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Mushroom spine dynamics in medium spiny neurons of dorsal striatum associated with memory of moderate and intense training

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A growing body of evidence indicates that treatments that typically impair memory consolidation become ineffective when animals are given intense training. This effect has been obtained by treatments interfering with the neural activity of several brain structures, including the dorsal striatum. The mechanisms that mediate this phenomenon are unknown. One possibility is that intense training promotes the transfer of information derived from the enhanced training to a wider neuronal network. We now report that inhibitory avoidance (IA) induces mushroom spinogenesis in the medium spiny neurons (MSNs) of the dorsal striatum in rats, which is dependent upon the intensity of the foot-shock used for training; that is, the effect is seen only when high-intensity foot-shock is used in training. We also found that the relative density of thin spines was reduced. These changes were evident at 6 h after training and persisted for at least 24 h afterward. Importantly, foot-shock alone did not increase spinogenesis. Spine density in MSNs in the accumbens was also increased, but the increase did not correlate with the associative process involved in IA; rather, it resulted from the administration of the aversive stimulation alone. These findings suggest that mushroom spines of MSNs of the dorsal striatum receive afferent information that is involved in the integrative activity necessary for memory consolidation, and that intense training facilitates transfer of information from the dorsal striatum to other brain regions through augmented spinogenesis.

dendritic spines | dorsal striatum | accumbens | memory consolidation | intense training

A growing body of evidence indicates that treatments that commonly produce amnesia become ineffective when animals are given enhanced training (i.e., a high number of training sessions or relatively high levels of aversive stimulation). This protective effect against amnesia induced by interference with normal activity of brain nuclei has been found in a wide variety of learning tasks (1). Extensive evidence indicates that the dorsal striatum is intimately involved in the acquisition, consolidation, and retrieval memory for many kinds of training experiences (2–5). Interference with cholinergic activity of the dorsal striatum and reversible inactivation of this structure induced after moderate training impair memory consolidation. However, notably, these treatments are ineffective in impairing memory when animals are overtrained (6–10). Such findings suggest that intense training may induce functional changes within the dorsal striatum that prevent the impairment of memory. Although the dorsal striatum seems histologically homogeneous, there is a functional differentiation along its medial-lateral axis related to memory consolidation (11). Prior studies have shown that the dorsomedial striatum (DMS) is predominantly involved in spatial/contextual learning, influencing goal-directed behaviors (12, 13). In contrast, the dorsolateral striatum (DLS) enables the formation of procedural learning (4, 14).

Previous research suggests that memory consolidation may involve changes in the density and morphology of dendritic spines in the hippocampus, amygdala, and cerebral cortex (15–23). Regarding striatal spinogenesis, it has been reported that rearing rats in a rich environment produced an increase in spine density in medium spiny neurons (MSNs), which is indicative of experience-dependent neuronal modifications in this region (24, 25).

Other lines of research have shown that stressful events induce the release of corticosterone, which facilitates memory consolidation of a variety of learning tasks, and that corticosterone release correlates positively with the intensity of training (26–32). There is compelling evidence that activation of corticosterone receptors in the dorsal striatum, amygdala, hippocampus, and insular cortex facilitate memory consolidation of inhibitory avoidance (IA) learning (33–36).

Although there are no reports on the mechanisms underlying synaptogenesis in the striatum, related work shows that corticosterone administration to dorsal hippocampal slices induces dose-dependent spinogenesis within the first hour after treatment. This effect was impeded by blocking NMDA receptors and PI3K, MAPK, PKC, or PKA (37). Similarly, dexamethasone, a specific agonist of glucocorticoid receptor, increased the density of mushroom and thin spines, also within 1 h. These effects were blocked by RU-38486, an antagonist of the glucocorticoid receptor (38). Thus, these quick effects on mushroom genesis indicate that they are not genomic and that spinogenesis was induced via membrane-localized glucocorticoid receptors (39) and multiple kinase pathways (40–42).

Based on the foregoing, we hypothesized that in both DMS and DLS, intense training of IA would induce enhanced genesis of mushroom spines, which are thought to be a substrate of

Significance

Interference with activity of cerebral structures, including the dorsal striatum, produces amnesia after moderate levels of training; such interference, nonetheless, becomes ineffective when animals are subjected to intense training procedures. The mechanisms that mediate the protective effect of intense training are unknown. This report shows an increase in relative density of mushroom spines in medium spiny neurons (MSNs) of the dorsal striatum, which depends upon the intensity of training; relative density of mushroom spines in MSNs of nucleus accumbens, on the other hand, is augmented after noncontingent administration of a foot-shock alone. These findings suggest that increased mushroom spinogenesis produced by intense training strengthens memory consolidation and facilitates transfer of information from the dorsal striatum to other cerebral structures.

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long-term memory (LTM) because of their longevity and structural and functional characteristics (43–45). If such spinogenesis occurred, then it might represent the basis of the protective effects of intense training against amnestic treatments that are administered to the dorsal striatum, as is the case with the administration of cholinergic and sodium channel blockers (8–10), which impede the activation of acetylcholine receptors and the production of action potentials, respectively, therefore inhibiting synaptic transmission. Thus, increased mushroom spine production could constitute a mechanism to store and relay information derived from the enhanced learning experience; by contrast, low or moderate levels of training would induce a limited increase of mushroom spines, which would not be sufficient to overcome the amnestic effects of treatments that interfere with neuronal activity of the dorsal striatum. Once normal activity is recovered, the increased density of mushroom spines would allow for strengthened consolidation of the task and for facilitated afferent activation of MSNs from other regions of the brain. In turn, when the animals are later tested for retention, afferent signals to the dorsal striatum would more easily activate MSN mushroom spines and their efferent connections.

The aim of this study was to determine whether low and intense foot-shock used in IA training induces distinct patterns of changes in dendritic spine density and morphology in the dorsal striatum. It was expected that both the DMS and DLS regions of the dorsal striatum would show the same patterns of spine dynamics because the IA task entails both spatial and procedural components (46). It was also expected that stronger learning would induce additional changes in spine density and morphology than weaker learning.

Results

Consolidation and LTM. Because one of the main objectives of this study was to evaluate possible changes in spine density and shape after moderate and intense training, it was important to demonstrate that training with 3.0 mA produces stronger learning than training with 1.0 mA. To this end, we measured resistance to extinction, which is stronger when a learning experience is also stronger. We found that animals trained with 3.0 mA took significantly longer to achieve extinction than animals trained with 1.0 mA (Fig. S1*A*).

We then proceeded to study dendritic spine dynamics associated with consolidation and LTM of moderate and enhanced IA training. Independent groups of rats were trained with 0.0, 1.0, or 3.0 mA (groups T-0, T-1, and T-3, respectively); half of the groups were tested for retention at 6 h, and the other half were tested at 24 h after training. These retention intervals have been shown to fall within the limits of consolidation and LTM of IA in the rat, respectively (47).

At the 6-h interval, significant differences in retention latencies among the groups became evident [$H_{(2)} = 24.03$, $P < 0.0001$]; pairwise comparisons yielded no significant differences between T-1 and T-3, which had perfect retention scores (600 s), but each of them differed from T-0 (24.5-s retention score) ($P < 0.001$ for each comparison). Essentially the same results were found at 24 h after training. A statistical difference was found in retention latencies among the groups [$H_{(2)} = 26.02$, $P < 0.0001$]. T-1 and T-3 had top scores, significantly higher than the score of T-0 (21.8-s retention score) ($P < 0.001$ for each comparison) (Fig. S1*B*).

Golgi–Cox Staining. Immediately after retention testing, the brains of these rats were processed according to the Golgi–Cox procedure (an explanation of the detailed procedure may be found in *SI Materials and Methods*). In addition to T-0, T-1, and T-3, two other groups were studied to factor out the effect of the foot-shock on spine dynamics. The animals of one control group were put inside the shock compartment, and a 5.6-s foot-shock of 3.0 mA (median duration of foot-shock received by the animals that had been trained with 3.0 mA) was delivered [shock-only (SO) group ($n = 8$)]; half of the animals were killed 6 h later, and the other half were killed 24 h later. To determine the density and types of dendritic spines in basal conditions, the animals of the other control group ($n = 4$) were kept under identical living conditions as the animals used for the behavioral study, but they never left the bioterium, except for euthanasia [intact (INT) group]. Fig. 1 shows photomicrographs of a representative Golgi–Cox-stained slice with MSNs from DLS, DMS, nucleus accumbens core (NAcC), and nucleus accumbens shell (NAcS), as well as the different types of spines that were analyzed.

Spine Densities in Dorsal and Ventral Striatum. To find out if the spine populations in basal conditions were different across the striatal regions, we performed an analysis of spine density in DLS, DMS, NAcC, and NAcS of the INT group. There were significant differences among the four regions [$F_{(3,15)} = 5.62$, $P < 0.05$]. The Bonferroni test showed that NAcC had significantly higher spine density (18.84 ± 2.26) than NAcS (15.56 ± 0.67), DMS (15.13 ± 1.44), and DLS (14.95 ± 1.38) ($P < 0.05$ for each comparison) and that the latter three did not differ from each other (INT groups in Fig. 2). These results agree well with the results of Meredith et al. (48), who also found a higher density of spines in NAcC than in NAcS.

Changes in Spine Density in Dorsolateral Striatum. Significant differences in spine density among the groups were found during the consolidation period [$F_{(4,15)} = 18.206$, $P < 0.0001$]. Pairwise comparisons revealed that there were no significant differences between the INT and SO groups. INT had a significantly lower spine density than T-0, T-1, and T-3 ($P < 0.05$, $P < 0.005$, and $P < 0.0001$, respectively). SO had fewer spines than T-0, T-1, and T-3 ($P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively). T-0 did not differ from T-1, but it had a lower spine density than T-3 ($P < 0.001$ and $P < 0.0001$, respectively). By the same token, there were significant differences in spine density 24 h after training [$F_{(4,15)} = 7.676$, $P = 0.005$]. There were no significant differences between INT and SO, and the former had fewer spines than T-0, T-1, and T-3 ($P < 0.05$, $P < 0.01$, and $P < 0.0001$, respectively); SO, T-0, and T-1 did not differ from each other, but they had a lower density of spines

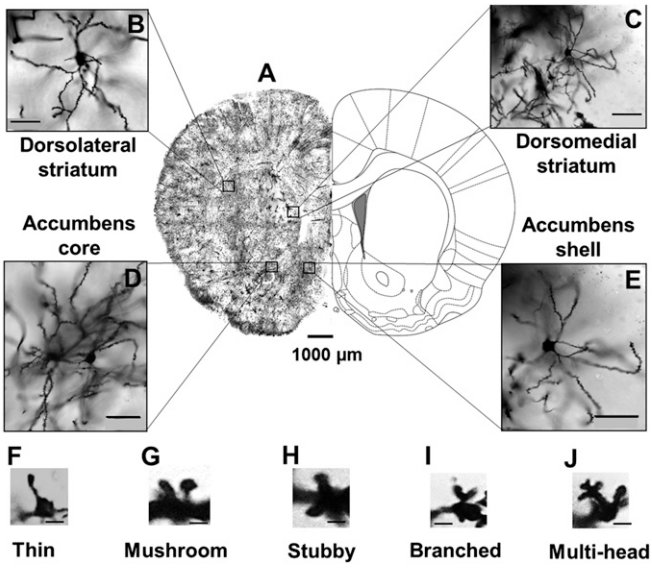

Fig. 1. Morphometric analysis. (*A*) Representative coronal slice impregnated with the Golgi–Cox stain. (*Insets*) Representative MSNs from the four regions that were analyzed. The DLS (*B*), DMS (*C*), NAcC (*D*), and NAcS (*E*) are shown. (Magnification: 40×, 0.8 N.A.) Also shown are microphotographs of the different types of spines that were studied: thin (*F*), mushroom (*G*), stubby (*H*), branched (*I*), and multihead (*J*). (Magnification: 100×, 1.25 N.A.) (Scale bar: 2 µm.)

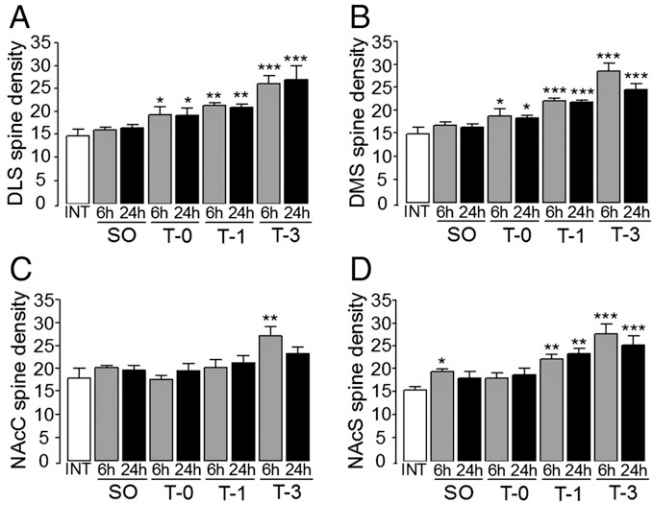

Fig. 2. Dendritic spine density in dorsal and ventral striatum observed during consolidation (6 h) and LTM (24 h) of IA training. The DLS (*A*), DMS (*B*), NAcC (*C*), and NAcS (*D*) are shown. The T-0, T-1, and T-3 groups were trained with 0.0 mA, 1.0 mA, and 3.0 mA, respectively. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$ vs. INT. Each bar represents mean ± SEM of 40 MSNs, obtained from four rats in each condition.

than T-3 ($P < 0.0005$, $P < 0.01$, and $P < 0.05$, respectively) (Fig. 2*A*). The two-way ANOVA (treatment × retention interval) showed that there was not a significant interaction between these two variables [$F_{(3,24)} = 0.09$, $P = 0.97$].

Changes in Spine Density in Dorsomedial Striatum. There were significant changes in dendritic spine density during the consolidation stage (i.e., at the 6-h retention interval [$F_{(4,15)} = 21.120$, $P < 0.0001$]). The Bonferroni test revealed that INT did not differ from SO. On the other hand, T-0, T-1, and T-3 showed a significant increase in spine density compared with INT ($P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively); T-3 also showed more spines than SO, T-0, and T-1 ($P < 0.0001$, $P < 0.0001$, and $P < 0.05$, respectively); and T-1 had a higher spine count than SO ($P < 0.05$). Practically the same differences observed among the groups at 6 h also occurred 24 h after training (LTM) [$F_{(4,15)} = 15.93$, $P < 0.0001$]. The post hoc analyses showed that INT did not differ from SO, whereas a higher spine density was observed in T-0, T-1, and T-3 compared with INT ($P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively). T-3 also had a higher spine density than SO and T-0 ($P < 0.0001$ and $P < 0.001$, respectively) (Fig. 2*B*). The two-way ANOVA (treatment × retention interval) indicated that the interaction between these two variables was not significant [$F_{(3,24)} = 1.73$, $P = 0.19$], indicating that there were no significant differences between the 6-h and 24-h groups in any of the treatment conditions.

Changes in Spine Density in Accumbens Core. There were significant changes in dendritic spine density at the 6-h retention interval [$F_{(4,15)} = 5.49$, $P < 0.01$]. The Bonferroni test showed that the spine density of T-3 was significantly higher than the rest of the groups (*P* values ranging between 0.05 and 0.0005); comparisons between the remaining pairs of groups were not significant. The ANOVA showed no significant differences in the groups at the 24-h interval [$F_{(4,15)} = 1.61$, $P = 0.22$] (Fig. 2*C*). The treatment × retention interval interaction was not significant [$F_{(3,24)} = 1.56$, $P = 0.23$].

Changes in Spine Density in Accumbens Shell. Dendritic spine density changed significantly among the groups during consolidation [$F_{(4,15)} = 14.65$, $P < 0.0001$]. There were no significant differences between INT and T-0, but INT had fewer spines than SO, T-1, and T-3 ($P < 0.05$, $P < 0.001$, and $P < 0.0001$, respectively); SO had fewer spines than T-3 ($P < 0.005$); T-0 had a lower score than T-1 ($P < 0.05$) and T-3 ($P < 0.0001$); and T-3 had more spines than T-1 ($P < 0.01$). Lastly, the comparisons between SO and T-0 and between SO and T-1 did not reach statistical significance. At the 24-h interval, the ANOVA also showed a significant difference among groups [$F_{(4,15)} = 8.38$, $P < 0.001$]. The post hoc analyses showed that INT did not differ from SO and T-0, but it had a lower spine density than T-1 ($P < 0.001$) and T-3 ($P < 0.001$). SO had a lower score than T-1 ($P < 0.05$) and T-3 ($P < 0.005$), but not T-0; T-0 also had a lower spine ratio than T-1 ($P < 0.05$) and T-3 ($P < 0.005$). T-1 and T-3 did not differ from each other (Fig. 2*D*). The treatment × retention interval interaction was not significant [$F_{(3,24)} = 0.71$, $P = 0.56$].

After having analyzed the changes in striatal spine density produced by the different experimental and control manipulations, we went on to investigate changes in spine morphology that might be produced by such manipulations.

Thin Spine Ratios in Dorsolateral Striatum. There were significant differences among the groups regarding spine ratios at the 6-h interval [$F_{(4,15)} = 8.77$, $P < 0.001$]. The Bonferroni analysis showed that INT did not differ significantly from SO and T-0; INT had a higher ratio than T-1 ($P < 0.01$) and T-3 ($P < 0.005$). T-0 was not significantly different from T-1, but it had a higher score than T-3 ($P < 0.01$). T-1 and T-3 did not differ from each other. When spine ratio was analyzed at the 24-h interval, significant differences among the groups were found [$F_{(4,15)} = 19.47$, $P < 0.0001$]. Pairwise comparisons revealed that INT did not differ significantly from SO, and each of these two groups had a higher ratio than T-0, T-1, and T-3 (*P* values ranging from 0.005 to 0.0001). T-0 did not differ from T-1, but it had a higher ratio of spines than T-3 ($P < 0.05$). T-3 and T-1 did not differ from each other (Fig. 3*A*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [($F_{(3,24)} = 0.40$, $P = 0.75$].

Mushroom Spine Ratios in Dorsolateral Striatum. The result of the ANOVA indicated significant differences among the groups at the 6-h interval [$F_{(4,15)} = 43.83$, $P < 0.0001$]. Pairwise comparisons showed that INT did not differ significantly from SO, and that these two groups had lower spine ratios than the rest of the groups (*P* values ranging from 0.05 to 0.0001). T-0 had a significantly lower spine ratio than T-1 ($P < 0.05$) and T-3 ($P < 0.001$), and T-3 had a higher spine ratio than T-1 ($P < 0.05$). At the 24-h interval, significant differences among the groups appeared [$F_{(4,15)} = 8.91$, $P < 0.001$]. INT and SO were not significantly different, and their low ratio differed significantly from T-0, T-1, and T-3 (*P* values ranging from 0.005 to 0.0001). Lastly, T-0, T-1, and T-3 did not differ significantly from each other (Fig. 3*B*). The two-way ANOVA showed a nonsignificant treatment × retention interval interaction [$F_{(3,24)} = 1.77$, $P = 0.18$].

Stubby Spine Ratios in Dorsolateral Striatum. There were no significant differences in stubby spine ratios among groups at the 6-h interval [$F_{(4,15)} = 1.02$, $P = 0.43$] or at the 24-h interval [$F_{(4,15)} = 2.21$, $P = 0.12$] (Fig. 3*C*). The two-way ANOVA showed a nonsignificant treatment × retention interval interaction [$F_{(3,24)} = 1.29$, $P = 0.30$].

Branched Spine Ratios in Dorsolateral Striatum. The one-way ANOVA showed that there were significant differences among groups at 6 h after training [$F_{(4,15)} = 54.56$, $P < 0.0001$]. INT, SO, and T-0 did not differ significantly from each other, and each of these groups had significantly lower scores than T-1 and T-3 (*P* values ranging from 0.05 to 0.0001); T-3 had a higher score than T-1 ($P < 0.0001$). The data collected at the 24-h interval yielded a significant treatment effect [$F_{(4,15)} = 3.43$, $P < 0.05$]. T-3 showed a significant difference compared with each of the other groups

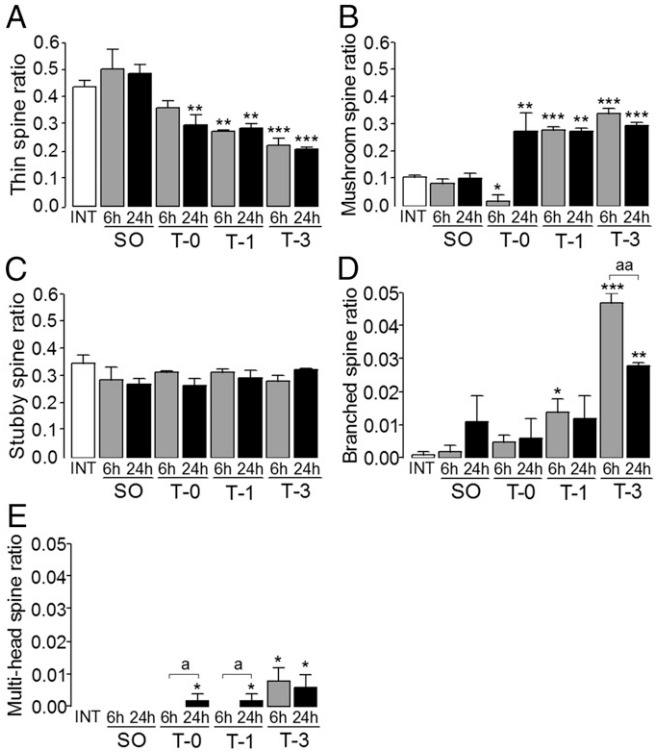

Fig. 3. Dendritic spine types in DLS: thin (*A*), mushroom (*B*), stubby (*C*), branched (*D*), and multihead (*E*). The T-0, T-1, and T-3 groups were trained with 0.0 mA, 1.0 mA, and 3.0 mA, respectively. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$ vs. INT. a, $P < 0.05$ between groups; aa, $P < 0.01$ between groups. Note differences in abscissa scales. Each bar represents mean ± SEM of 40 MSNs, obtained from four rats in each condition.

(P values ranging between 0.05 and 0.0005) (Fig. 3*D*). The two-way ANOVA indicated a significant treatment × retention interval interaction [$F_{(3,24)} = 3.04$, $P < 0.05$]. The Bonferroni test showed that in T-3, there was a higher ratio of branched spines at the 6-h interval than at the 24-h interval ($P < 0.05$).

Multihead Spine Ratios in Dorsolateral Striatum. There was a significant treatment effect at the 6-h interval [$F_{(4,15)} = 2.96$, $P < 0.005$]. The post hoc test showed that the only significant differences were between T-3 and each of the rest of the groups (P values ranging between 0.005 and 0.0005). When the spine ratio was analyzed at the 24-h interval, significant differences among the groups were found [$F_{(4,15)} = 1.35$, $P < 0.05$]. The ratio of multihead spines shown by INT did not differ from SO, but each of these groups had significantly lower scores than T-0, T-1, and T-3 (P values ranging between 0.05 and 0.0005). T-0 was not statistically different from T-1 and T-3, and the latter had a higher score than T-1 ($P < 0.05$) (Fig. 3*E*). The two-way ANOVA indicated a significant treatment × retention interval interaction [$F_{(3,24)} = 3.72$, $P < 0.05$], as shown by the higher relative ratio of spines at the 24-h interval relative to the 6-h interval in T-0 ($P < 0.05$) and in T-1 ($P < 0.05$).

Thin Spine Ratios in Dorsomedial Striatum. There were significant changes in the ratio of thin spines in DMS during consolidation [$F_{(4,15)} = 18.88$, $P < 0.0001$]. The Bonferroni analysis showed that INT and SO did not differ significantly from each other and that they had a higher ratio than T-0, T-1, and T-3 (P values ranging between 0.05 and 0.0001); T-0 also showed more thin spines than T-1 ($P < 0.01$) and T-3 ($P < 0.0005$), although the latter two did not differ from each other. The ANOVA also demonstrated a significant treatment effect at the 24-h interval [$F_{(4,15)} = 43.72$, $P < 0.0001$]. At this interval, INT and SO did not show significant differences; in turn, each of them had significantly higher scores than the rest of the groups ($P < 0.0001$ for each comparison). T-0 did not differ from T-1, but it had a higher thin spine ratio than T-3 ($P < 0.005$); T-3 also differed from T-1 ($P < 0.05$) (Fig. 4*A*). The two-way ANOVA indicated that the foot-shock intensity × retention interval interaction was not significant [$F_{(3,24)} = 0.92$, $P = 0.44$].

Mushroom Spine Ratios in Dorsomedial Striatum. The result of the ANOVA indicated significant differences among the groups at the 6-h interval [$F_{(4,15)} = 22.89$, $P < 0.0001$]. Pairwise comparisons revealed that INT did not differ from SO; each of these two groups showed a lower ratio than T-0, T-1, and T-3 (P values ranging between 0.05 and 0.0001). The mushroom spine ratio of T-0 was lower than T-1 ($P < 0.05$) and T-3 ($P < 0.0001$), and the latter differed from T-1 ($P < 0.05$). The same pattern of results emerged at the 24-h interval [$F_{(4,15)} = 48.80$, $P < 0.0001$]. There were no significant differences between INT and SO, and each of them had a significantly lower ratio than T-0, T-1, and T-3 ($P < 0.0001$ for each comparison). T-0 did not differ from T-1, but it differed from T-3 ($P < 0.005$); T-3 had a significantly higher score than T-1 ($P < 0.05$) (Fig. 4*B*). The two-way ANOVA indicated that there was a nonsignificant foot-shock intensity × retention interval interaction [$F_{(3,24)} = 0.94$, $P = 0.44$].

Stubby Spine Ratios in Dorsomedial Striatum. There were no significant differences among groups in the ratio of stubby spines 6 h after training [$F_{(4,15)} = 1.12$, $P = 0.38$] or 24 h after training [$F_{(4,15)} = 0.79$, $P = 0.55$] (Fig. 4*C*). The two-way ANOVA indicated that there was no significant interaction between foot-shock intensity × retention interval [$F_{(3,24)} = 0.24$, $P = 0.87$].

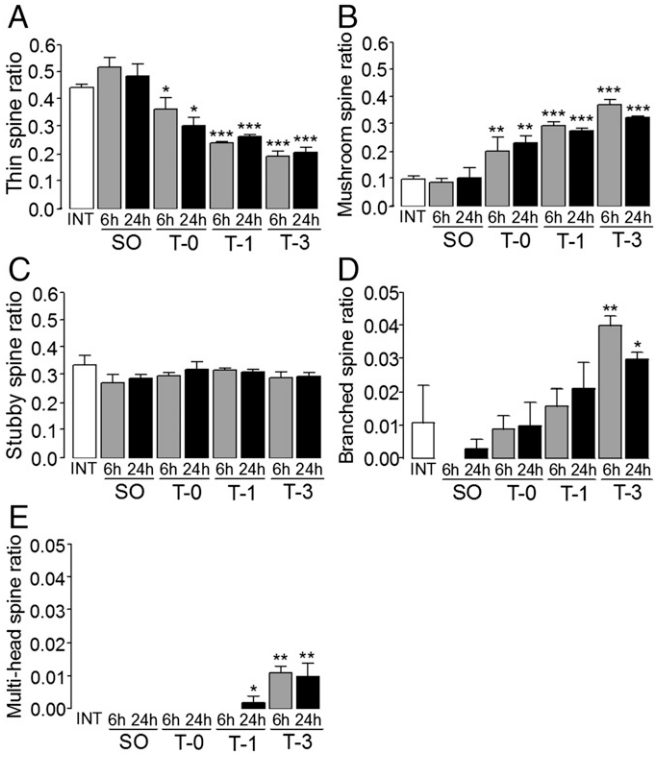

Fig. 4. Dendritic spine types in DMS: thin (*A*), mushroom (*B*), stubby (*C*), branched (*D*), and multihead (*E*). The T-0, T-1, and T-3 groups were trained with 0.0 mA, 1.0 mA, and 3.0 mA, respectively. $*P < 0.05$, $**P < 0.01$, $***P < 0.0001$ vs. INT. Note differences in abscissa scales. Each bar represents mean ± SEM of 40 MSNs, obtained from four rats in each condition.

Branched Spine Ratios in Dorsomedial Striatum. There were significant differences among groups regarding the ratio of branched spines during consolidation [$F_{(4,15)} = 6.95$, $P < 0.005$]. There were no significant differences between any of the groups, except for T-3, which had a higher ratio that differed significantly from the rest of the groups (P values ranging between 0.005 and 0.0001). Significant differences among groups were also found at the 24-h interval [$F_{(4,15)} = 8.89$, $P < 0.05$]. Pairwise comparisons revealed that the only significant differences were the differences between T-3 compared with INT ($P < 0.05$) and with SO ($P < 0.0001$) (Fig. 4*D*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 1.05$, $P = 0.39$].

Multihead Spine Ratios in Dorsomedial Striatum. The one-way ANOVA showed that there were significant differences among groups 6 h after training [$F_{(4,15)} = 19.38$, $P < 0.0001$]. Pairwise comparisons proved that the only significant differences were the differences between T-3, which had a higher ratio of this type of spine, and each of the rest of the groups ($P < 0.0005$ for each comparison). At the 24-h interval, there were also significant differences among groups [$F_{(4,15)} = 3.64$, $P < 0.05$]. T-3 had a higher score than the rest of the groups (P values ranging from 0.005 to 0.0005), and T-1 also scored higher than INT and T-0 ($P < 0.05$ for both comparisons) (Fig. 4*E*). The two-way ANOVA indicated that there were no significant differences produced by the treatment × retention interval interaction [$F_{(3,24)} = 0.27$, $P = 0.85$].

Thin Spine Ratios in Accumbens Core. There were significant changes in the ratio of thin spines in NAcC during consolidation [$F_{(4,15)} = 7.29$, $P < 0.005$]. Pairwise comparisons proved that the thin spine ratio was significantly higher in INT than in SO, T-1, and T-3, the foot-shocked groups (P values ranging between 0.005 and 0.0001), but it did not differ from T-0, the nonshocked group. SO had a lower spine ratio than T-0 ($P < 0.05$), and it did not differ significantly from T-1 and T-3; spine ratios were not significantly different between T-0 and T-1 or between T-1 and T-3, but there was a higher ratio in T-0 than in T-3 ($P < 0.01$). Measurements made at the 24-h interval revealed significant differences among groups [$F_{(4,15)} = 6.47$, $P < 0.005$]. As in the case of the evaluation at the 6-h interval, INT had a significantly higher thin spine ratio than SO, T-1, and T-3 (P values ranging between 0.005 and 0.0001), but it did not differ from T-0. SO had a lower spine ratio than T-0 ($P < 0.001$) and T-1 ($P < 0.001$); T-0 and T-1 did not differ from each other, although the former had a higher spine ratio than T-3 ($P < 0.01$). There were no significant differences between T-1 and T-3 (Fig. 5*A*). The treatment × retention interval interaction was not significant [$F_{(3,24)} = 0.40$, $P = 0.75$].

Mushroom Spine Ratios in Accumbens Core. The results of the ANOVA indicated significant differences among the groups at the 6-h interval [$F_{(4,15)} = 15.63$, $P < 0.0001$]. The post hoc test showed that INT had a significantly lower ratio of mushroom spines than the groups than received a foot-shock [i.e., SO, T-1, T-3 (P values ranging from 0.005 to 0.0001)], but it did not differ from T-0. SO did not differ from T-1 and T-3, but it had more mushroom spines than T-0 ($P < 0.05$). T-0 had a lower score than T-1 ($P < 0.05$) and T-3 ($P < 0.001$). T-1 and T-3 did not differ from each other. The ANOVA showed that at the 24-h interval, there were significant differences among the groups [$F_{(4,15)} = 6.05$, $P < 0.005$]. The post hoc test revealed that INT had a significantly lower ratio of mushroom spines than the rest of the groups (P values ranging between 0.01 and 0.0001). SO had a higher ratio of mushroom spines than T-0 ($P < 0.05$), and it did not differ significantly from T-1 and T-3; T-0, T-1, and T-3 did not differ from each other (Fig. 5*B*). The two-way ANOVA showed that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 1.02$, $P = 0.40$].

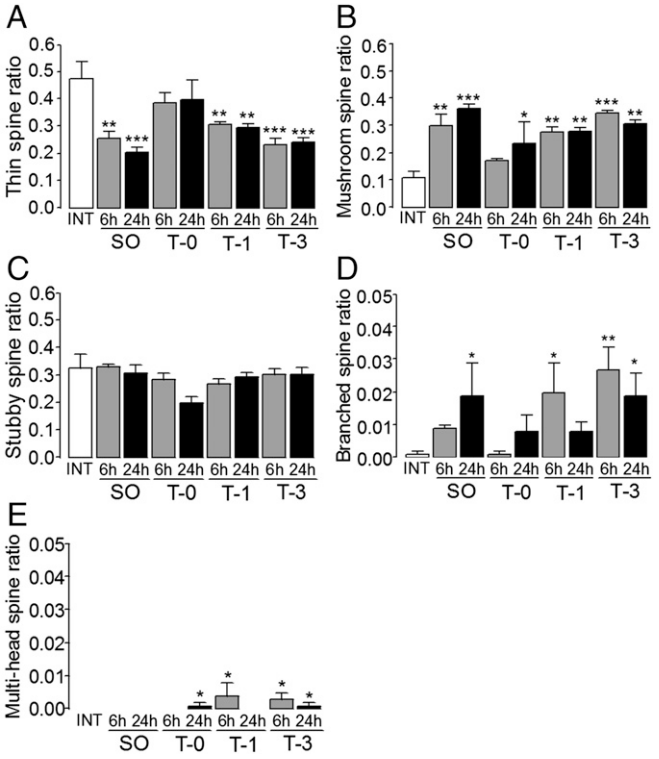

Fig. 5. Dendritic spine types in NacC: thin (*A*), mushroom (*B*), stubby (*C*), branched (*D*), and multihead (*E*). The T-0, T-1, and T-3 groups were trained with 0.0 mA, 1.0 mA, and 3.0 mA, respectively. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ vs. INT. Note differences in abscissa scales. Each bar represents mean ± SEM of 40 MSNs, obtained from four rats in each condition.

Stubby Spine Ratios in Accumbens Core. The ANOVA indicated that there were no significant differences in stubby spine ratios at either the 6-h interval [$F_{(4,15)} = 0.92$, $P = 0.48$] or the 24-h interval [$F_{(4,15)} = 2.53$, $P = 0.08$] (Fig. 5*C*). The two-way ANOVA indicated that there were no significant differences produced by the treatment × retention interval interaction [$F_{(3,24)} = 2.29$, $P = 0.10$].

Branched Spine Ratios in Accumbens Core. The one-way ANOVA showed that there were significant differences in spine ratios among groups at 6 h posttraining [$F_{(4,15)} = 4.55$, $P < 0.01$]. INT showed a significantly lower ratio of branched spines than T-1 ($P < 0.05$) and T-3 ($P < 0.005$), whereas SO had a lower ratio than T-3 ($P < 0.01$). There were no significant differences between T-1 and T-3, and each of them had a higher spine ratio than T-0 ($P < 0.05$ and $P < 0.005$, respectively).

Regarding the measurements made at the 24-h interval, the ANOVA revealed a significant difference among groups [$F_{(4,15)} = 3.81$, $P < 0.05$]. INT did not differ significantly from T-0 and T-1, but it had a significantly lower ratio of branched spines than SO ($P < 0.05$) and T-3 ($P < 0.05$). The pairwise comparisons demonstrated that there were no significant differences among the rest of the groups (Fig. 5*D*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 1.49$, $P = 0.24$].

Multihead Spine Ratios in Accumbens Core. The ANOVA indicated significant differences among the groups at the 6-h interval [$F_{(4,15)} = 2.38$, $P < 0.05$]. The spine ratio of INT was not statistically different from the spine ratios of SO and T-0, but it was lower than the spine ratios of T-1 ($P < 0.05$) and T-3 ($P < 0.0005$). SO did not differ from T-0 and T-1, but its spine ratio was significantly lower than the spine ratio of T-3 ($P < 0.01$). T-0 had a lower spine ratio

than T-1 ($P < 0.05$) and T-3 ($P < 0.0005$); T-1 and T-3 did not differ from each other. At the 24-h interval, the groups also differed significantly [$F_{(4,15)} = 2.38$, $P < 0.05$]. The post hoc test showed that INT did not differ significantly from S0 and T-1, and it had a lower spine ratio than T-0 ($P < 0.05$) and T-3 ($P < 0.0005$). Likewise, SO did not differ significantly from T-1, and it had a lower spine ratio than T-0 ($P < 0.05$) and T-3 ($P < 0.005$). T-0 had a higher spine ratio than T-1 ($P < 0.05$), and it did not differ from T-3, and T-1 also had a lower ratio than T-3 ($P < 0.05$) (Fig. 5*E*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 0.93$, $P = 0.44$].

Thin Spine Ratios in Accumbens Shell. Highly significant differences among the groups at the 6-h interval were revealed by the ANOVA [$F_{(4,15)} = 9.54$, $P = 0.0005$]. INT showed a higher spine ratio than the foot-shocked groups (SO, T-1, and T-3; P values ranging from 0.0005 to 0.0001), but it did not differ from T-0. SO showed a lower ratio than T-0 ($P < 0.05$), but its spine ratio was not significantly different from T-1 and T-3. T-0 had a higher spine ratio than T-1 and T-3 ($P < 0.05$ for both comparisons). Also, the foot-shocked groups did not differ significantly from each other. The ANOVA also showed a significant treatment effect at the 24-h interval [$F_{(4,15)} = 2.38$, $P < 0.05$]. Pairwise comparisons produced, basically, the same results as the results obtained at the 6-h interval. INT showed a higher spine ratio than the foot-shocked groups (SO, T-1, and T-3; P values ranging from 0.05 to 0.0005), but it did not differ from T-0. SO had a significantly lower spine ratio than T-0 ($P < 0.005$). T-0 did not differ from T-1, and it had a higher spine ratio than T-3 ($P < 0.01$). These two foot-shocked groups did not differ significantly from each other (Fig. 6*A*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 0.12$, $P = 0.95$].

Mushroom Spine Ratios in Accumbens Shell. The one-way ANOVA showed that there were significant differences in spine ratios among groups 6 h after training [$F_{(4,15)} = 13.72$, $P < 0.0001$]. INT showed a significantly lower mushroom spine ratio than the foot-shocked groups [SO, T-1, and T-3; P values ranging from 0.001 to 0.0001], but it did not differ from T-0. The foot-shocked groups (SO, T-1, and T-3) had higher scores than T-0 (P values ranging from 0.05 to 0.005), and these foot-shocked groups did not differ significantly from each other. Regarding the measurements made at the 24-h interval, the ANOVA revealed a significant difference among groups [$F_{(4,15)} = 5.26$, $P < 0.01$]. INT showed a significantly lower mushroom spine ratio than the rest of the groups (P values ranging from 0.05 to 0.0001). T-0 also had a lower spine ratio than T-3 ($P < 0.05$), but it did not differ significantly from T-1. The pairwise comparisons demonstrated that SO, T-1, and T-3 did not differ from each other (Fig. 6*B*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 0.86$, $P = 0.48$].

Stubby Spine Ratios in Accumbens Shell. The ANOVA indicated that there were no significant differences in stubby spine ratios at the 6-h interval [$F_{(4,15)} = 1.26$, $P = 0.33$] and 24-h interval [$F_{(4,15)} = 5.03$, $P = 0.06$] (Fig. 6*C*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 4.48$, $P = 0.12$].

Branched Spine Ratios in Accumbens Shell. The ANOVA indicated that there were significant differences in branched spine ratios at the 6-h interval [$F_{(4,15)} = 4.05$, $P < 0.05$]. The only significant difference between groups was the difference between T-0 and T-3 ($P < 0.05$). At the 24-h interval, no differences among groups were found [$F_{(4,15)} = 0.77$, $P = 0.56$] (Fig. 6*D*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 1.26$, $P = 0.31$].

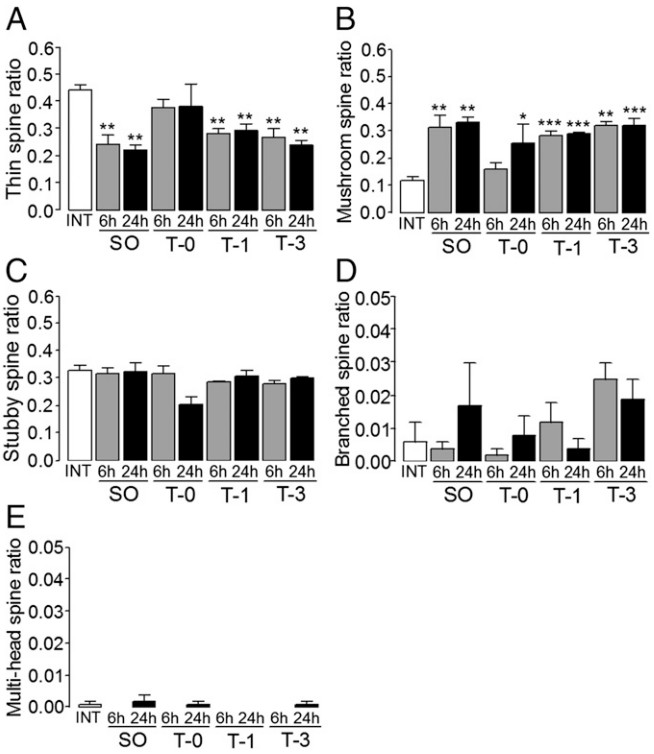

Fig. 6. Dendritic spine types in NAcS: thin (*A*), mushroom (*B*), stubby (*C*), branched (*D*), and multihead (*E*). The T-0, T-1, and T-3 groups were trained with 0.0 mA, 1.0 mA, and 3.0 mA, respectively. *$P < 0.05$, **$P < 0.01$, ***$P < 0.0001$ vs. INT. Note differences in abscissa scales. Each bar represents mean ± SEM of 40 MSNs, obtained from four rats in each condition.

Multihead Spine Ratios in Accumbens Shell. The ANOVA indicated that there were no significant differences in multihead spine ratios at the 6-h interval [$F_{(4,15)} = 1.00$, $P = 0.44$] and the 24-h interval [$F_{(4,15)} = 0.27$, $P = 0.90$] (Fig. 6*E*). The two-way ANOVA indicated that the treatment × retention interval interaction was not significant [$F_{(3,24)} = 0.35$, $P = 0.79$].

Discussion

The main findings of this work were that moderate training of IA induced increased spine density and an augmented ratio of mushroom spines in both the DLS and DMS. Intense training of this task further increased spine density in both striatal regions, as well as in the ratio of mushroom spines, but only in the DMS. This spinogenesis was specific to the association of the context and the foot-shock, because exposure to the context alone produced smaller changes and the foot-shock by itself produced no changes. These results strongly suggest that the effects are due to changes mediating memory.

The extinction results indicated that the higher foot-shock intensity used in training induced enhanced learning; that is, the 3.0-mA group showed significantly higher resistance to extinction in comparison to rats trained with the moderate foot-shock intensity (1.0-mA group), thus confirming earlier work from our group in which the same IA task was studied (49, 50).

At 6 h and 24 h after training, the increase in dendritic spine density in the MSNs of DLS and DMS was directly proportional to the foot-shock intensity administered during training (Fig. 2 *A* and *B*). Exposure to the context provided by the conditioning box without the administration of foot-shock (T-0 group) also increased spine density. However, this increase was significantly lower than the increase in the trained groups. Interestingly, the foot-shock by itself did not induce a significant change in spine density (SO group).

As we had expected, there were no significant differences in spine density between DLS and DMS. These results indicate that MSNs of DLS and DMS are responsive to both the contextual and procedural information induced by the learning experience (4, 13, 14). In contrast, the finding that foot-shock administered alone did not induce changes in spine density suggests that MSNs of the dorsal striatum are not involved in processing nonassociative aversive stimulation. These patterns of spine dynamics seen at 6 h were maintained at 24 h, suggesting that the relatively early changes brought about by both procedural and contextual learning are persistent.

A different picture emerged from analysis of the accumbens. In the NAcS, an intensity-dependent increase in the amount of dendritic spines occurred after training with 1.0 mA and 3.0 mA, both at the 6-h and 24-h intervals, as was the case in dorsal striatum. Also, a significant increase in spine density was found at the 6-h interval after stimulation with the foot-shock alone. However, no changes were produced in the 0-mA (only context) group. These data seem to imply that spinogenesis in NAcS in the trained animals is only related to the effects of the foot-shock, rather than to the associative processes involved in IA learning. The only change in spine density found in the accumbens core was an increase when the 3.0-mA foot-shock was administered during IA training. This result also suggests that dendritic spines of this region of the accumbens are not sensitive to the complex interaction of stimuli and responses involved in IA training (Fig. 2 *C* and *D*), because no changes were produced in the context-alone situation or after training with the lower foot-shock.

In relation to the different types of spines that were studied, three very consistent findings emerged. First, in DLS and DMS, there was a significant reduction in the ratio of thin spines in the groups that were only exposed to the conditioning box (T-0 group) and in the groups that had been trained with 1.0 and 3.0 mA (T-1 and T-3 groups), both at the 6-h and 24-h intervals. In contrast, the ratio of mushroom spines of DMS increased significantly in T-0, T-1, and T-3 at 6 h and 24 h after training; this increase was dependent upon the intensity of the foot-shock that had been administered (i.e., the T-3 group showed a greater increment than the other groups). There was also a significant intensity-dependent increment of mushroom spine ratios in DLS in the context (T-0) and trained (T-1 and T-3) groups during the consolidation period. At variance with the intensity-dependent changes in mushroom spine ratios just described, the increments in mushroom ratios seen in DLS did not differ significantly between the T-1 and T-3 groups (i.e., intense training did not induce further mushroom spinogenesis). Interestingly, the foot-shock alone (SO group) did not induce changes in the number of thin and mushroom spines in these two regions at either interval. These data agree well with the assumption that thin spines are involved in learning and that as this process gives way to storing of information, there is a reduction in the number of thin spines and a correlative increase in the population of mushroom spines. Such effects may be involved in enabling LTM (43, 51–53).

Second, thin spine ratio declined significantly in both the accumbens core and shell at both time intervals in all foot-shocked groups, whether they had been trained or not. No changes in the ratio of thin spines were observed in the nonshocked groups. Mushroom spine ratios increased significantly in both the accumbens core and shell at both time intervals in all foot-shocked groups whether they had been trained or not, and no changes were observed in the nonshocked groups. These results suggest that, as in the case of spine density in these two regions, the changes in thin and mushroom spines are related to the aversive component of the learning experience rather than the complex associative processes derived from the interaction among the context, foot-shock, and motor performance involved in IA.

Third, in the two dorsal and two ventral striatal regions, stubby spine ratios remained unchanged despite the different manipulations that were enforced. The role of stubby spines remains unknown, although this spine type is thought to regulate neuronal excitability (54), because the stubby spines facilitate the diffusion of calcium ions to the parental dendrite (53).

Branched spine ratio was increased in DMS only after training with the higher foot-shock intensity, both at the 6-h and 24-h intervals. This increase in branched spine ratio also happened in the DLS, where, additionally, there was an increase in the group that had been trained with 1.0 mA at the 6-h interval. These results indicate that high-intensity IA training, but not simple exposure to context or to the foot-shock alone, induced development of branched spines in both DMS and DLS. Thus, in these striatal regions, branched spines appear to participate in the afferent activity derived from the associative processes involved in intense training. In regard to the NAcC, the ratio of this type of spine was increased after training with 1.0 mA and 3.0 mA, as well as in the group that received the foot-shock alone; however, exposure to the context, by itself, did not modify this ratio. Although these results might suggest that branched spines in the accumbens core may also be engaged in memory of avoidance training, the fact that they were increased by foot-shock alone, and that no change was produced by exposure to the context, clearly argues against this interpretation. Surprisingly, branched spines in NAcS were not changed by any of the experimental manipulations.

Multihead spine dynamics showed some variability. There was an increase in the ratio of multihead spines in DLS after training with 1.0 mA at the 24-h testing interval, with 3.0 mA at the two testing intervals, and in the 0-mA (context) group at the 24-h interval. The same was true for DMS, except that the ratio remained unchanged in the 0-mA group. The foot-shock by itself did not produce changes in the ratio of multihead spines. Thus, development of multihead spines can be detected when LTM has already been formed after moderate and intense training, but only intense training promotes multihead spinogenesis during consolidation.

Multihead spine ratios in NAcC did not change at the 6-h interval in the SO and T-0 groups, but they were increased in the T-1 and T-3 groups; at 24 h, there was an increment in the ratio in the T-0 and T-3 groups. A remarkable feature of multihead spines in the NAcC was that, unlike all other spine types in this region and in the NAcS, their ratio did not change as a consequence of the administration of the foot-shock alone. However, this ratio increased after exposure to the context and after training with both low and high foot-shock intensities. It thus seems possible that the formation of new multihead spines may underlie the reported involvement of the accumbens in memory of aversive events (55–57). In view of the lack of consistency in the dynamics shown by the multihead spines, it is difficult at this time to determine a clear function of these spines in memory formation.

An important premise underlying this study was that dorsal striatum participates in the integration of information derived from the learning experience, which is provided by a number of structures. In general, the expectations of this study were met, namely, that both the DMS and DLS regions of the dorsal striatum would show the same patterns of spine dynamics after IA training (i.e., that stronger learning would induce increased spine density compared with weaker learning). Also, there was an increase in the ratio of mushroom spines in these two regions. Mushroom dendritic spines are stable; they can persist for months (58, 59). Mushroom dendritic spines have a greater head volume than other spines, which correlates positively with the area of postsynaptic density (60), the amount of presynaptic vesicles (60), the size of the presynaptic active zone (61), the number of postsynaptic receptors (62), and the current injected into the synapse (62, 63); all these features are important for generating long-term modifications in the efficiency of excitatory signals and lend support to the notion that mushroom spines represent a physical substrate of LTM.

As described above, in both DLS and DMS, the ratio of mushroom spines was augmented in an intensity-dependent fashion

during consolidation, indicating that the transfer from short-term memory to LTM is facilitated in intense training through increased mushroom spinogenesis. On the other hand, there was an important difference between DMS and DLS in relation to the behavior of this type of spine during LTM. Whereas in the former, training with the foot-shock of high intensity induced a higher ratio of this type of spine than training with the low-intensity foot-shock, in DLS, intense training did not increase the ratio of mushroom spines more than moderate training did. This dissimilar outcome may be due to the different types of learning these regions mediate (spatial/contextual vs. procedural), as well as their dissimilar connectivity. The DMS receives afferent fibers, directly or indirectly, from the dorsal hippocampus and medial prefrontal cortex, whereas the afferents to DLS originate mainly in somatosensory and motor cortical areas (64).

It is reasonable to postulate that in the INT animals, as the intensity of the training is increased and a stronger association between the context and the aversive stimulation is formed, a heightened glutamatergic synaptic activation of DMS MSNs by hippocampus and medial prefrontal cortex takes place, thus promoting a larger mushroom spinogenesis than produced by moderate training. Concurrent, stress-dependent activation of glucocorticoid receptors activates kinase pathways, which results in further mushroom spinogenesis, within an appropriate time frame (<6 h). When normal activity of the dorsal striatum is importantly disturbed, as when its synaptic activity is disrupted by sodium channel blockers, an amnestic state ensues in those animals that have been subjected to moderate training. Consolidation may take place, however, after intense training because the enhanced release of corticosterone promotes an increased nongenomic spinogenesis of mushroom spines.

In the case of DLS, an augmented ratio of mushroom spines was produced by moderate training of the one-trial IA task, indicating that a single stimulus–response association is sufficient to induce changes in mushroom spinogenesis. Increased intensity of training did not induce an additional increase in DLS mushroom spine density, maybe because there was not a further increase in the afferent signals from the neocortex. One can hypothesize that additional sessions of IA training (i.e., a higher number of stimulus–response associations) would produce a habitual type of response, which is characteristic of procedural learning. Thus, after additional training, an increase in mushroom spines in DLS would be induced by heightened activation of the cortical-striatal connections. Previous work from our laboratory, where cats and rats were trained to perform an instrumental lever-press response, showed that infusion of cholinergic blockers (65) or sodium channel blockers (7) into the caudate nucleus and dorsal striatum, respectively, produced amnesia when the animals were treated during the early phases of learning, but no deficits were seen after extended training (i.e., when the response had become habitual). We can hypothesize that this extended training should have induced enhanced spinogenesis and an increased ratio of mushroom spines in DLS, at least in the rat.

These findings also suggest that increased mushroom spinogenesis produced by intense training strengthens memory consolidation and facilitates the transfer of information from the dorsal striatum to other cerebral regions. Such increased spinogenesis may account for the protective effect against amnestic treatments. These mechanisms might be relevant for understanding why highly emotional memories, such as the ones causing posttraumatic stress disorder, are so enduring.

Materials and Methods

Animals. We studied male Wistar rats (250–350 g) bred at the Instituto de Neurobiología, Universidad Nacional Autónoma de México. They were maintained on a 12-h/12-h light/dark cycle (lights on at 0700 h) at a temperature between 22 °C and 23 °C, housed individually with food and tap water ad libitum. Behavioral observations were carried out between 0900 h and 1300 h. All procedures were in compliance with the *Guide for the Care and Use of Laboratory Animals* of the NIH (66) and were approved by the Animal Ethics Committee of the Instituto de Neurobiología, Universidad Nacional Autónoma de México.

Inhibitory avoidance training apparatus. Subjects received 5-min daily handling sessions for three consecutive days before training in an IA task (details are provided in *SI Materials and Methods* and ref. 67). The apparatus is an alley with two distinct compartments separated by a guillotine door. The safe compartment has a floor of stainless-steel bars, and a 10-W light bulb located in the center of its lid illuminated it. The nonilluminated shock compartment was made of electrifiable stainless-steel plates.

Extinction. On the day of training, each animal was put inside the lit compartment; 10 s later, the door between the compartments was opened and the latency to cross to the dark shock compartment was measured (training latency). Upon crossing, the door was closed and a foot-shock of 1.0 mA (n = 10) or 3.0 mA (n = 10) was delivered. Five seconds after shock onset, the door was reopened, allowing the animal to escape to the lit compartment; this latency was also measured (escape latency). An additional group was subjected to the same training procedure except that the foot-shock was omitted (n = 10). Starting 24 h after training, extinction of the task was measured on seven consecutive days; the same procedure of training was followed except that the foot-shock was omitted.

Consolidation and LTM. Assessment of spine density and morphology was carried out at 6 h and 24 h after training; these retention intervals fall within the limits of consolidation and LTM of IA in the rat (47). Two groups of rats were trained as described above using 1.0 mA (T-1 group, n = 20) and 3.0 mA (T-3 group, n = 20), respectively. A third group did not receive the foot-shock (T-0 group, n = 20); this group controlled for exploratory activity inside the conditioning chamber. Half of the animals in each group were given a test of retention at 6 h after training, and the other half were given a test of retention at 24 h after training; during these sessions, the same procedure of training was followed except that the electric foot-shock was omitted. Immediately after the retention session, four animals from each group were killed to perform the histological analyses.

Morphometric analysis. Upon euthanasia, the rats' brains were removed and processed according to the modified Golgi–Cox staining method, as described by Gibb and Kolb (68) (details are provided in *SI Materials and Methods*). The MSNs were identified by their soma size, dendritic extensions, and numerous dendritic spines. A total of 1,440 MSNs were analyzed: five neurons per hemisphere × four brain regions × nine groups × four rats per group. Dendritic spines were classified into five different types: thin, mushroom, stubby, branched, and multihead (25, 69, 70). The total density of dendritic spines per region was expressed as the mean of dendritic spines in 10 neurons for each animal; spine counting was carried out in a single secondary dendrite in each neuron, along a 30-μm segment, starting 130 μm away from the soma. On the other hand, the quantification of dendritic spines according to shape was expressed as a ratio in relation to total dendritic spines (i.e., number of spines of a given shape/total number of spines in the 10 segments per region in each animal). This procedure would allow us to detect potential changes in the dynamics of spine formation as a function of the diverse independent variables that were studied (details are provided in *SI Materials and Methods*).

Statistical Analyses. Independent Kruskal–Wallis ANOVAs were computed for acquisition, escape, and retention latencies. When appropriate, the Mann–Whitney U test was used to make comparisons between any two groups.

The Friedman test was used in analyzing retention latencies across the 7 d of extinction, followed, when appropriate, by the Wilcoxon signed-rank test to make comparisons between any 2 d along the extinction sessions in each group. To compare retention scores among the three groups on each day of extinction testing, the Kruskal–Wallis and Mann–Whitney U tests were computed. One-way ANOVAs were used to determine if there were significant effects of treatments on total dendritic spines and ratios of spine shapes in each region at the 6-h retention interval; the same was done for the data at the 24-h retention interval. To compare spine density and ratios of spine shapes in each region obtained at 6 h vs. 24 h, two-way ANOVAs were run, excluding the INT group. Only the results of the treatment × retention interval interactions were taken into account, because the one-way ANOVAs served to find potential differences induced by the treatments at each retention interval. In every case, the post hoc Bonferroni test was used when appropriate.

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