($n = 5$ rats, 65 neurons) exhibited an average of 1.2 ± .2 spontaneously active DA neurons per electrode track that fired at an average rate of 3.8 ± .2 Hz and with 33.4 ± 3.0% of the action potentials occurring in a burst discharge pattern. Helpless rats ($n = 5$ rats, 24 neurons) showed a significant decrease in DA neuron population activity and in the firing rate compared with nonhelpless rats (population activity: $F_{1,9} = 10.115$, $p < .05$; firing rate: $F_{1,87} = 5.176$, $p < .05$), which was restored by ketamine injected 24 hours before recording (population activity: $F_{1,8} = 10.809$,

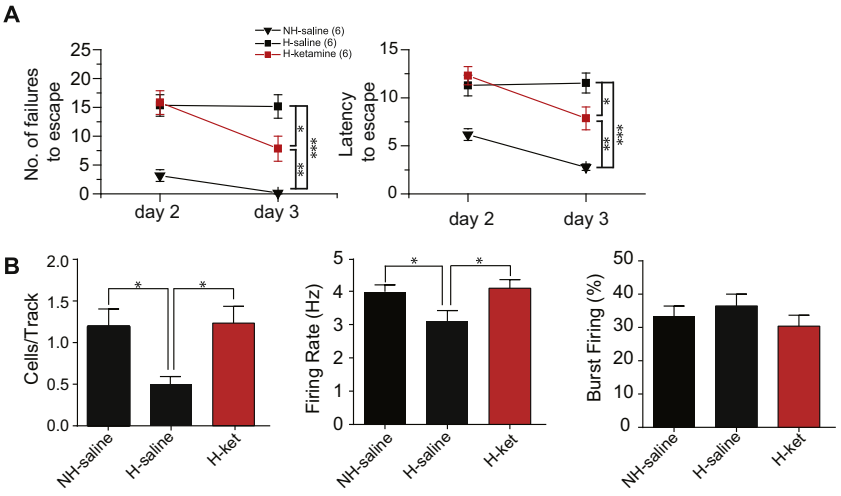

Figure 3. Acute injection of ketamine restores sustainably of escape behavior and ventral tegmental area dopamine (DA) activity. **(A)** Number of failures (left) and latency (right) to escape across 2 consecutive days of escapable shock sessions. Injection of ketamine (ket) (red) after the first escapable session induces a significant decrease in the number of failures and the latency to escape (nonhelpless rats [NH]: triangle; helpless rats [H]: square). **(B)** Number of spontaneously active DA neurons per electrode track (top). Helpless rats showed a 50% decrease in number of active DA neurons and a decrease in firing rate (middle), which was reversed by ketamine 24 hours postinjection (red bar). No change in the bursting activity (bottom) was observed between nonhelpless rats, helpless rats, and helpless rats treated with ketamine. *$p < .05$, **$p < .01$, ***$p < .001$. Error bars are ± SEM.

$p < .05$; firing rate: $F_{1,69} = 5.323$, $p < .05$). No difference in burst firing between nonhelpless and helpless rats was observed after saline (H = 2.413, $p = .12$) or ketamine injection (H = 3.195, $p = .072$). The effect of ketamine injection on escape behavior and DA neuron recordings in nonhelpless rats is shown in Figure S3 in Supplement 1.

Therefore, ketamine injection 24 hours before recordings/behavioral task in helpless rats restored escape behavior in parallel with restoration of dopaminergic activity in the VTA.

Shell and Core Segments of the Nucleus Accumbens Show Opposite Responses to HFS of the Fimbria

To examine potential changes in synaptic transmission in the vSub-NAc pathway in helpless rats, hippocampal-evoked activity was assessed in the NAc after HFS of the fimbria (Figure 4A) (32). As previously described (19,20), fimbria HFS induced long-term potentiation (LTP) in the vSub-NAc pathway of home cage control animals (Figure 4B, C, left), whereas long-term depression (LTD) was induced in helpless rats (Figure 4C, right). The baseline evoked spike probability in home cage control animals was .47 ± .05 and increased to .60 ± .08 15 minutes post-HFS ($n = 6$; $F_{5,31} = 2.993$, $p < .05$), whereas in helpless animals, baseline spike probability was .47 ± .04 and decreased to .29 ± .07 15 minutes post-HFS ($n = 8$; $F_{7,45} = 8.095$, $p < .001$). Neurons were located either in the NAc core or shell (Figure 4D).

In nonhelpless rats, HFS to the fimbria induced two types of responses: an LTP (baseline spike probability: .46 ± .02; post-HFS: .58 ± .05; $F_{5,35} = 4.477$, $p < .05$; $n = 6$; Figure 4E, top left) or an LTD (baseline spike probability: .49 ± .03; post-HFS: .32 ± .03; $F_{5,28} = 2.925$, $p < .05$; $n = 6$; Figure 4E, bottom left). Interestingly, the majority of the electrode placements for LTP were located in the NAc shell and the majority of the LTD placements were located in the NAc core (Figure 4F). No-shock rats had the same responses, with neurons located in the NAc shell showing a significant increase of the spike probability (baseline spike probability: .53 ± .03; post-HFS: .66 ± .03; $F_{4,28} = 4.432$, $p < .01$; $n = 5$; Figure 4E, top right) and neurons in the core a significant decrease (baseline spike probability: .59 ± .07; post-HFS: .41 ± .06; $F_{4,14} = 4.828$, $p < .05$; $n = 5$; Figure 4E, bottom right).

Therefore, in the LH model, LTD in the vSub-shell NAc, but not in the vSub-core NAc, appears to be a marker of helplessness.

Ketamine Restores Long-Term Potentiation in the Hippocampus-Accumbens Pathway of Helpless Rats

When ketamine was injected 20 minutes before HFS, LTP of the vSub-NAc pathway was restored ($n = 6$; Figure 5A, left). This effect was not due to the injection, since preinjection of saline had no effect on the HFS-induced LTD ($n = 4$, data not shown). Neurons were located either in the NAc core or shell (Figure 5A, right). Therefore, besides restoring escape behavior and dopaminergic activity in the VTA, ketamine restored synaptic plasticity in the hippocampus-accumbens pathway.

The HFS-induced LTP in control rats is, at least in part, dopamine-dependent (33). Thus, in a separate group of helpless rats ($n = 6$), the D1 antagonist SCH23390 was infused into the NAc 15 minutes before recording baseline activity. In these neurons, ketamine induced a decrease of fimbria-evoked spiking activity, and after HFS of the fimbria, an LTD was observed ($F_{5,27} = 3.769$, $p < .01$; $n = 6$). This effect was not due to infusion in the NAc, since preinfusion of the vehicle Dulbecco's phosphate buffered saline had no effect on the HFS-induced LTP previously described ($n = 3$, data not shown). Therefore, ketamine restores synaptic plasticity in the NAc via activation of D1 receptors. (Figure 6)

As previously mentioned, ketamine had no effect on escape behavior in a subset of helpless rats. In these neurons, ketamine before HFS did not restore LTP of the vSub-NAc pathway and induced an LTD ($F_{4,25} = 3.385$, $p < .01$). Therefore, ketamine restores escape behavior likely via reinstatement of adequate response to fimbria HFS (synaptic plasticity) in the NAc.

Discussion

In this study, we examined the effect of repeated and acute ketamine injections in a behavioral model of depression. We find that ketamine reverses helpless behavior, restores normal DA neuron population activity, and restores LTP in the vSub-NAc pathway. Our findings indicate that a normal LTP in the NAc shell correlates with a failure to induce helplessness. Moreover, this study shows that the effect of ketamine on synaptic plasticity is, at least in part, due to activation of D1 receptors in the NAc. Therefore, our results suggest that the antidepressant action of ketamine acts via the DA system, at least in part, and the NAc shell (summarizing schematic Figure 6).

Several models of depression, such as the chronic mild stress model (30) and the LH model (9), induce anhedonia in rats, thought to involve disruptions of the DA system (8,30). In particular, inhibition of VTA DA neurons can acutely induce multiple depression-like behaviors, and activation of VTA DA neurons can rescue stress-induced depression-like phenotype (30). In the present study, helpless rats exhibited a decrease in the population activity specifically in the central tracks of the VTA, with no change in the firing rate or the bursting activity of single DA neurons. This is consistent with our study showing that chronic stress selectively decreases DA neuron population activity in the medial and central tracks of the VTA (34). Therefore, in helpless animals, a decrease in the number of spontaneously active DA neurons is consistent with a decrease in VTA activity inducing stress-induced depressive-like behavior (30).

In the present study, repeated injections of ketamine restored escape behavior in helpless rats independent of the timing of the injection (20 minutes or 2 hours), confirming the antidepressant effect of this agent at low doses. We also showed that in these rats, 20 minutes after the injection, the DA population activity in the VTA was restored to what was observed in control animals. Two hours after the injection, ketamine not only restored the population activity in helpless rats but also induced a significant increase both in helpless and nonhelpless rats, which is consistent with that observed in rodent models of psychosis (35) and might correspond to the dissociative and psychotic symptoms described in patients one half hour after intravenous infusion of ketamine (2). These restorative actions of ketamine were maintained 24 hours postinjection with respect to both decreased escape failures and restoration of VTA DA neuron activity, albeit the escape behavior remained below control levels. This is consistent with recent studies highlighting the beneficial effect of repeated doses of ketamine, in comparison with acute injection, in sustainably treating major depressive disorder in patients (3).

One of the challenges in treating depression is not only to restore sensitivity to positive rewarding events but also to diminish negative mood state. In depressed patients, ruminative self-focus is associated with hyperactivity of limbic regions such as the medial prefrontal cortex, the amygdala, and the hippocampus (36). In our study, we showed that nonhelpless rats displayed normal synaptic plasticity in the vSub-shell pathway but disruption of the vSub-core pathway. However, helpless rats

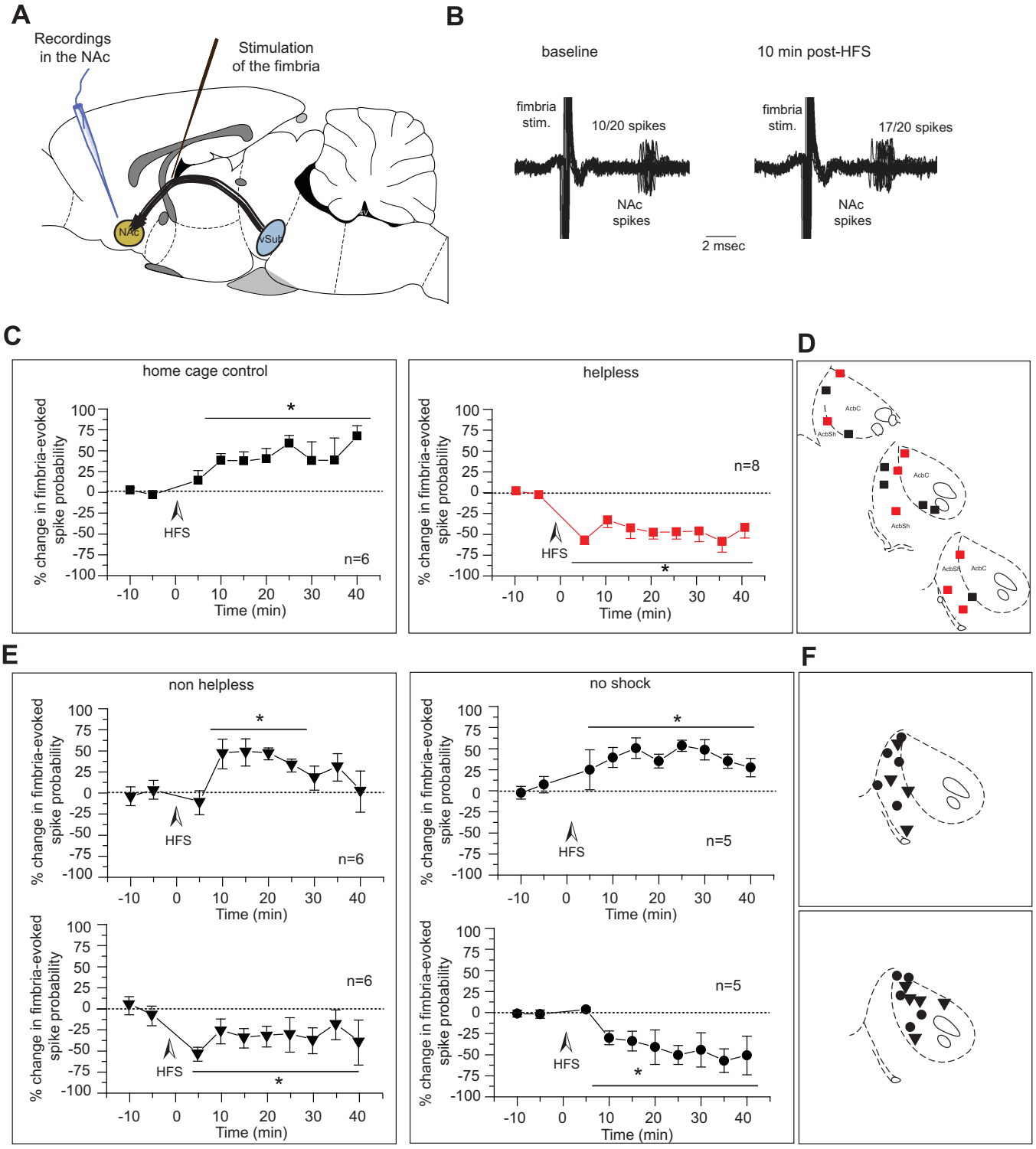

Figure 4. Shell and core segments of the nucleus accumbens (NAc) show different responses to high-frequency stimulation (HFS) of fimbria in helpless versus nonhelpless rats. **(A)** Schematic of recording and stimulating electrode placements. **(B)** Extracellular recording trace showing a representative example of the increased fimbria-evoked spike probability recorded from a NAc neuron in a control animal 10 minutes after high frequency stimulation (stim.). Twenty overlaid consecutive traces are shown with the numbers demonstrating the number of evoked spikes for 20 stimulations. **(C)** High-frequency stimulation of the fimbria produced long-term potentiation in control rats (black squares) but produced long-term depression in helpless rats (red squares). **(D)** Recording electrode placements in the NAc of home cage control (black squares) and helpless rats (red squares) animals shown as coronal sections of the rat brain. **(E)** High-frequency stimulation of the fimbria produced long-term potentiation in the accumbens shell in nonhelpless and no-shock rats (top) but produced long-term depression in the accumbens core (bottom). Plots show mean percent change (± SEM) in fimbria-evoked spike probability, normalized to the baseline. **(F)** Recording electrode placements in the NAc of nonhelpless (triangles) and helpless rats (circles), shown as coronal sections of the rat brain. *$p < .05$; arrow indicates the time of stimulation. AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell; vSub, ventral subiculum of the hippocampus. [Reproduced with permission from Paxinos and Watson (51)].

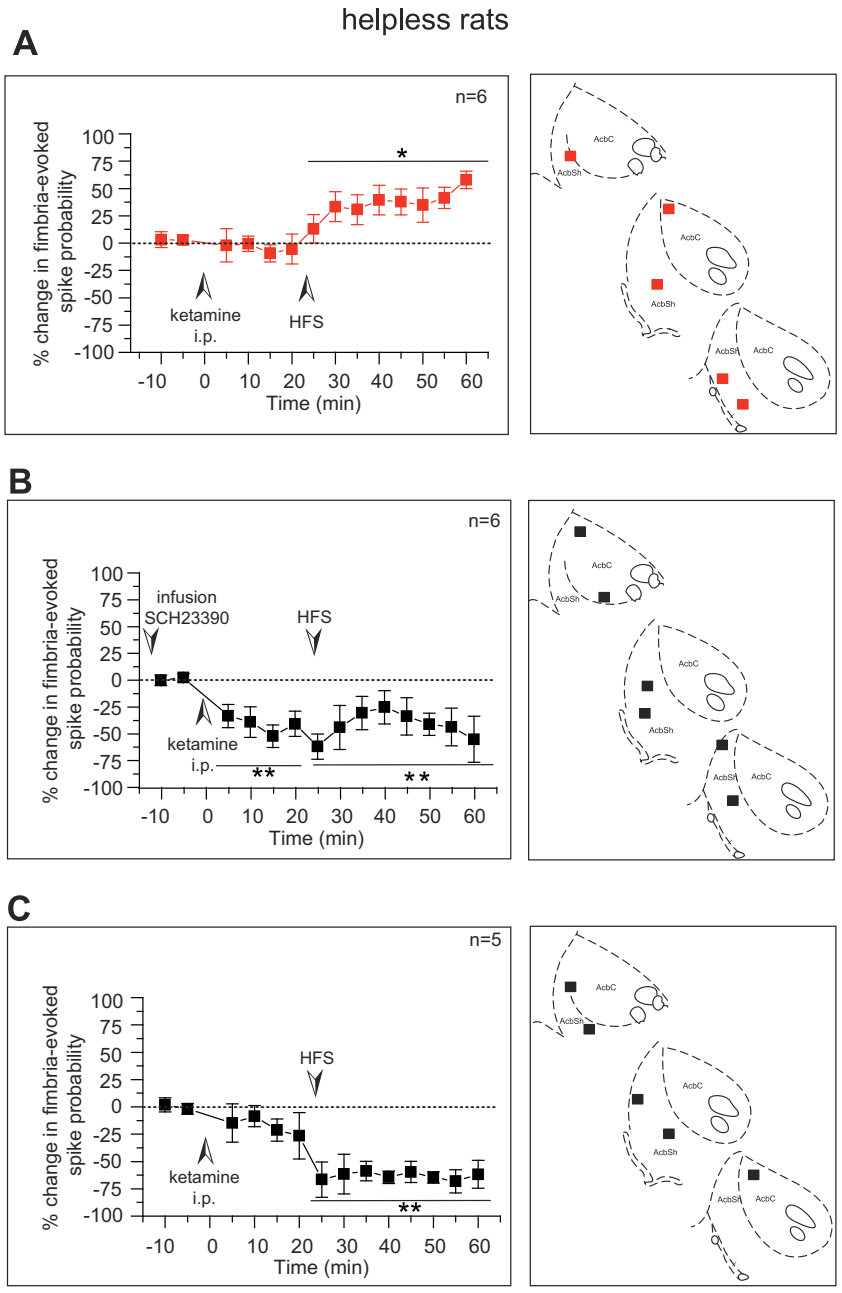

Figure 5. Ketamine restores long-term potentiation (LTP) in the hippocampus-accumbens pathway of helpless rats that depends on D1 receptors. Left: **(A)** Injection of ketamine (intraperitoneal [i.p.] 5 mg/kg) in helpless animals restored fimbria high-frequency stimulation (HFS)-induced LTP in the accumbens shell and **(B)** infusion of the D1 antagonist SCH23390 in the nucleus accumbens (.5 μg/.5 μL) prevented ketamine (i.p. 5 mg/kg) from restoring LTP in the fimbria-nucleus accumbens pathway. **(C)** Ketamine did not affect HFS-induced long-term depression in rats that did not show behavioral improvement. Plots show mean percent change (± SEM) in fimbria-evoked spike probability, normalized to baseline. Right: recording electrode sites shown as coronal sections of the rat brain. $*p < .05$, $**p < .01$. AcbC, nucleus accumbens core; AcbSh, nucleus accumbens shell. [Reproduced with permission from Paxinos and Watson (51)]

showed disruption of plasticity in both the vSub-shell and vSub-core pathways. All groups of rats received footshocks during escape behavior testing; therefore, this footshock stress appears to correspond to vSub-core LTD across groups. Indeed, stress can enhance synaptic strength (37) and elevate DA levels (38) in the NAc shell but not the core (37,38). In contrast, helpless rats differed from nonhelpless and no-shock rats by an LTD in the vSub-shell pathway. Thus, in no-shock and nonhelpless rats, LTP in the vSub-shell pathway produced by footshock-induced stress may protect them from helpless behavior. Ketamine injected before HFS restored normal plasticity in the vSub-shell and vSub-core of helpless rats. Although it is not clear why an NMDA antagonist like ketamine would restore LTP, ketamine induces an increase in glutamate and aspartate release by a presynaptic action (39). This increase may then activate glutamatergic neurotransmission at non-NMDA receptors, including α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors. Ketamine also increases the release of DA (40) in the striatum, which is a potent modulator of synaptic plasticity (41). Therefore, ketamine could act to increase LTP via an indirect activation of AMPA and DA D1 receptors, since blockade of D1 receptors prevents ketamine actions in helpless rats. Consistent with previous studies (33), ketamine or SCH23390 injected alone have no effect on the basal fimbria-evoked spiking activity in the NAc. However, with preinfusion of SCH23390, ketamine had an inhibitory effect, consistent with studies showing that D1–NMDA interactions in the NAc affect the excitability of NAc neurons (42).

In contrast, when ketamine has no effect on escape deficit in helpless rats, it does not restore normal synaptic plasticity in the vSub-NAc pathway, suggesting that normal plasticity in the

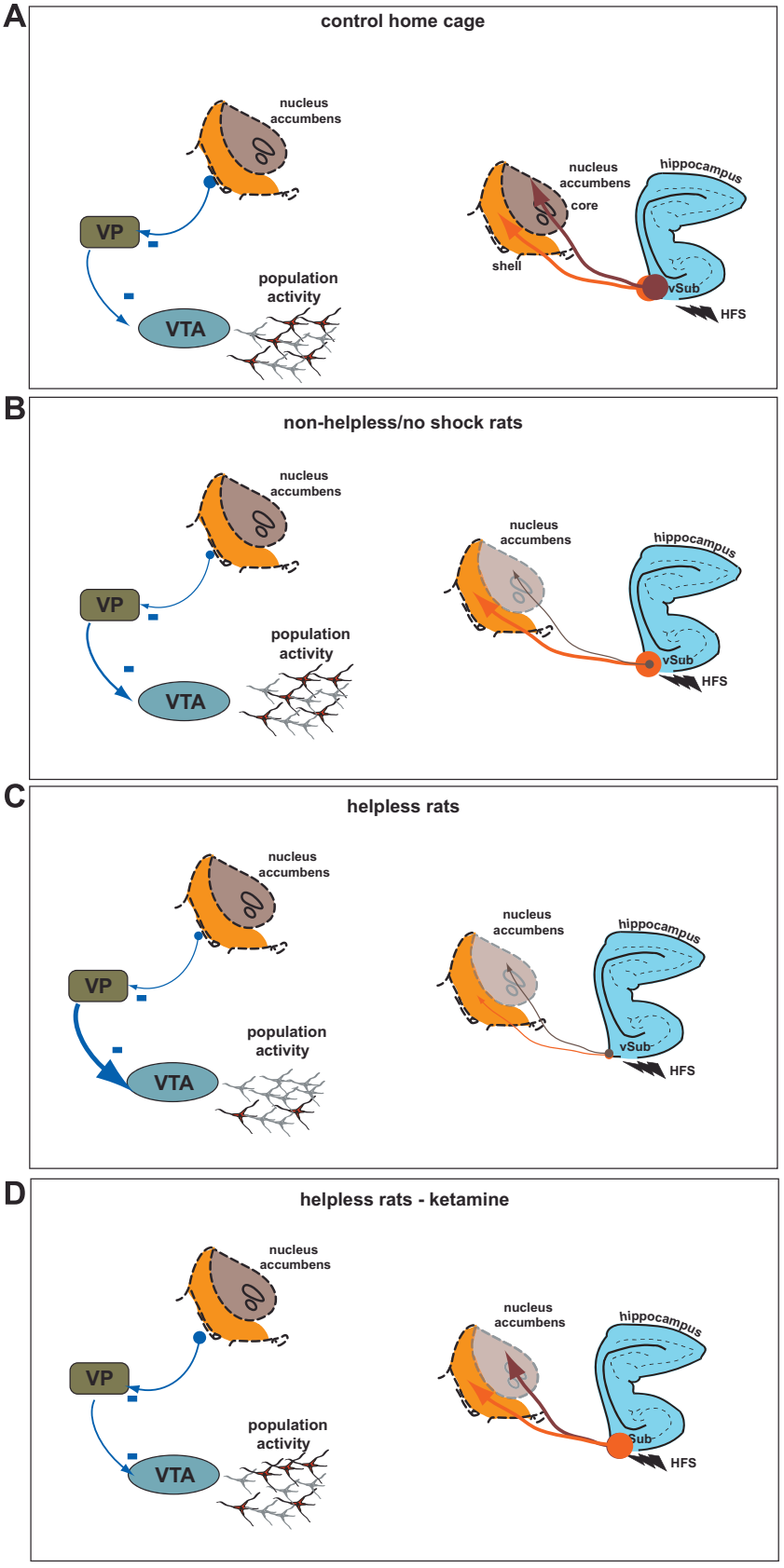

Figure 6. Summarizing schematic. Schematic summarizing the population activity in the ventral tegmental area (VTA) (left) and effect of high-frequency stimulation (HFS) on the ventral subiculum of the hippocampus (vSub)-nucleus accumbens (NAc) shell and core (right). **(A)** In home cage control rats, HFS of the vSub produces long-term potentiation (LTP) in the NAc core and shell (right). **(B)** Following inescapable shock, rats that did not show helplessness showed HFS-induced long-term depression (LTD) in the vSub-NAc core projection, with the vSub-shell projection showing normal HFS-induced LTP (right) and a dopamine (DA) neuron population activity comparable with control rats. **(C)** In contrast, in helpless rats, the vSub-shell and core pathways show LTD in response to HFS (right), which corresponds to a decrease in DA neuron population activity (left). **(D)** Following injection of ketamine, both DA neuron activity (left) and vSub-NAc LTP (right) are restored in both core and shell regions in helpless rats. Thin arrow, LTD; thick arrow, LTP. VP, ventral pallidum.

vSub-NAc pathway is necessary for escape behavior. This is also consistent with recent studies (29,43) showing that ketamine rapidly increases m-Tor-dependent synaptogenesis in the prefrontal cortex (4,6) and the hippocampus (5) and increases hippocampal brain-derived neurotrophic factor and mammalian target of rapamycin levels during the forced swim test in rats (44). Moreover, the rapid and sustained antidepressant-like effect of ketamine in the LH model also involves stimulation of AMPA receptors (45). Since LTD involves internalization of AMPA receptors (46), it is feasible that, in response to HFS, ketamine reverses the altered synaptic plasticity of helpless rats via increased glutamate and DA release and activation of AMPA receptors. In the social defeat stress model, a decrease in AMPA function in the NAc is proposed to mediate resilience (47). However, in the present study, the effect of ketamine was examined after LTP induction, which by itself induces changes in AMPA function (48). Moreover, ketamine has been injected IP, which we expect will have an effect on structures other than the NAc such as the hippocampus, the prefrontal cortex (4,5), or the basolateral amygdala (49), which play an important role in depressive-like behavior (50) and have a critical influence on synaptic plasticity in the NAc.

Taken together, the present data provide strong evidence that both repeated and acute injections of ketamine may be effective in reversing depression symptoms by restoring an abnormally attenuated dopaminergic population activity, which we predict will reverse hyposensitivity to rewarding events. Ketamine may also be effective in treating ruminative behavior by restoring normal information processing in the vSub-NAc shell pathway via activation of D1 receptors in the NAc. These two processes may be related, in that the decrease in vSub-NAc drive could contribute to the decrease in DA neuron activity, since activation of this circuit increases DA neuron population activity (23,35). Such information is a significant step toward the understanding of the mechanisms of ketamine at a systems level for the treatment of depression and may point to a direction for more effective treatments.

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