Zebrafish: Social behaviour test

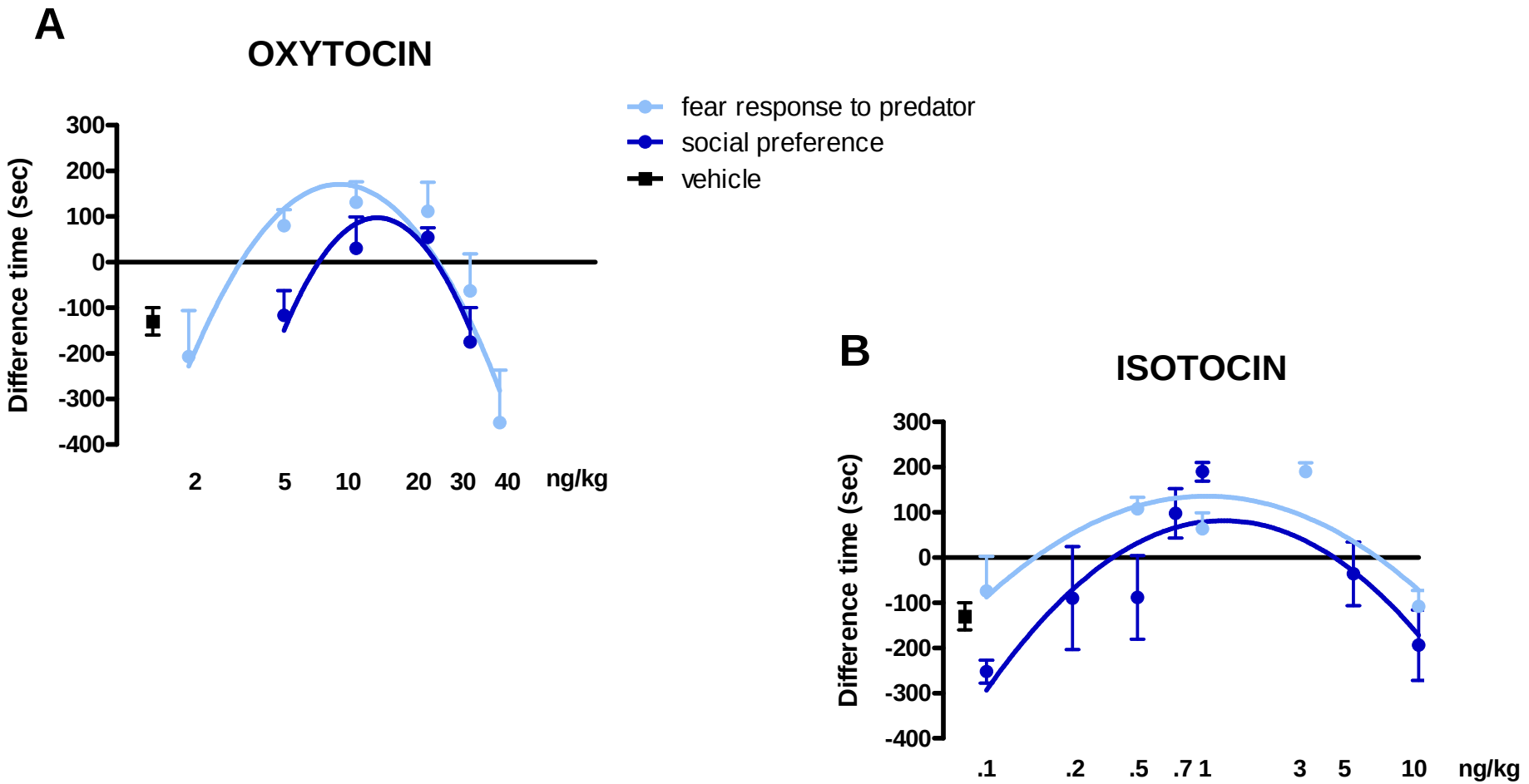


Figure 1: Social behaviour (social preference and fear response to predator test) (mean ± SEM) evaluated 10 minutes following i.m. administration of vehicle and different doses of oxytocin (panel A) and isotocin (panel B).

Zebrafish: Social behaviour test

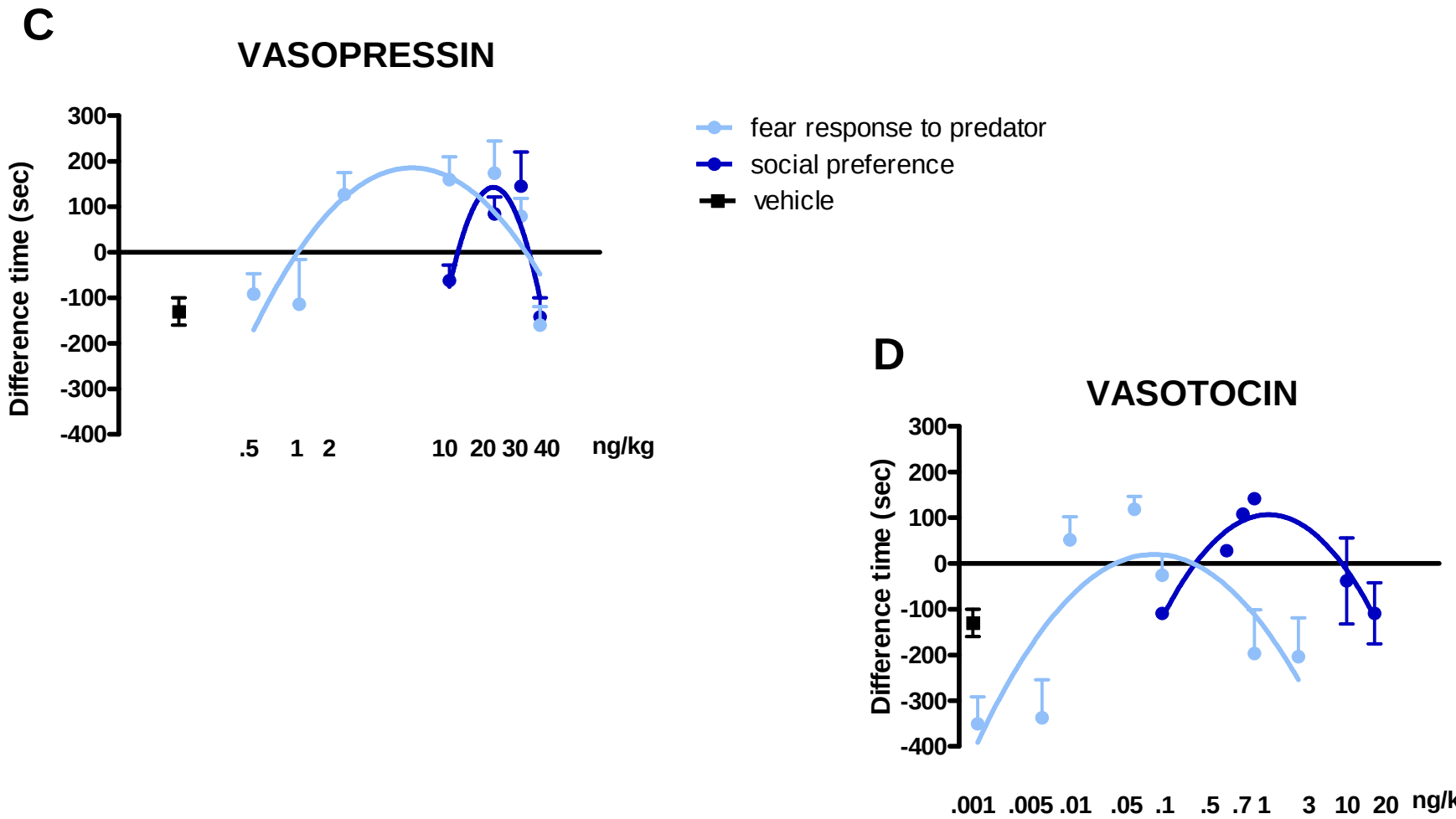


Figure 2: Social behaviour (social preference and fear response to predator test) (mean ± SEM) evaluated 10 minutes following i.m. administration of vehicle and different doses of vasopressin (panel A) and vasotocin (panel B).

ED 50 and CONFIDENCE LIMITS (CL)

Drug	ED50 (95% CL) (ng/kg) (Social preference)	ED50 (95% CL) (ng/kg) (Fear response)	Ratio
ISO	0.59 (0.5–0.8)	0.21 (0.1–0.48)	2.80
AVT	0.32 (0.29–0.34)	0.01 (0.005–0.09)	32.00
OT	10.69 (5.9–19.0)	4.2 (2.6–6.75)	2.54
AVP	13.35 (10.0–17.0)	1.29 (0.8–2.07)	10.35

Figure 3: ED50 (ng/kg) and 95% confidence limits (CL) values for different peptides were calculated on the basis of score difference between time spent close to Nacre and to WT (for social preference test) or to aggressor and empty compartment (for fear response to predator). All the peptides were injected i.m.

Zebrafish: Social preference test

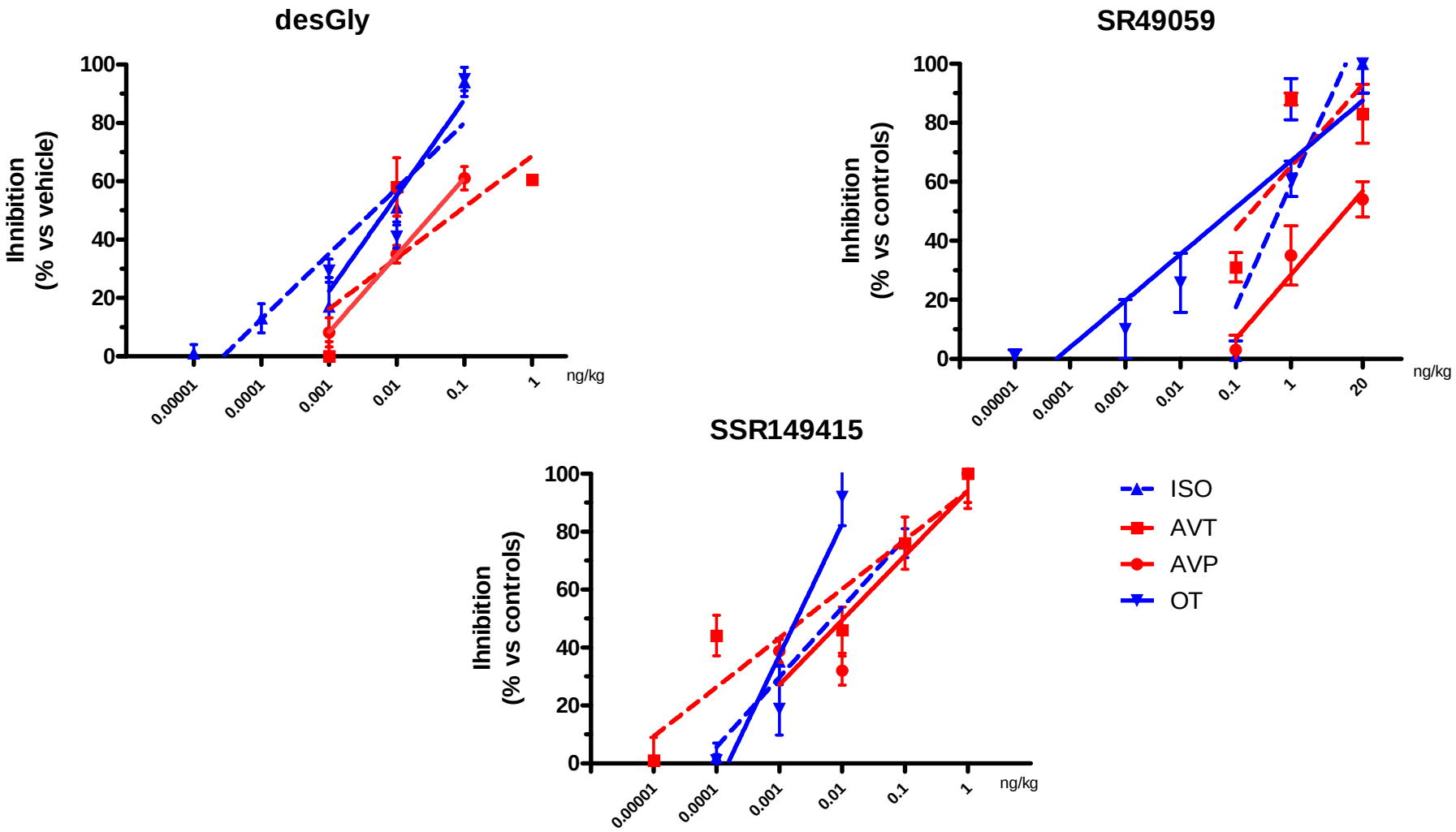


Figure 4: Effect of the DesGly, SR 49059 and SSR 149415 antagonists on social preference induced by neuropeptides; the antagonists were injected i.m. 10 min before the neuropeptides at maximally active doses in social preference test. The data are expressed as mean ± SEM of 10 animals for group.

Zebrafish: Fear response to predator test

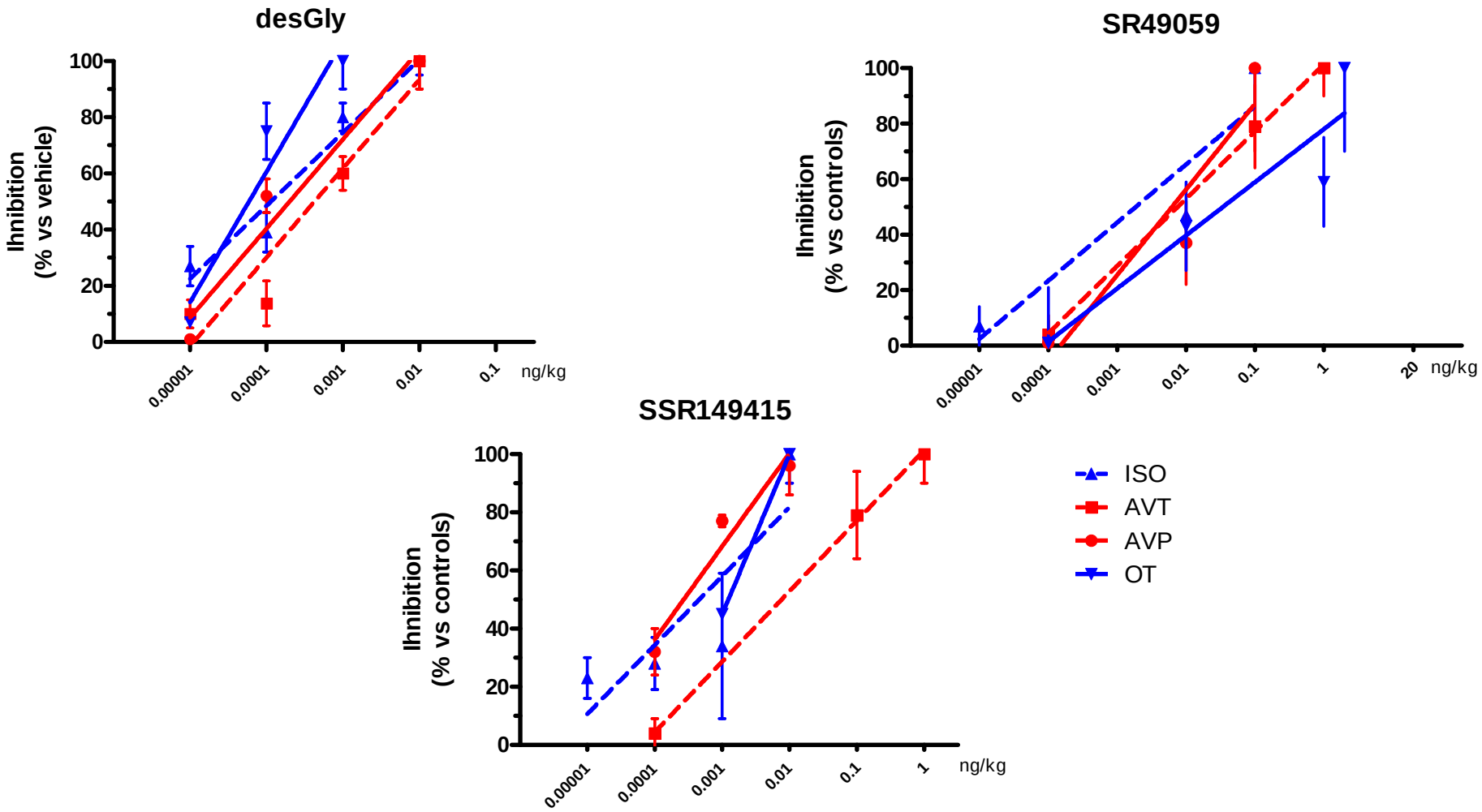


Figure 5: Effect of the DesGly, SR 49059 and SSR 149415 antagonists on fear response induced by neuropeptides; the antagonists were injected i.m. 10 min before the neuropeptides at maximally active doses in fear response to predator test. The data are expressed as mean ± SEM of 10 animals for group.

ED 50 and CONFIDENCE LIMITS (CL)

Drug	ED50 (95% CL) (ng/kg) ($\times 10^{-3}$) (Social preference)			Drug	ED50 (95% CL) (ng/kg) ($\times 10^{-3}$) (Fear response)		
	desglyDTyrOVT	SR49059	SSR149415		desglyDTyrOVT	SR49059	SSR149415
ISO	7.7 (4.6–13.0)	498 (93.0–620.0)	7.9 (1.3–46.0)	ISO	0.1 (0.06–0.2)	4.0 (0.7–30.0)	0.4 (0.2–1.1)
AVT	9.2 (3.8–22.0)	198 (116.0–336.0)	2.7 (0.6–13.0)	AVT	0.4 (0.3–10.0)	9.0 (2.7–36.0)	6 (1.8–21.0)
OT	8.0 (4.9–13.0)	167 (48.0–560.0)	2.4 (1.2–43.0)	OT	0.05 (0.02–0.09)	38.0 (14.0–84.0)	0.9 (0.5–1.8)
AVP	39.0 (24.0–64.0)	10,500 (3,100–35,000)	12 (3.7–39.0)	AVP	0.09 (0.07–0.1)	13.0 (4.5–40.0)	0.2 (0.1–0.4)

Figure 6: ED50 and 95% confidence limits (CL) (ng/kg) values for different antagonists were calculated on the basis of regression lines of score difference (%) vs control value. All the antagonists were injected i.m. 10 min before each neuropeptides.

Zebrafish: Swimming activity test

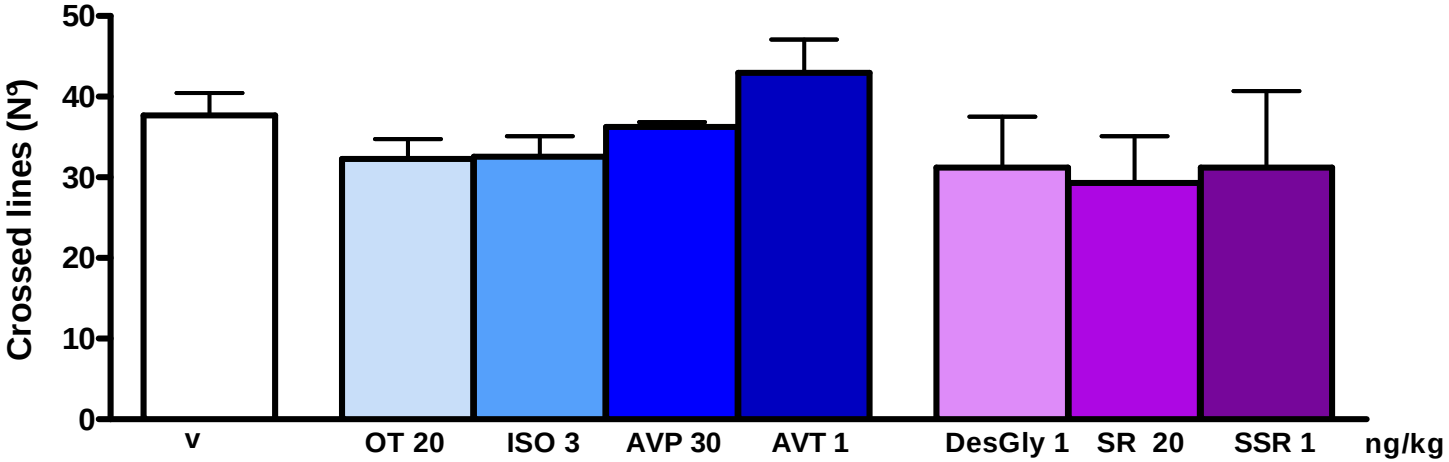


Figure 7: Number of line crossings of WT zebrafish during 30 min of swimming activity test, after treatment with vehicle (V), oxytocin (OT 20 ng/kg), isotocin (ISO 3 ng/kg), vasopressin (AVP 30 ng/kg), vasotocin (AVT 1 ng/kg), desglyDTyrOVT (DesGly 1 ng/kg), SR49059 (SR 20 ng/kg), SSR149415 (SSR 1 ng/kg) at the maximally active doses (found either on social preference or fear response to predator). Data are mean ± SEM. N=10 fish for each group.

Zebrafish : T-maze

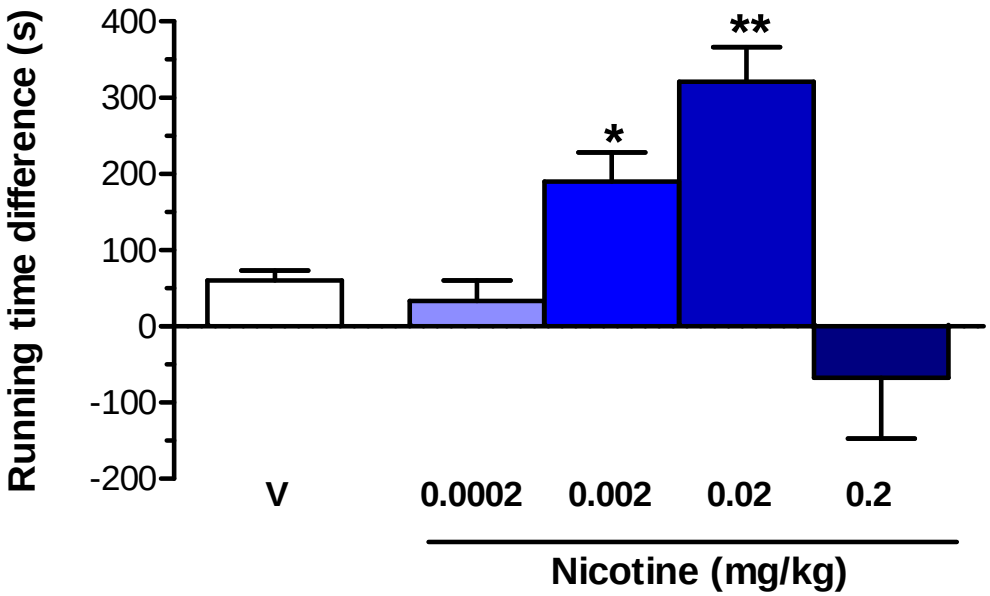


Figure 8: Cognitive performance of zebrafish in terms of difference (s) of pre-training (at 0 h) minus post-training running time (at 24 h) to reach the reservoir following nicotine or vehicle (V) i.p. treatment in theT-maze test. Data are expressed as mean ± SEM. N= 10 fish for each group. * P< 0.05, **P<0.01 vs corresponding vehicle group (Tukey's test).

Zebrafish: T-maze

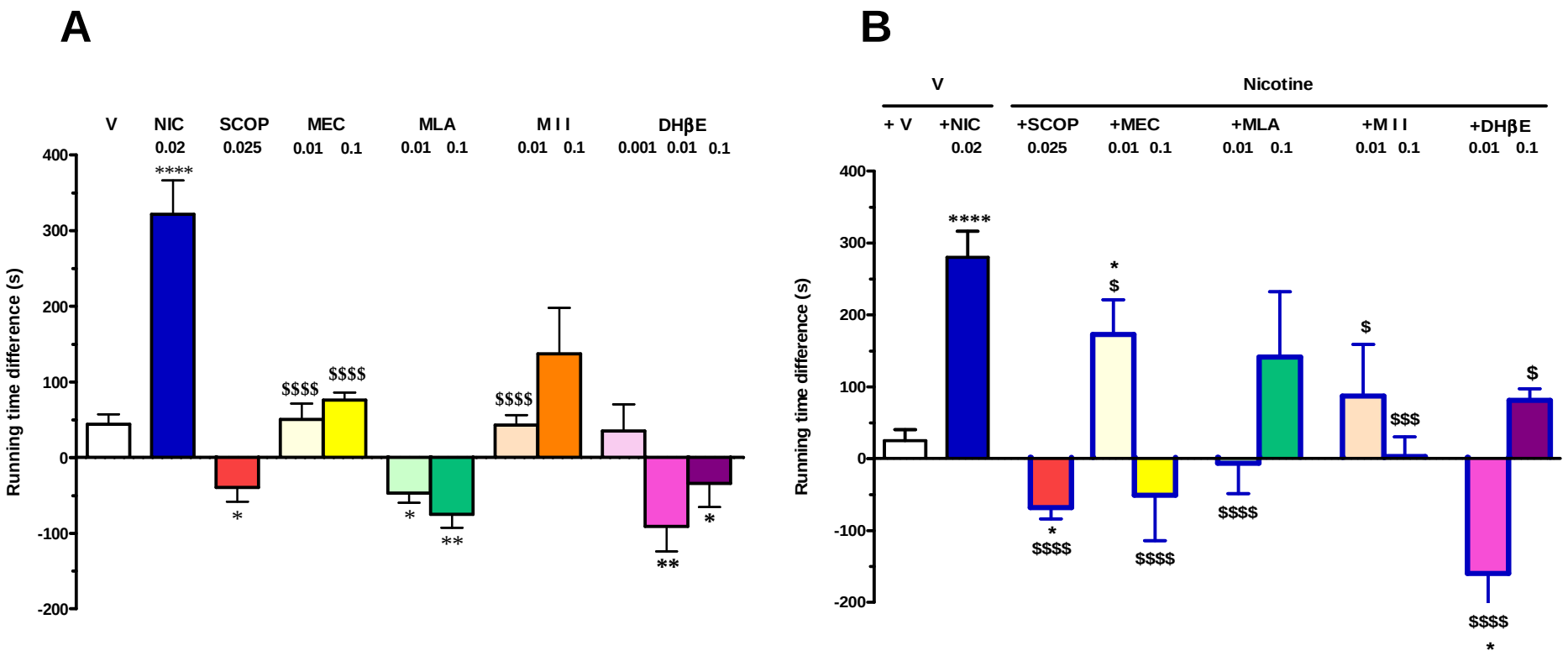


Figure 9: Effect of different non-selective or selective nAchRs subtype receptors antagonists given alone or 10 min before nicotine (NIC) in terms of difference (s) of pre-training (at 0 h) and post-training running time (at 24 h) in a T-maze. Scopolamine (SCOP), mecamylamine (MEC), methyllycaconitine (MLA), α-conotoxin (MII) and dihydro-β-erythroidine (DHβE) were administered alone (A) or 10 min before nicotine (B). The doses are expressed as mg/kg. Each value represents the mean ± SEM of ten observations per group. V= vehicle. * P<0.05, ** P<0.01, **** P<0.0001 compared to corresponding vehicle group; $ P<0.05, $$$ P<0.001, $$$$ P<0.0001 compared to corresponding NIC.

Zebrafish: Swimming activity test

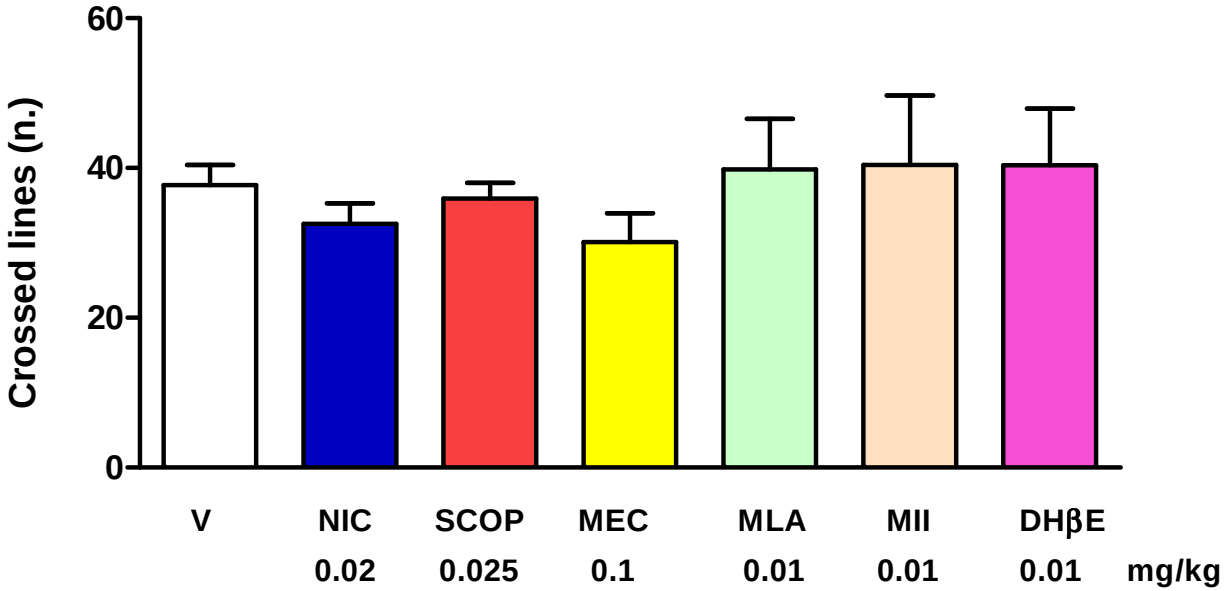


Figure 10: Number of line crossings of WT zebrafish during 30 min of swimming activity test, after i.p. treatment with vehicle (V), nicotine (NIC 0.02 mg/kg), scopolamine (SCOP 0.025 mg/kg), mecamylamine (MEC 0.1 mg/kg), methyllycaconitine (MLA 0.01 mg/kg), α-conotoxin (MII 0.01 mg/kg), dihydro-β-erythroidine (DHβE 0.01 mg/kg) at the maximally active doses. Data are mean ± SEM. N=10 fish for each group.

Zebrafish: VORT

Virtual object recognition test

(stationary shapes)

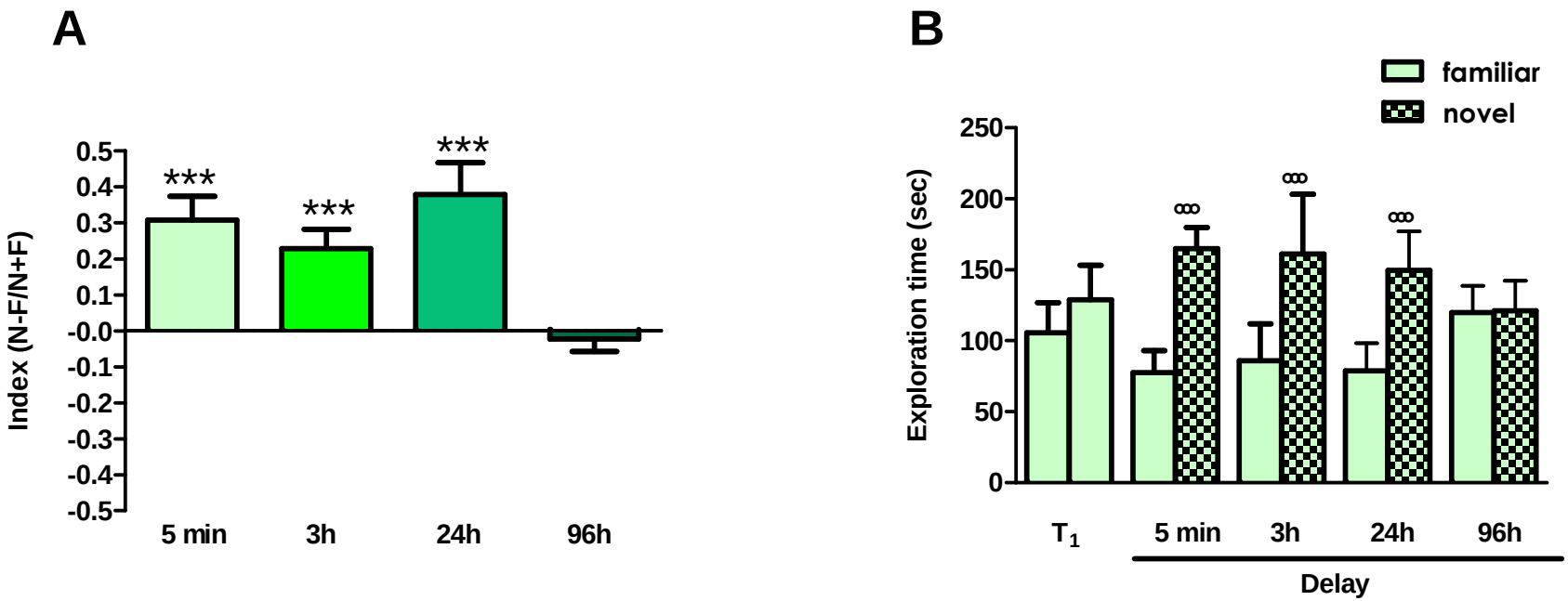


Figure 11: Discrimination index (N-F/N+F) (A) and exploration time (B) in VORT using geometrical 2D shapes. Data are expressed as mean ± SEM. N= 10 mice for each group. ***P<0.001 vs corresponding 96 h group; °°°P<0.001 vs corresponding familiar shape (Tukey's test).

Zebrafish: VORT

(stationary shapes)

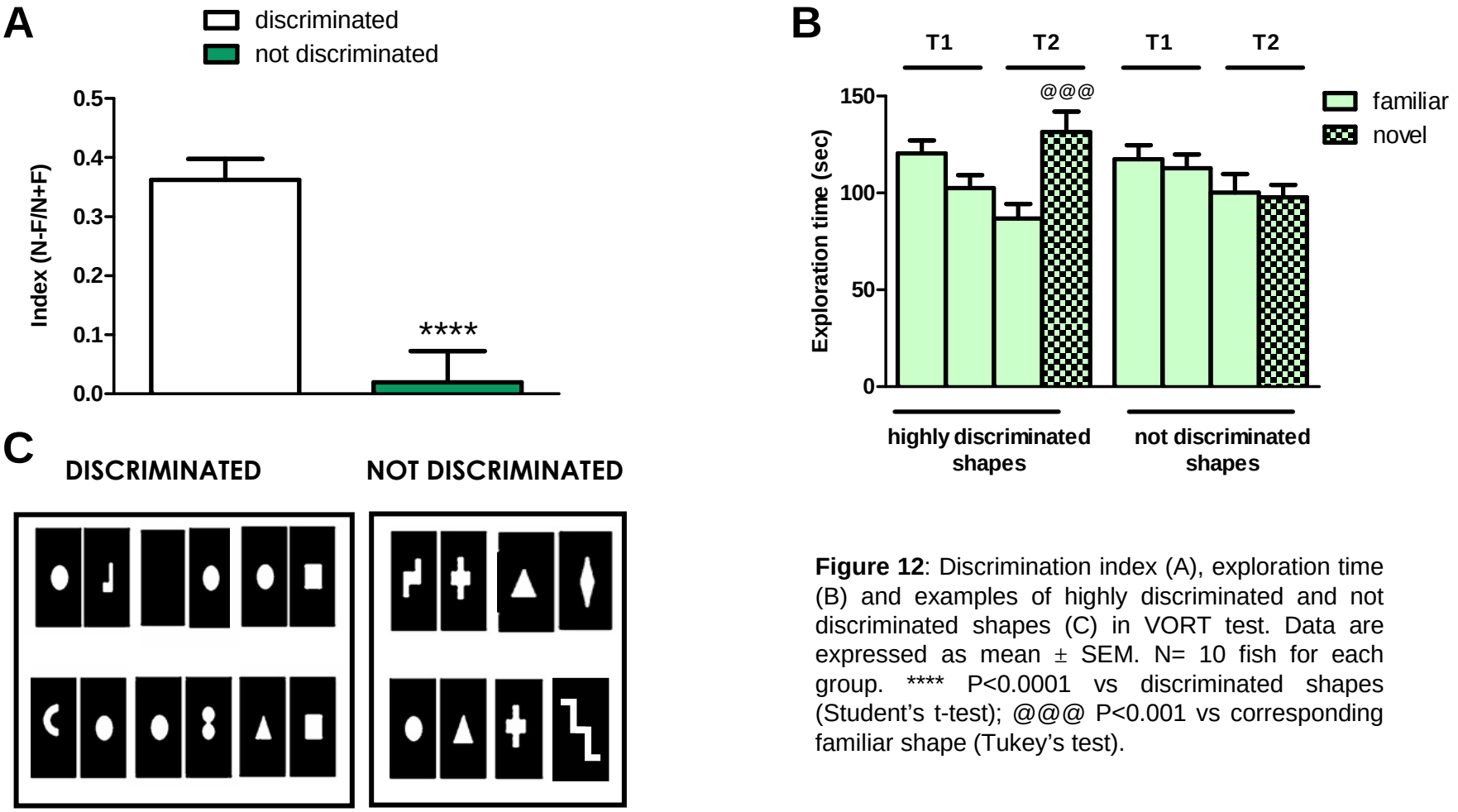


Figure 12: Discrimination index (A), exploration time (B) and examples of highly discriminated and not discriminated shapes (C) in VORT test. Data are expressed as mean ± SEM. N= 10 fish for each group. **** P<0.0001 vs discriminated shapes (Student's t-test); @@@ P<0.001 vs corresponding familiar shape (Tukey's test).

Zebrafish: VORT

(stationary shapes)

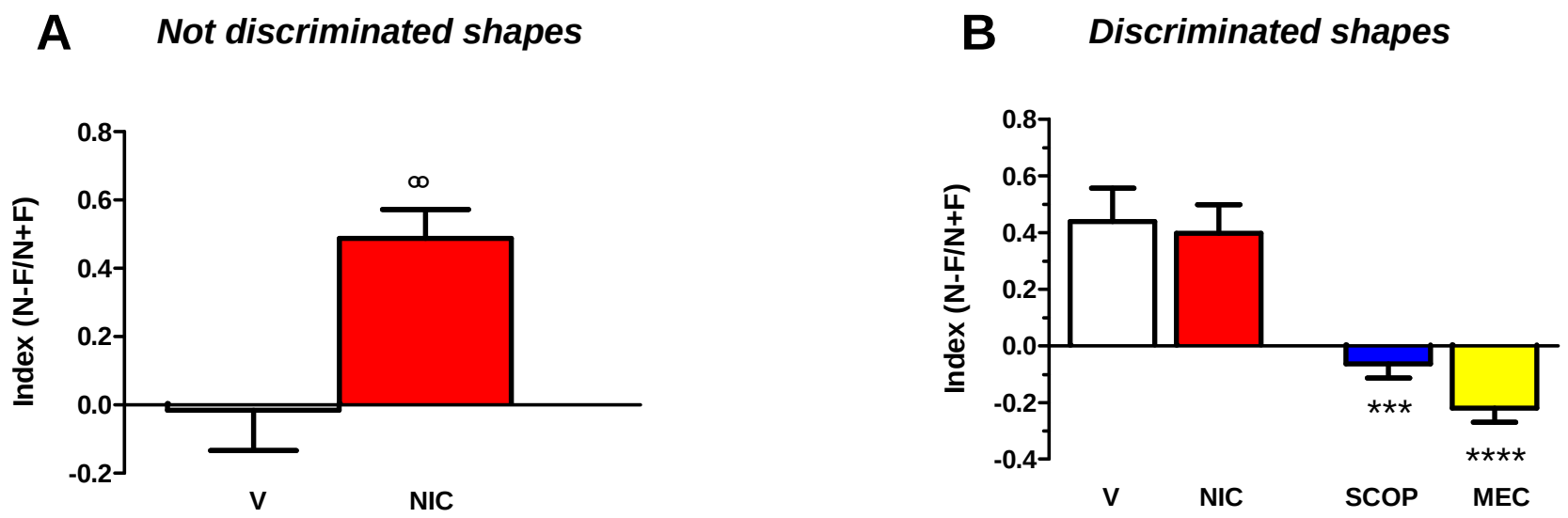


Figure 13: Discrimination index (mean ± SEM) in VORT, using not discriminated (A) or discriminated (B) geometric shapes after injection of nicotine and cholinergic antagonists. Nicotine (NIC 0.02 mg/kg) or vehicle (V) were injected 20 min before T1; scopolamine (SCOP 0.025 mg/kg) and mecamylamine (MEC 0.1 mg/kg) or vehicle (V) were injected 20 and 30 min respectively before T1. N= 10 fish for group. °° P<0.01 vs corresponding vehicle group (Student's t-test); ***P< 0.001, ****P<0.0001 vs corresponding vehicle and nicotine group (Tukey's test).

Zebrafish: VORT

(stationary shapes)

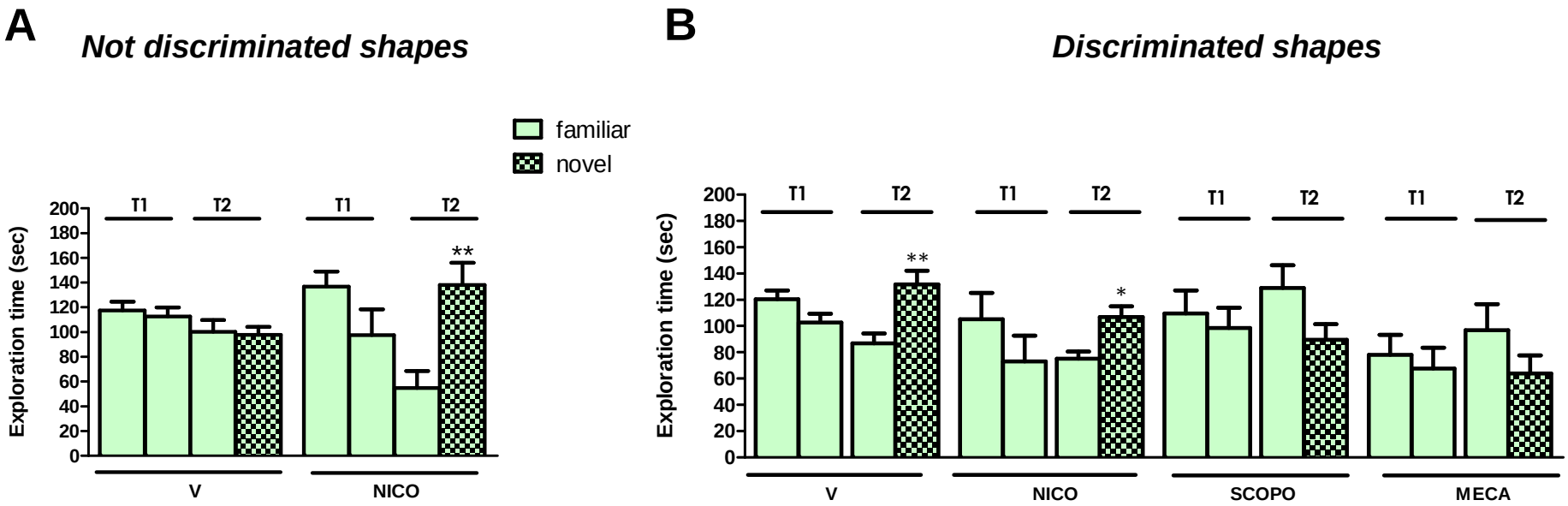


Figure 14: Exploration time (mean ± SEM) in VORT, using not discriminated (A) or discriminated (B) geometric shapes after injection of nicotine and cholinergic antagonists. Nicotine (NIC 0.02 mg/kg) or vehicle were injected 20 min before T1; scopolamine (SCOP 0.025 mg/kg) and mecamylamine (MEC 0.1 mg/kg) or vehicle were injected 20 and 30 min respectively before T1. N= 10 fish for group. * P<0.05, ** P< 0.01 vs corresponding familiar shape (Tukey's test).

Zebrafish: VORT

(Moving shapes)

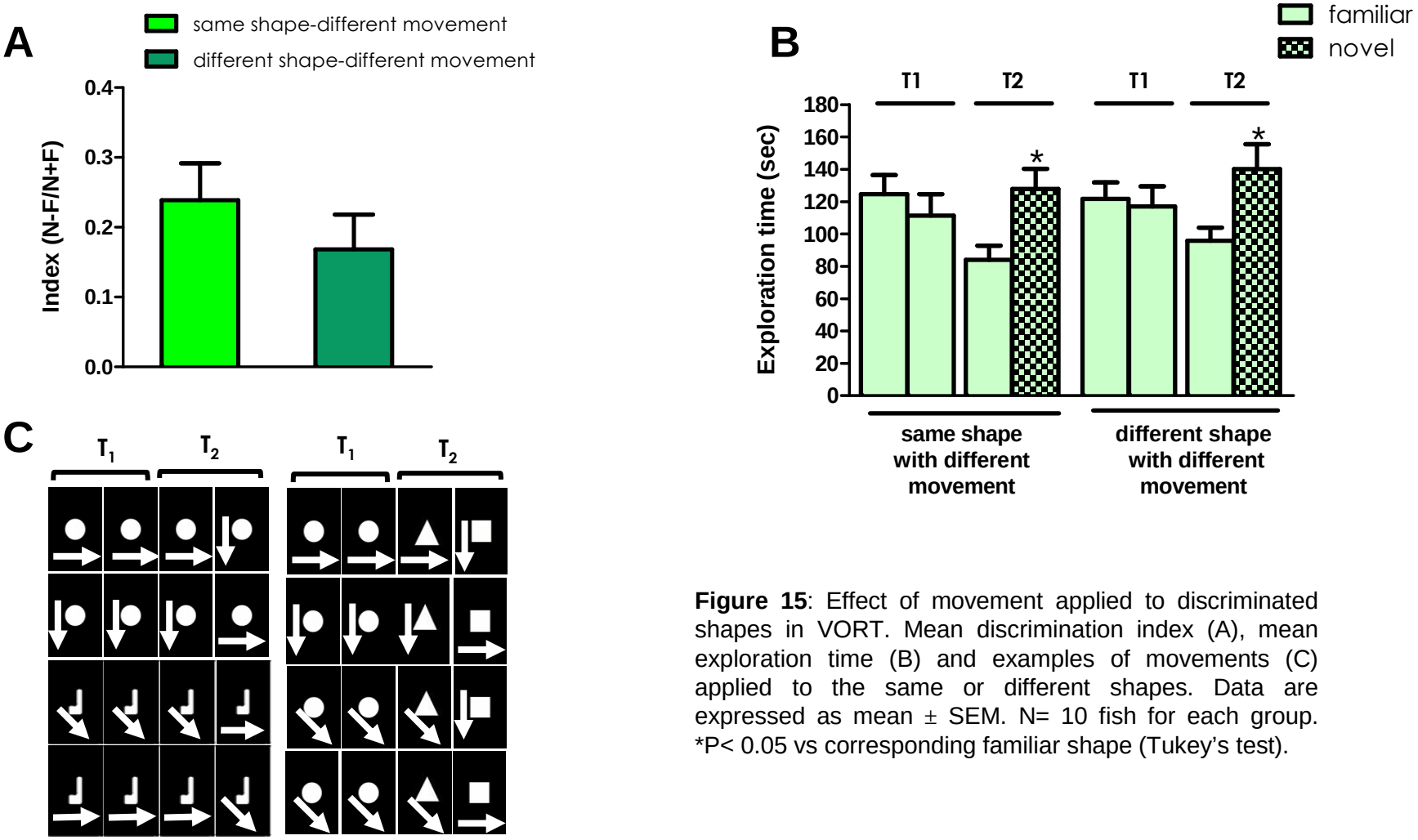


Figure 15: Effect of movement applied to discriminated shapes in VORT. Mean discrimination index (A), mean exploration time (B) and examples of movements (C) applied to the same or different shapes. Data are expressed as mean ± SEM. N= 10 fish for each group. *P< 0.05 vs corresponding familiar shape (Tukey's test).

Zebrafish: VORT

(Moving shapes)

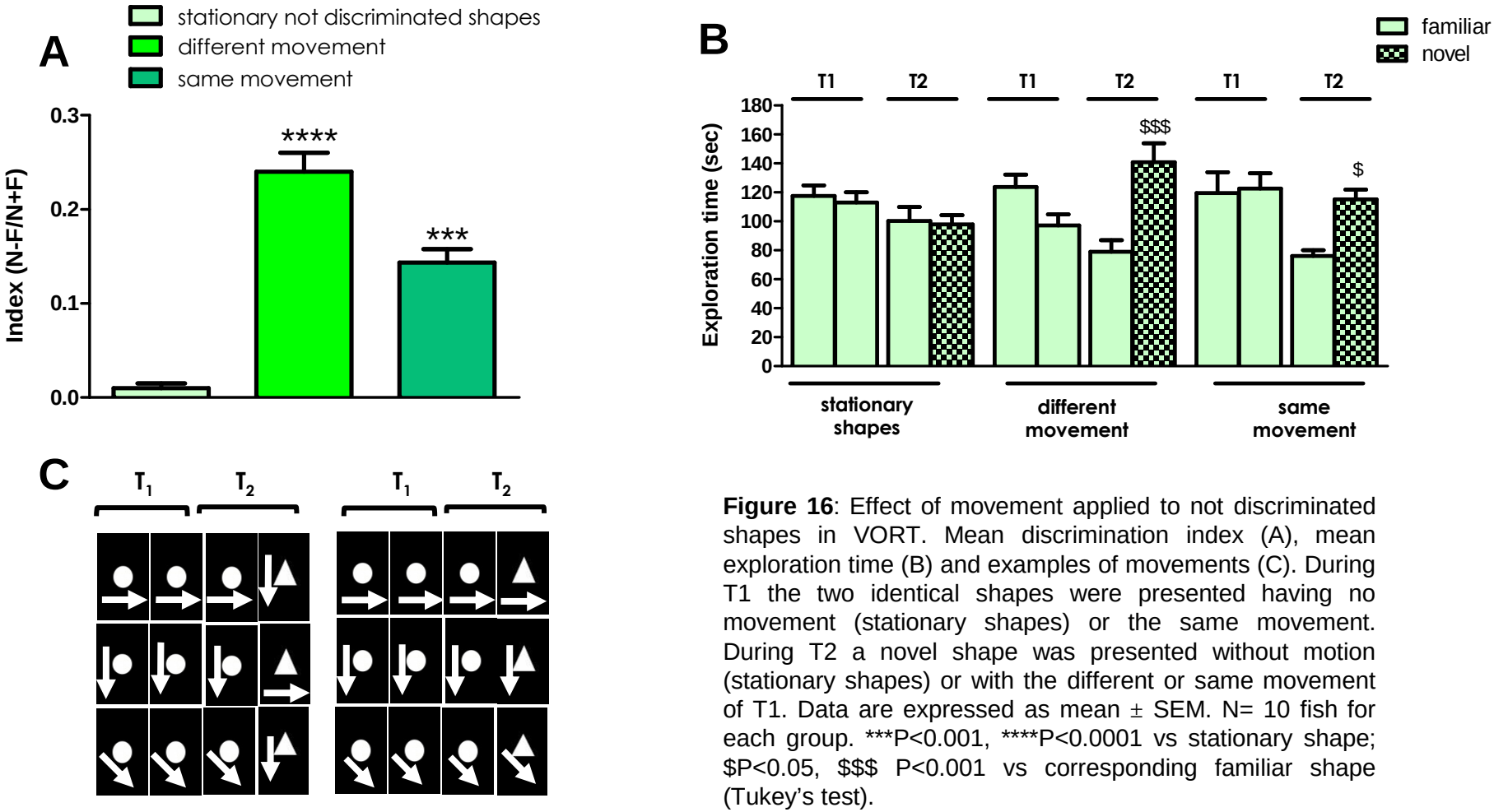


Figure 16: Effect of movement applied to not discriminated shapes in VORT. Mean discrimination index (A), mean exploration time (B) and examples of movements (C). During T1 the two identical shapes were presented having no movement (stationary shapes) or the same movement. During T2 a novel shape was presented without motion (stationary shapes) or with the different or same movement of T1. Data are expressed as mean ± SEM. N= 10 fish for each group. ***P<0.001, ****P<0.0001 vs stationary shape; $P<0.05, $$$ P<0.001 vs corresponding familiar shape (Tukey's test).

Mice: NOR vs VORT

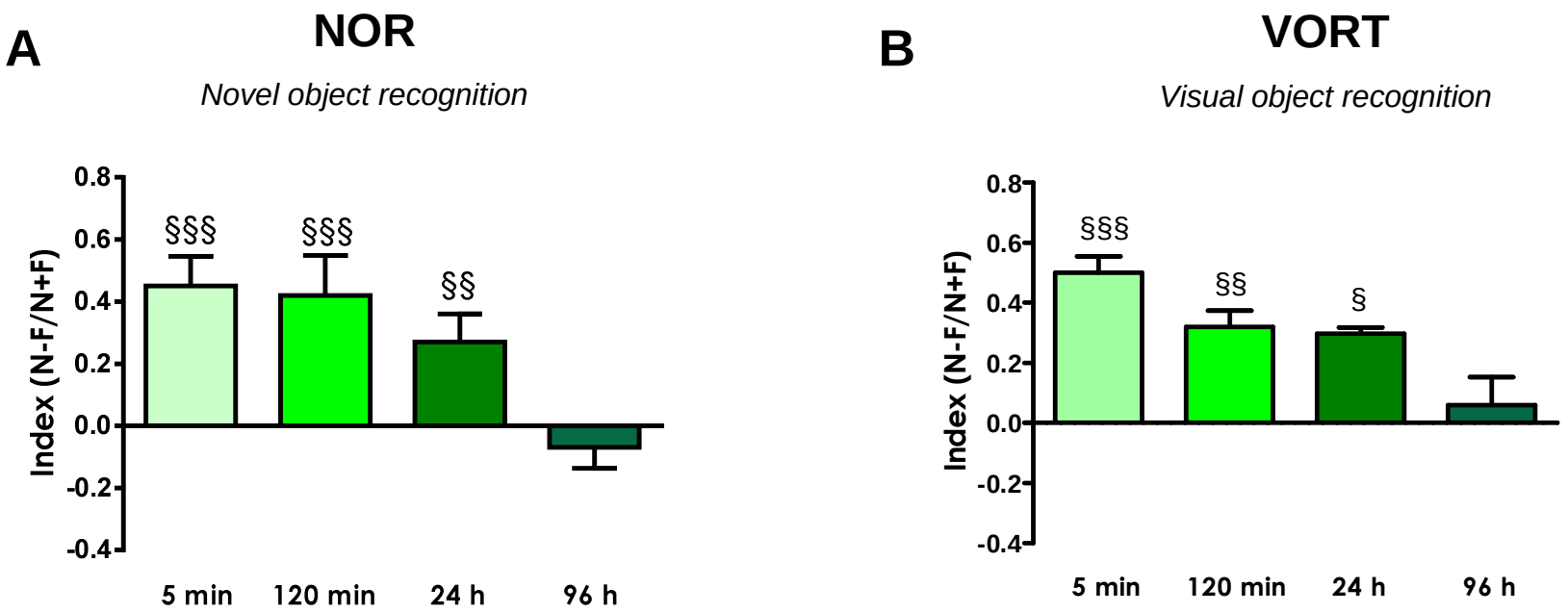


Figure 17: Discrimination index (N-F/N+F) in the NOR test with 3D objects (A) and in VORT using geometrical discrimination 2D shapes (B). Data are expressed as mean ± SEM. N= 10 mice for each group. §P<0.05, §§P<0.01, §§§P<0.001 vs corresponding 96 h group (Bonferroni's test).

Mice: NOR vs VORT

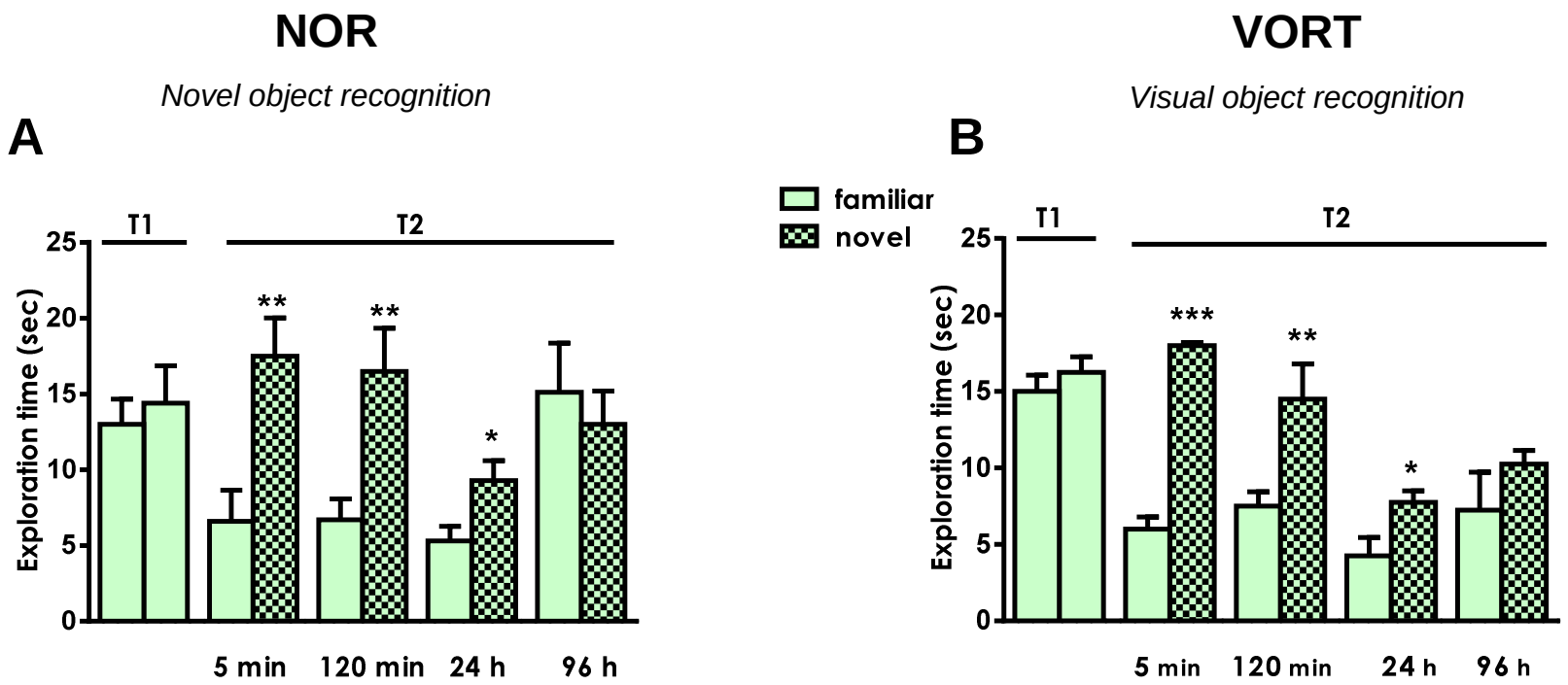


Figure 18: Exploration time in the NOR test with 3D objects (A) and in VORT using geometrical discrimination 2D shapes (B). Data are expressed as mean ± SEM. N= 10 mice for each group. *P<0.05, **P<0.01, ***P<0.001 vs corresponding familiar object/shape group (Tukey's test).

Mice: VORT

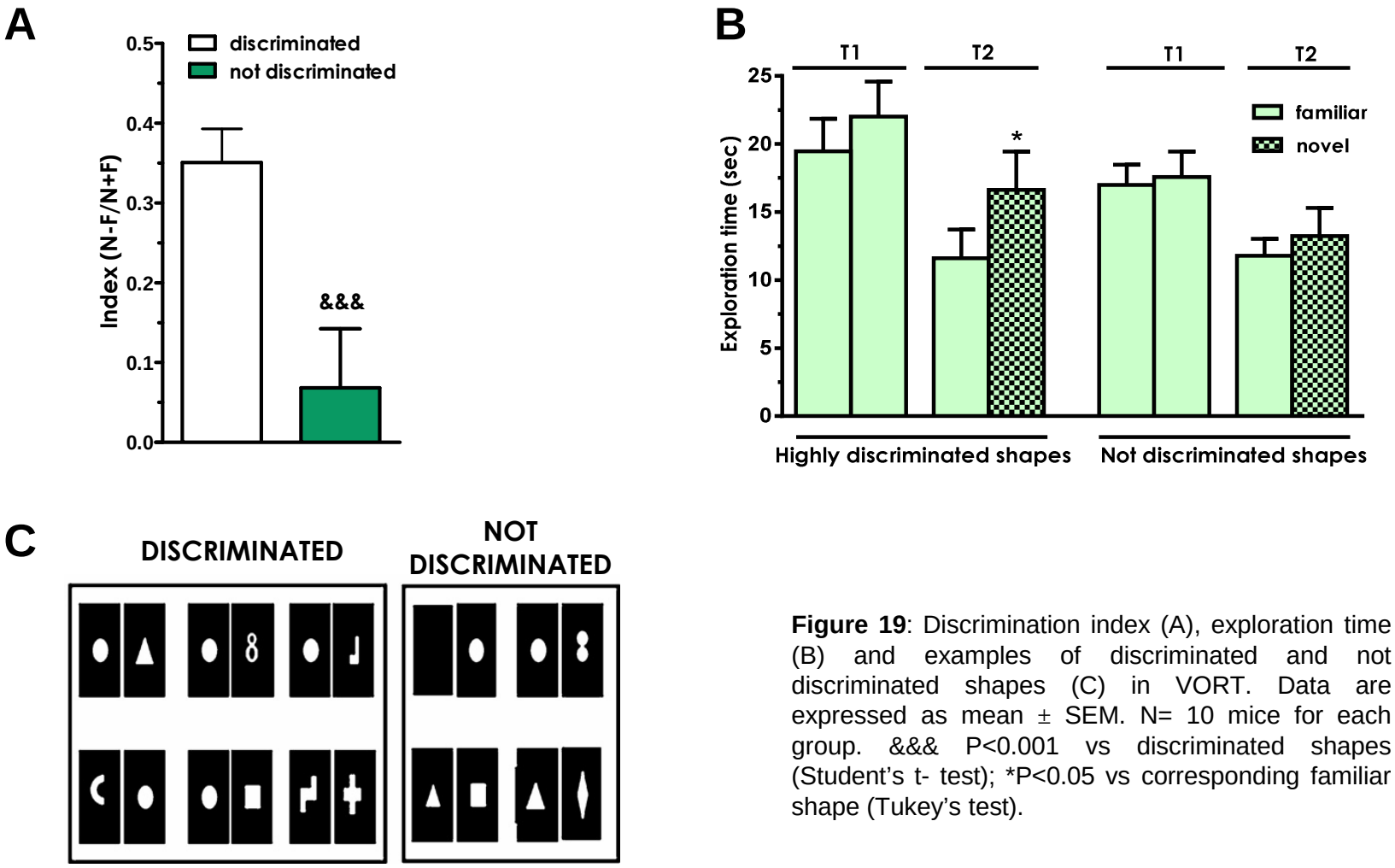


Figure 19: Discrimination index (A), exploration time (B) and examples of discriminated and not discriminated shapes (C) in VORT. Data are expressed as mean ± SEM. N= 10 mice for each group. &&& P<0.001 vs discriminated shapes (Student's t- test); *P<0.05 vs corresponding familiar shape (Tukey's test).

Mice: VORT

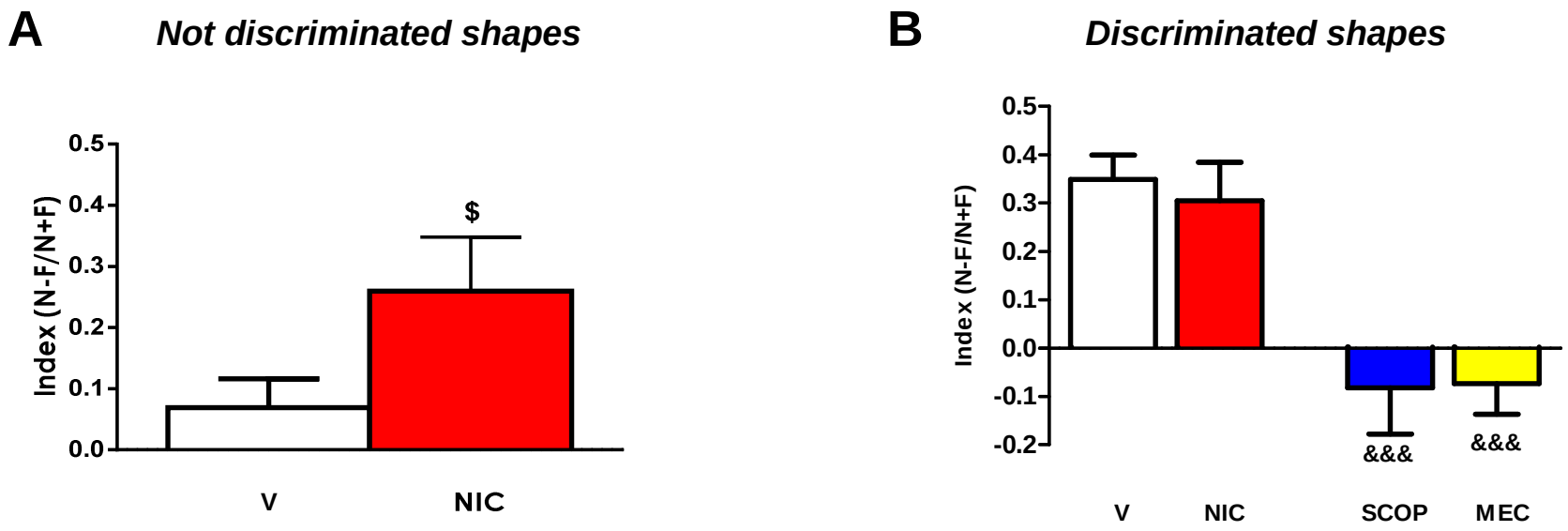


Figure 20: Discrimination index (mean ± SEM) in VORT, using not discriminated (A) or discriminated (B) geometric shapes after injection of nicotine and cholinergic antagonists. Nicotine (NIC 0.1 mg/kg) or vehicle (V) were injected 5 min before T1; scopolamine (SCOP 0.25 mg/kg) and mecamylamine (MEC 1 mg/kg) or vehicle (V) were injected 20 and 30 min respectively before T1. N= 10 mice for group. $ P<0.05 vs corresponding vehicle group (Student's t-test); &&& P<0.001 vs corresponding vehicle and nicotine group (Tukey's test).

Mice: VORT

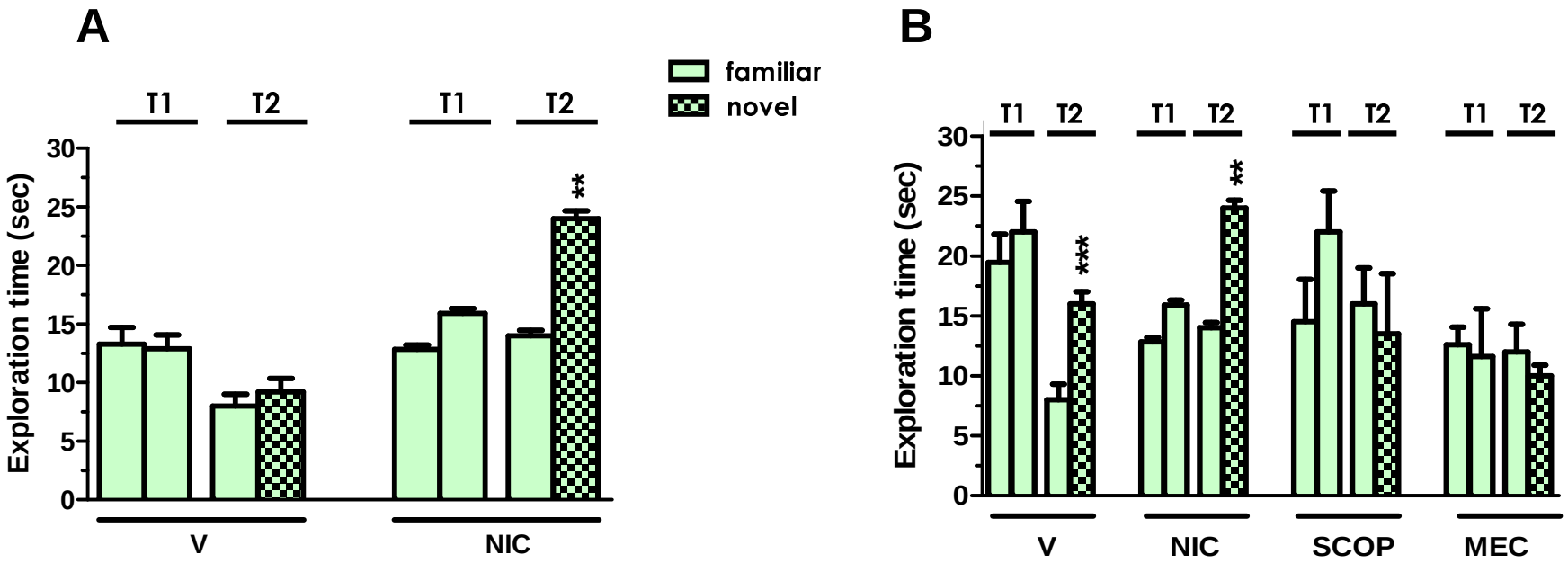
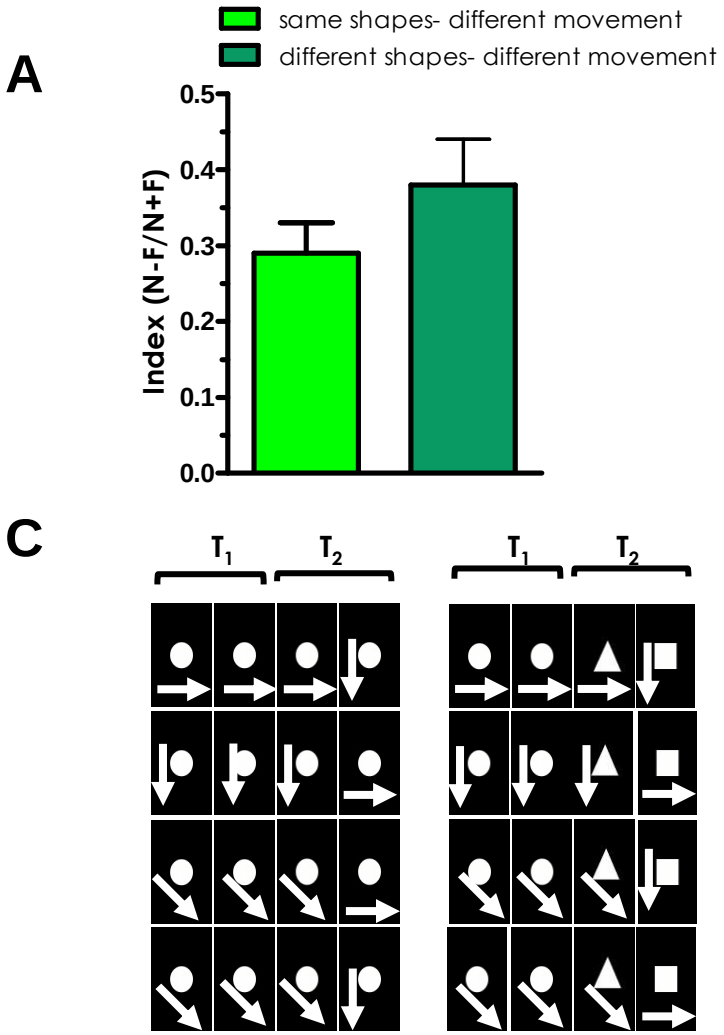


Figure 21: Exploration time (mean ± SEM) in VORT, using not discriminated (A) or discriminated (B) geometric shapes after injection of nicotine and cholinergic antagonists. Nicotine (NIC 0.1 mg/kg) or vehicle (V) were injected 5 min before T1; scopolamine (SCOP 0.25 mg/kg) and mecamylamine (MEC 1 mg/kg) or vehicle (V) were injected 20 and 30 min respectively before T1. N= 10 mice for group. ** P<0.01, *** P< 0.001 vs corresponding familiar shape (Tukey's test).

Mice: VORT

(Moving shapes)



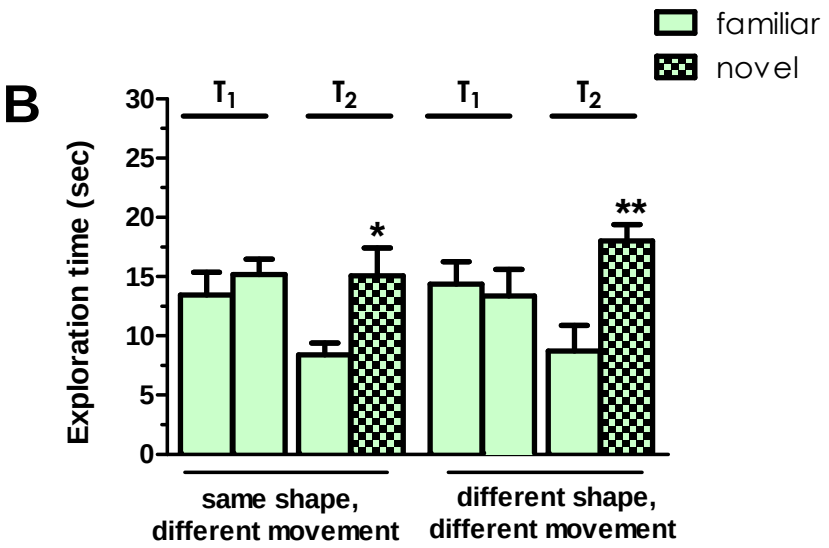


Figure 22: Effect of movement applied to discriminated shapes in VORT. Mean discrimination index (A), mean exploration time (B) and examples of movements (vertical, horizontal and oblique) (C) applied to the same or different shapes. Data are expressed as mean ± SEM. N= 10 mice for each group. *P<0.05, ** P<0.01 vs corresponding familiar shape (Tukey's test).

Mice: VORT

(Moving shapes)

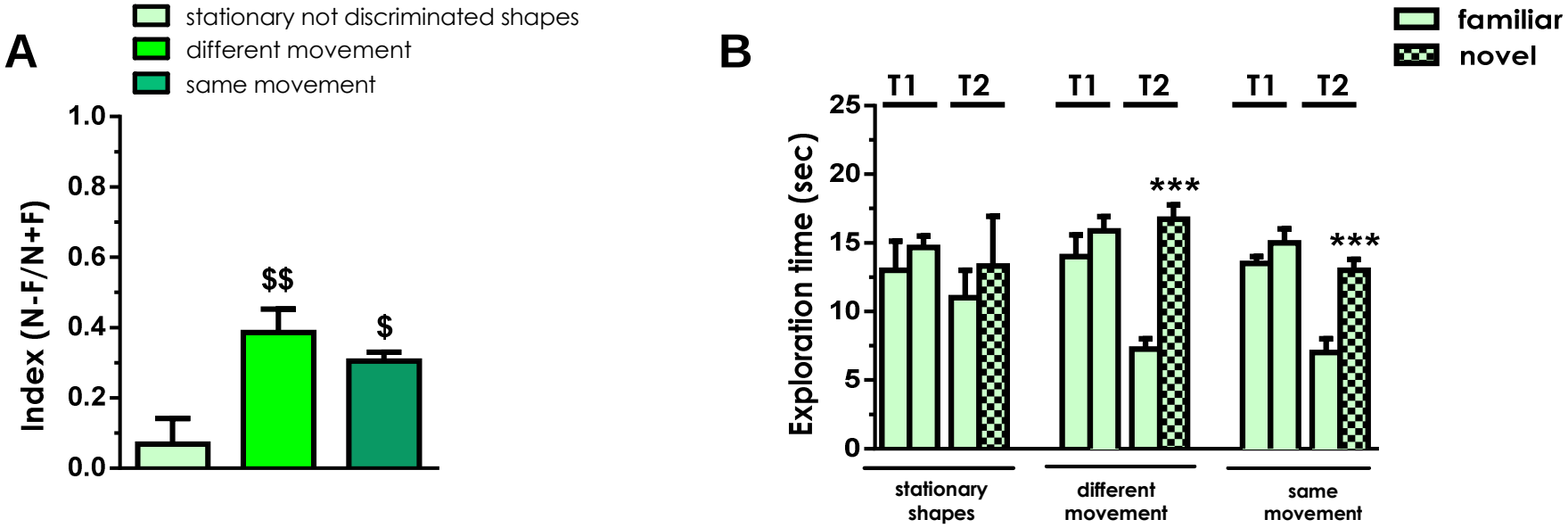


Figure 23: Effect of movement applied to not discriminated shapes in VORT. Mean discrimination index (A), mean exploration time (B) and examples of movements (C). During T1 the two identical shapes were presented having no movement (stationary shapes) or the same movement. During T2 a novel shape was presented without motion (stationary shapes) or with the same or different movement from T1. Data are expressed as mean ± SEM. N= 10 mice for each group. $ P<0.05, $$ P<0.01 vs corresponding stationary shape; *** P<0.001 vs corresponding familiar shape (Tukey's test).