Oxytocin Selectively Gates Fear Responses Through Distinct Outputs from the Central Amygdala
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Fear can be severely immobilizing but can also be a major driving force for some of humans’ most heroic acts. In both cases, the internal emotional experience may be similar, although it may lead to substantially different behavioral outcomes (1–3). Studies on human emotions often use autonomic nervous system parameters to assess arousal, because of the role of our internal organs in the emotional state (4–5). Projections from the central nucleus of the amygdala (CeA) to the hypothalamus and different brain stem nuclei coordinate behavioral and physiological fear expression (6). It has been postulated that different fear responses, characterized by more active or passive behavioral coping strategies, can be triggered by a neuronal switch within the CeA (7). The question thus arises whether fear responses only vary in intensity, or whether different qualities of fear responses exist, reflected in different associations between behavioral and physiological components. We investigated whether a neurophysiological basis for such a distinct regulation could be found in the CeA.

**References**


**Supporting Online Material**

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Materials and Methods

SOM Text

Figs. S1 to S5

Tables S1 to S3

References

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Most projections from the CeA to the hypothalamus and brain stem nuclei originate from the medial part of the CeA (CeM) (6). Oxytocin can inhibit neurons in the CeM through its excitatory effects on γ-aminobutyric acid (GABA) inhibitory (GABAergic) projections that originate from the lateral and capsular part of the CeA (8) (henceforth referred to as CeL). The CeL contains distinct neuronal populations (7–10) whose individual activation may differentially regulate active versus passive fear responses (7). Can a similar distinction of neuronal populations be found in the CeM (10)? Do projections from the CeM to selective targets in the hypothalamus and brain stem arise from distinct neuronal populations and, if so, are these under a specific inhibitory control from the CeL? Such specificity might provide a neurophysiological basis within the CeA to selectively regulate behavioral and physiological components of the fear response.

We first evaluated the target specificity of CeM projections by double fluorescent retrograde tracing of the ventrolateral column of the periaqueductal gray (PAG), which is implicated in the freezing response (6), and the dorsal vagal complex (DVC), which modulates cardiovascular responses (11). We injected green and red fluorescent latex microspheres, respectively, into the PAG and DVC of 3- to 4-week-old Sprague-Dawley rats (12, 13). After allowing 48 hours of retrograde transport, we killed the animals, verified the injection sites (Fig. 1, A and B), and assessed retrograde label in horizontal brain slices of the CeM. Both green and red microspheres were present throughout the CeM (Fig. 1, A and B), yet in separate neurons that were intermingled without any obvious clusters (Fig. 1C and fig. S1). Confocal quantification revealed 5.9% colabeling (n = 680 neurons, Fig. 1D, fig. S1, and table S1). Injecting a mixture of green and red microspheres in the DVC resulted in their copresence in all labeled CeM neurons (Fig. 1C), confirming sensitivity to detect colabeling.

We next compared electrophysiological properties by whole-cell recordings from fluorescently labeled PAG- and DVC-projecting neurons (henceforth called CeM→PAGs and CeM→DVCs, respectively). CeM→PAGs (n = 42) were, on average, significantly more depolarized (−59.6 ± 1.6 versus −65.4 ± 1.2 mV) and had lower membrane resistance (37.3 ± 5.6 versus 58.5 ± 4.0 pF) than CeM→DVCs (n = 43, Fig. 1E). In cell-attached configuration, average basic spiking frequencies of CeM→PAGs (n = 22) were significantly higher than CeM→DVCs (4.1 ± 0.5 versus 2.5 ± 0.4 Hz, n = 28).

Prompted by these anatomical and electrophysiological differences, we also tested their pharmacological characteristics. Although both projection neurons were similarly excited or inhibited by a range of neuropeptides (table S2), oxytocin—known to increase spontaneous inhibitory }

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**Fig. 2.** Distinct effects of TGOT on CeM→DVC and CeM→PAG neurons. (A and B) Representative traces of sIPSCs (top) and their average frequencies (bottom) recorded before (control) and after (TGOT) application of (A) CeM→PAGs and (B) CeM→DVCs. (C and D) The same for spontaneous spiking activity (*F_{2,36} = 8.4, P < 0.005; **F_{2,352} = 17.8, P < 0.0001, n = 20 to 34 neurons) Error bars indicate SEM.

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**Fig. 3.** Local stimulation reveals inhibitory projections on TGOT-insensitive CeM→DVCs. (A) Local perfusion of neurons that project to CeM→DVCs (red) and CeM→PAGs (green) in a horizontal brain slice of the CeA with ACSF flow away from CeM, GABA-R, GABAₐ receptor; OT-R, oxytocin receptor; BLA, basolateral amygdala; LA, lateral amygdala; LC, intercalated neurons. (B) Average sIPSC frequencies in CeM→DVCs recorded with patch pipette “(1)” after bath-perfused TGOT followed by locally puffed KCl (n = 6), subsequently, in the same slice, in CeM→PAGs recorded with pipette “(2)” after bath-perfused TGOT (n = 6). (C) Action potential and sIPSC frequencies in respective CeL (n = 5) and CeM→DVC neurons (n = 5) after local perfusion with glutamate. (*P < 0.05, **P < 0.005) Error bars indicate SEM.
itory postsynaptic current (sIPSC) frequencies of CeM neurons (8) (fig. S2A)—only affected CeM→PAGs. Thus, bath perfusion of the oxytocin agonist [Thr4,Gly7]-oxytocin (TGOT) specifically and reversibly increased sIPSC frequencies in CeM→PAGs (from 2.2 ± 0.3 to 5.6 ± 0.7 Hz, n = 34) (Fig. 2A) but not in CeM→DVCs (from 1.5 ± 0.3 to 1.7 ± 0.2 Hz, n = 32) (Fig. 2B) (see also fig. S3). In cell-attached recordings, this translated into a selective inhibition of spontaneous spiking frequencies of CeM→PAGs (from 5.6 ± 0.7 to 3.1 ± 0.2 Hz, n = 10) (Fig. 2C) versus CeM→DVCs (from 3.5 ± 1.0 to 3.7 ± 0.9 Hz, n = 10) (Fig. 2D) (8).

To verify whether the failure of TGOT to inhibit CeM→DVCs was caused by the absence of inhibitory projections, we locally depolarized by puffing KCl (35 mM) from a patch pipette (13).
spinal fluid (ACSF) 400 similar for all groups [baseline: artificial cerebro-
rate before reexposure and the initial elevation 
ther discussed in (i)]. To activate the amygdala, we 
monitored heart rate. To activate the amygdala, we 
performed a 2-day contextual fear-conditioning pro-
tocol (13) that resulted in >90% freezing after conditioning and >80% freezing responses upon reexposure to the context in all vehicle-injected 
animals (Fig. 4, A and C, and fig. S2B). Bilateral 
injection of TGOT or GABA_A receptor agonist 
muscimol decreased these freezing responses to <50% (Fig. 4C). In rats where TGOT had not 
decreased freezing, the injection sites, identified 
with fluorescent muscimol (Fig. 4B), were outside 
the CeA (fig. S5), which confirmed the CeA role in these contextual freezing responses [further 
discussed in (13)]. Baseline heart rate before reexposure and the initial elevation 
in heart rate upon reexposure to the context were 
similar for all groups [baseline: artificial cerebro-
spinal fluid (ACSF) 400 ± 10; TGOT 394 ± 10; 
muscimol 394 ± 12 beats per min (bpm)] (Fig. 4D), 
the typical decrease that followed in the second 
10-min period was inhibited by muscimol but 
not by TGOT. Bombesin reduced freezing and 
preserved the decrease in heart rate (Fig. 4C2 
and 4D2) consistent with its inhibition of both 
CeM→PAGs and CeM→DVCs. Together, these data 
not only support the selective action of oxytocin 
on freezing behavior via CeM→PAGs, but also 
suggest a critical role of CeM→DVC neurons in 
the control of cardiovascular changes to fearful 
stimuli. Finally, heart rate variability (HRV) anal-
ysis (13) revealed an absence of increase in the high frequency band in the muscimol-treated rats 
(Fig. 4E), which indicated an inhibition of the 
parasympathetic activation (13) (table S3 and fig. 
S7). The failure of TGOT to affect this para-
sympathetic cardiovascular response is consistent 
with the absence of TGOT effects on CeM→DVCs (11, 15).

The present findings provide evidence that specific behavioral and physiological compo-
nents of the fear response are controlled by dist-
inct neuronal populations in the CeM (Fig. 4F). 
These project to the PAG and the DVC; exhibit 
unique electrophysiological characteristics; and 
despite being spatially intermingled, are selec-
tively modulated by oxytocin through inhibitory 
projections from the CeL. The functionality of 
this selectivity was further revealed at the behav-
ioral and physiological level by oxytocin’s 
inhibition of freezing responses without affect-
ing cardiovascular changes. Previous studies 
have distinguished neuronal populations in the 
CeL on the basis of expression of CRF or opioids 
(16) or on mutually inhibitory connections (10). 
These may play a specific role in the inhibition of 
CeM→PAGs, and, in combination with the dis-
tinct electrophysiological characteristics of CeM 
neurons, affect further local information processing 
[see e.g., (17)].

A specific regulation by the CeL of CeM neu-
rons with different projections could have impor-
tant implications for the mechanisms underlying 
the expression of fear. Distinct neuronal popu-
lations in the CeL are activated or inhibited dur-
ing the expression of fear (9, 10) and this might 
represent a switch between active versus passive 
fear and associated coping strategies (7). Our 
recent findings imply that the CeM differentially 
controls expressions of the fear response through separate projections to the brain stem. Our find-
ings, instead of supporting a rigid association be-
 tween behavioral and physiological expressions 
of fear (1, 2), suggest that these expressions may 
be specifically controlled by the CeL. This under-
lines, first, the importance of considering mul-
tiple parameters in the correct assessment of fear 
responses in animals, but it also opens the poten-
tial for new therapeutic applications (fig S8). 
In humans, panic disorder can manifest itself at the 
visceral level predominantly by increases in heart 
rate, respiratory rhythm, or gastrointestinal mo-
tility (18). While its onset appears to be trig-
gered in the lateral and basal amygdala (19), its specific expression may result from a differen-
tial gating within the CeA. Although panic and other anxiety disorders are typically treated with 
benzodiazepines, future neuropeptide-based ther-
apies might offer a more precise inhibition of their expression.

The amygdala orchestrates behavioral re-
sponses to both negative (fearful) and positive 
(rewarding) salient stimuli (20, 21), although the 
precise underlying circuits are still unclear. Dis-
tinct, intercalated CeL and CeM populations, tar-
targeted by projections from the basolateral amygdala 
(22) and brain stem (11), could play a role in 
regulating behavioral and physiological expres-
sions associated with different emotions (3–5). 
Furthermore, the CeA expresses a multitude of 
neuropeptide receptors that can specifically affect 
local activity (8, 23–25). Levels of oxytocin and 
its receptors can vary between individual rats ac-
cording to genetic background (26), early life 
experience (27), internal state (28), or environ-
ment (24, 29) and have been associated with 
different degrees of anxiety and fear (27). As we 
found that oxytocin decreases freezing responses, 
but leaves cardiovascular responses unaffected,
this specific regulation could preserve the in-
ternal, visceral expression of fear, but alleviate 
the behavioral inhibition that leads to freezing. 
Such individual regulation may provide the most 
dequate reaction in circumstances when a pro-
aactive behavioral response is required, while 
preventing an internal, visceral adaptive response to fear.